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Clair H. Hershey

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Cassava

Genetic Improvement: Theory and Practice

CGIAR

The Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) delivers research-based solutions that harness agricultural biodiversity and sustainably transform food systems to improve people's lives. Alliance solutions address the global crises of malnutrition, climate change, biodiversity loss, and environmental degradation.

The Alliance is part of CGIAR, a global research partnership for a food-secure future.

About the author

Clair Hershey led a dedicated and talented research and support team as cassava breeder at the International Center for Tropical Agriculture (CIAT) (now part of the Alliance of Bioversity International and CIAT) in Cali, Colombia, from 1978 to 1991. Much of the content of this book derived from that experience. He got PhD training for this position in the Department of Plant Breeding, Cornell University, Ithaca, NY, with a degree in 1978. During his tenure, he managed the field genebank, completed extensive agronomic and consumer-related trait evaluation, and oversaw a doubling its size. The breeding program collaborated extensively in the Americas, Asia and Africa, with training, variety development and germplasm exchange. A key innovation was the establishment of an agro-ecosystem and market-oriented breeding system for global germplasm development.

After leaving Colombia, Dr. Hershey entered a family farm business in Pennsylvania, USA, as crop and financial manager. For 11 years, he edited *Plant Breeding News*, a monthly e-newsletter with over 1,500 subscribers, sponsored by the Food and Agriculture Organization of the United Nations (FAO) and Cornell University. During 2009–2010, he was a visiting scientist at FAO headquarters in Rome, supporting development of the Global Partnership Initiative for Plant Breeding Capacity Building. In 2011, he returned to CIAT as leader of the Cassava Program and as leader of the Crop Discovery Research flagship project for the CGIAR Research Program on Roots, Tubers and Bananas (RTB). He held these positions until retirement in 2016. Currently, he lives with his wife near his roots in central Pennsylvania. Along with periodic consulting, he enjoys time with the families of his three children and eight grandchildren, as well as local travel, biking, canoeing and woodworking.

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A NOTE TO THE READER

This book was first developed in 1986, and has undergone mostly minor updates and revisions since that time, the latest in 2010. As such, it does not pretend to include the vast advances in cassava breeding of the past decade. It has been distributed through multiple forums over the years, and we wish now to create a home for it in CGSpace, a joint repository of several CGIAR Centers, as a legacy publication for a new generation of cassava breeders. Although not up to date in some areas, I hope this knowledge will serve as a valuable baseline for future research and methodologies.

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Several people reviewed early drafts of this book. Kazuo Kawano, Tan Swee Lian and Carlos Iglesias deserve special thanks for their detailed input. FAO in Rome provided professional style editing to a version of this book in 2010. Any errors or omissions are, of course, my own responsibility.

Clair H. Hershey

INTRODUCTION

Cassava breeders rely on a wide range of information to succeed in their task of developing genetically superior varieties for adoption in the production, processing and market value chains. The published literature on cassava has risen exponentially in the past quarter century, and provides a fundamental basis for application of scientifically sound research practices. Yet, most publications are rather narrowly focused, and the task of integrating knowledge and techniques across a vast literature for purposes of planning and executing a breeding program can be overwhelming. Unlike for most major crops, there has not been a comprehensive treatment of the fundamentals of cassava breeding from a practical and theoretical standpoint. This field guide aims to fill that gap, as a tool aimed mainly at applied cassava breeders. It will also provide support for those many research programs that collaborate with breeders, such as physiologists, agronomists, soil scientists, plant pathologists, entomologists, economists and social scientists, among others.

The book is structured to provide a broad general background, and a framework for developing and managing a comprehensive cassava breeding program. Chapters 1 to 3 describe the work of a cassava breeder, provide an overview of the cassava plant and its products, and look at goals, strategy and research management for cassava breeding. Chapters 4 and 5 focus on germplasm resources, from evolution and the description of resulting diversity, to management and exchange of germplasm. Chapters 6 and 7 review breeding and selection methods for defining and describing the target areas for developing and testing new genetic materials. Chapter 8 describes systems to develop and execute comprehensive information management. Chapter 9 is an introduction to quantitative genetics, with examples from cassava. Chapter 10 covers the strategy and the techniques to appropriately select parents in a crossing program. Chapters 11 to 13 get to the core of field activities in a cassava breeding program – making pollinations, seed and seedling management, and the management of preliminary through advanced trials. Chapters 14 to 18 review key categories of selection objectives, including adaptation and stability in the target agro-ecosystem, yield potential and canopy characteristics, pest and disease resistance, root form and quality, and finally, the all-important balancing among all these objectives. Chapter 19 reviews marker-assisted selection. Chapters 20 to 22 describe key ways to assure that varieties meet end-user needs, through participatory breeding and regional trial networks, and finally through appropriate varietal release and multiplication schemes. Finally, Chapters 23 and 24 cover the various ways to measure success in a cassava breeding program, to assure that goals are being met, and give a forward-looking appraisal of the future for cassava breeding.

This book is derived primarily from my experiences and learning during the thirteen years that I was a cassava breeder at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia (1978-1991). The content evolved somewhat over time, but there is certainly the need for updates in many areas. While most of the fundamentals endure, there has been remarkable progress in cassava research in the past 20 years, with impact on the potential to accelerate genetic gains through cassava breeding.

Feel free to distribute to colleagues who may have interest in the contents of the book for research or training purposes. Use of this material for commercial purposes is prohibited.

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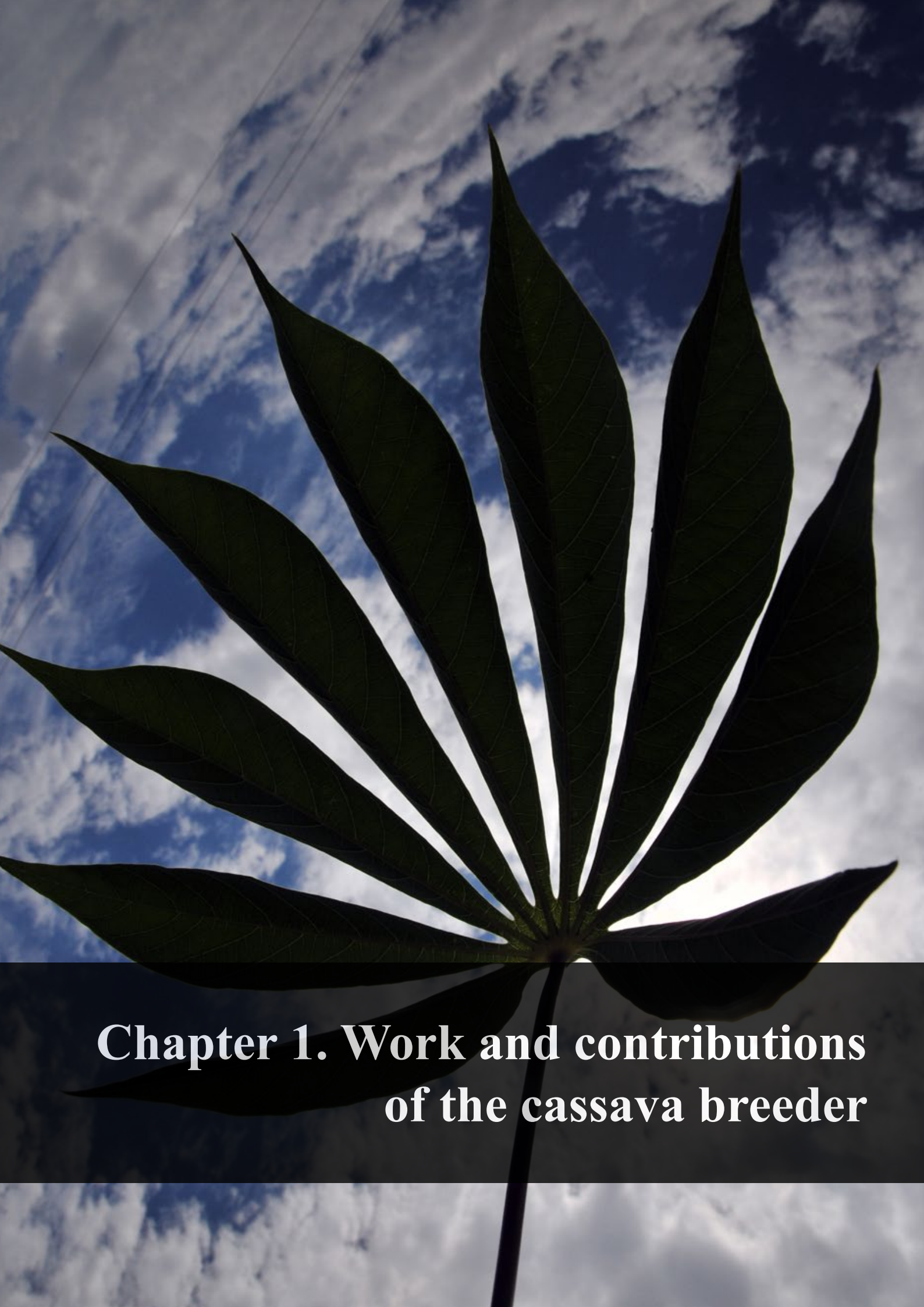
ACRONYMS AND ABBREVIATIONS

ALFP	Amplified fragment length polymorphism
AMMANET	African Molecular Marker Network
AMMI	Additive main and multiplicative interaction model
ARTS	African Root and Tuber Scale
AYT	Advanced yield trials
BA	Benzylaminopurine
BAC	Bacterial artificial chromosome
BlLS	Blight Leaf Spot
BrLS	Brown Leaf Spot
BSA	Bulked segregant analysis
BT	Bacillus thuringensis
CAD	Cassava Anthracnose Disease
CBB	Cassava Bacterial Blight
CBSD	Cassava Brown Streak Disease
CCMV	Cassava common mosaic virus
CEMSA	Centro de Mejoramiento de Semillas Agamicas
CENARGEN	EMBRAPA Genetic Resources and Biotechnology Centre, Brasilia, Brazil
CET	Clonal evaluation trial
CFSD	Cassava Frogskin Disease
CGM	Cassava Green Mite
CGR	Crop Growth Rate
chs	Chalcone synthase
CIAT	Centro Internacional de Agricultura Tropical [International Center for Tropical Agriculture] (now part of the Alliance of Bioversity International and CIAT)
CLAYUCA	Latin American and Caribbean Consortium for Support of Cassava Research and Development (both abbrev. appear in chapter 1)
CMD	Cassava Mosaic Disease
CNP	Cyanogenic potential of cassava
CNPMF	Centro Nacional de Pesquisa de Mandioca e Fruticultura
CORPOICA	Corporación Colombiana de Investigación Agropecuaria, Colombia (currently known as AGROSAVIA)
COSCA	Collaborative Studies of Cassava in Africa
cpDNA	Chloroplast DNA
CRLS	Concentric-ring Leaf Spot
CsCMD	Cassava common mosaic disease
CTCRI	Central Tuber Crops Research Institute
DarT	Diversity array technology
DM	Dry matter
DSC	Differential Scanning Calorimetry
dsRNA	Double-stranded RNAs
EARRNET	Eastern Africa Root Crops Research Network
ECZ	Edaphoclimatic zone
ELISA	Enzyme-linked Immunosobent Assay
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária (Brazil)
EMPASC	Empresa Catarinense de Pesquisa Agropecuaria Santa Catarina, Brazil
EMS	Ethyl methane sulfonate
EST	Expressed Sequence Tag
ETH	Swiss Federal Institute of Technology
FAO	Food and Agriculture Organization of the United Nations

FAOSTAT	FAO Statistical Database
GBSS	Granule-bound starch synthase
GCA	General combining ability
GCDS	Global Cassava Development Strategy
GCP-GI	Global Cassava Partnership for Genetic Improvement
G-E	Genotype by environment interaction
HCN	Hydrocyanic acid
HI	Harvest index
HNL	Hydroxynitrile lyase
IAA	Indole acetic acid
IAC	Instituto Agronómico do São Paulo, Brazil
IARCs	International Agricultural Research Centres
ICA	Instituto Colombiano Agropecuario, Colombia
IDIAP	Instituto de Investigación Agropecuaria de Panamá
IFAD	International Fund of Agricultural Development
IITA	International Institute for Tropical Agriculture
ILTAB	International Laboratory for Tropical Agricultural Biotechnology
INEAC	Institut National pour l'Étude Agronomique du Congo Belge
INIA	Instituto Nacional de Investigaciones Agropecuarias
INIFAP	Instituto Nacional de Investigaciones Forestales y Agropecuarias
INIVIT	Instituto Nacional de Investigaciones en Viandas Tropicales
IPB/UPLB	No definition
IPGRI (formerly IBPGR)	International Plant Genetic Resources Institute (currently Bioversity International, now part of the Alliance of Bioversity International and CIAT)
ISSRs	Inter-Simple Sequence Repeats
ISTRC	International Society of Tropical Root Crops
ISTRC-AB	International Society of Tropical Root Crops - Africa Branch
LAI	Leaf area index
MAS	Molecular-assisted selection
MBL	Medical Biotechnology Laboratories
MLO	Mycoplasma-like organisms
MTA	Material transfer agreement
NAA	Napthalene acetic acid
NARS	National Agricultural Research Systems
NRCRI	National Root Crops Research Institute
PAL	Phenylalanine lyase
PCA	Principal component axes
PCR	Polymerase Chain Reaction
PHD	Post-harvest root deterioration
PPB	Participatory plant breeding
PR	Participatory research
PRONAM	Programme National Manioc (Dem. Republic of Congo)
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction Fragment Length Polymorphisms
SACCAR	Southern African Centre for Cooperation in Agricultural Research and Training
SADC	Southern African Development Community
SARRNET	Southern Africa Root Crops Research Network
SCA	Specific combining ability
SCATC	South China Academy of Tropical Crops

SCRI	Scottish Crop Research Insitute
SED	Superelongation Disease
SI	Selection index
SINGER	System-wide Information Network for Genetic Resources (CGIAR)
SLU	Swedish Agricultural University
SMTA	Standard Material Transfer Agreement
SNP	Single nucleotide polymorphisms
SSR	Simple sequence repeats
TMS	Tropical Manioc Selection – (IITA designation)
UPLB	University of the Philippines, Los Baños
VISCA	Visayas State College of Agriculture, Leyte, the Philippines
WLS	White Leaf Spot



Chapter 1. Work and contributions of the cassava breeder

Society expects substantial benefits from the work of scientists who dedicate themselves to improving the world's food supply. These expectations have largely paralleled the ability in modern times to meet the food needs of rapidly expanding and urbanizing populations. The prerequisites to this success are complex and multifaceted. Understanding the genetic and physiological basis for plant behaviour was a key catalyst that allowed the remarkable advances of the twentieth and early twenty-first centuries. However, the entire system of plant production came into play as methods were developed to make soils better suited to high productivity, to manage water, and to control weeds, insects and pathogens. Clearly, the successes have not been complete, with periodic or even chronic food shortages still plaguing some areas. New information and technologies give promise to the fact that advances in crop productivity will continue well into the future.

Although agriculture has realized exponential gains in productivity in the past century, crop genetic improvement[1] is far from being a new science; it is in fact one of the most ancient of agricultural activities. As hunter-gatherer societies evolved to sedentary agriculture, human selection added to the forces of natural selection, to create crops and crop varieties that better served the purposes of humankind. Early plant domesticators certainly had no concept of genes, nor of their manipulation by crossing and selection. However, they obviously achieved considerable genetic advance over centuries by continually choosing superior plants in environments and conditions modified to support improved productivity.

Even in modern times, in spite of rapid advances in genetics and the molecular basis of variation, plant breeders often have a very incomplete understanding of which specific genes are being recombined, expressed and selected. Capabilities for precise genomic characterization have only recently begun to emerge. There is a wide and ever-increasing range of tools at the disposal of breeders. These tools can accelerate the process of improvement far beyond that achieved in earlier times. It is also possible to bring together germplasm from diverse geographic origins, an option greatly enhanced by global communication and transportation systems. Pest and pathogen testing techniques are often very precise and allow germplasm exchange at very low levels of risk. At the same time, the legal constraints to free exchange are tightening, due to the application of intellectual property rights by private companies.

After seven years of negotiations, the FAO Conference adopted the International Treaty on Plant Genetic Resources for Food and Agriculture, in November 2001. The Treaty entered into force in 2004, and provides a multilateral system of access and benefit-sharing (www.planttreaty.org). This legally-binding Treaty covers all plant genetic resources relevant for food and agriculture. It is in harmony with the Convention on Biological Diversity. The Treaty is vital in ensuring the continued availability of the plant genetic resources that countries will need to feed their people. Under the Treaty, genetic resources are available under a Standard Material Transfer Agreement (SMTA) (ftp://ftp.fao.org/ag/cgrfa/gb1/SMTAe.pdf).

Developing new crop varieties is a lengthy process, even with the most advanced tools, and requires a sustained commitment of resources for many years. Despite progress in pinpointing and manipulating target genes, the essential multiple-site and multiple-year field testing are still essential, and time-consuming. In addition, variety development is increasingly subject to regulatory processes that can further delay release, especially for food crops. Some of the techniques for genetic improvement, especially gene transfer among widely divergent species, have met considerable consumer resistance. Ten to fifteen years is a typical period from selecting parental lines, to commercial production of a new crop variety. Most plant breeding programmes, both private and public, are based on the assumption that genetic progress can continue unabated, perhaps indefinitely. This optimism is backed up by the steady, long-term success in crops where there has been a stable, critical mass of scientific expertise and adequate funding.

[1] *Genetic improvement* and *breeding* are used interchangeably throughout the text.

In the strict sense, plant breeding is the manipulation of genes controlling characters of interest, followed by selection of resulting plants that most closely approach the desired combination of characters. Most successful plant breeders, however, view their work in much broader terms. Their work comprises a blending of science and art. The breeder's eye applied in selection derives from scientific training, experience, intuition, common sense, and sometimes a measure of good luck. The actual process of selection, usually considered the cornerstone of breeding, is often only a small part of what most plant breeders do. Their work also involves skills in obtaining funding, research design, personnel management, budget planning and management, and presenting research results in various forums. There is also some degree of political acumen required to prosper within the idiosyncrasies of any institutional environment, whether public or private.

Like most areas of science, plant breeding continues to become more complex and more dependent on the interactions of many disciplines. Breeding programmes do not succeed or fail only because of the quality of a scientist's training in plant breeding. Success comes from a focused and well-designed programme, and these traits are not exclusive to those that are well-funded. An appropriate focus may make poorly funded programmes efficient and productive. Networking can also improve efficiencies by drawing on outside knowledge and resources and solving problems collaboratively.

Plant breeding has been broadly viewed, for several decades at least, as a powerful tool for achieving not only economic but also social objectives. When Norman Borlaug received the 1970 Nobel Peace Prize for the benefits derived from new wheat varieties in developing countries, plant breeding attained considerable prominence for the general public. Sometimes expectations have been unrealistic. Societal inequities, at times widened by new technology, have been blamed on breeders being insensitive to the social implications of their research. Plant breeding can have significant social consequences, either positive or negative, but it is the larger socio-economic milieu that will set the stage for these effects. Plant breeding is in fact a rather blunt tool to achieve social change, and although it may be seen as an influence on many, it cannot realistically be seen as a major instrument toward these ends.

On the other hand, scientists do not work in a social or political vacuum. It is not the objective of this work to suggest ethically appropriate goals and strategies for cassava breeding; it is essential however, that the breeder recognizes that his or her efforts do have social implications and that these need to be taken into account at the outset. Selection under high fertility conditions may produce a variety that performs well only with high fertilizer input, which in turn may only be available to farmers near commercial centres. Protection of nurseries with pesticides may result in susceptible varieties that require chemical pest control, and the chemicals can introduce human health hazards. Selection of a few very broadly adapted varieties may lead to narrowing of the genetic base in a region, with potential loss of new sources of diversity for breeding.

These are but a few examples. Rarely are the decisions of a strictly right or wrong nature. Rather, the balance of expected positive and negative aspects of any pathway of choices will have to be weighed. One goal of this text is to present a range of options available in setting of objectives, and some of the criteria that can influence decisions. It is not proposed to outline any universal standards to which the breeder should adhere in making judgments about the expected profile of socio-economic impact.

1. CASSAVA AS A TARGET FOR GENETIC IMPROVEMENT

In their formal training, plant breeders usually receive considerable theoretical background (e.g. genetics and breeding, molecular biology, statistics, pathology, entomology and physiology). Most programmes also provide students with some experience in practical, applied breeding projects. These practical examples, however, usually do not include cassava, or include it very superficially. A breeder assigned to cassava often has his or her first real exposure to the improvement of the crop on the first day on the job.

Cassava's uniqueness as a subject for genetic improvement is highlighted by the combination of vegetative propagation (and its implications for genetic structure of a variety), monoecious flowering habit, a high degree of heterozygosity, and a long growing season. The physical and biological diversity of growing environments and a range of end uses, may complicate the setting of breeding objectives. Its position as a crop of the poor profoundly influences strategies for technology development, testing and diffusion. The rapid expansion of the market for cassava products can create both challenges and opportunities for breeding. While initially left behind in the adoption of molecular-assisted breeding, cassava is now amenable to most techniques that are applied to more generously funded crops.

2. GOALS OF PLANT BREEDING

A common goal of plant breeding is to increase the productive potential of a crop. This has been the most evident contribution of crop genetic improvement over time. However, yield potential can be an elusive goal. Most crops are affected by a wide range of yield-limiting factors, such as pests and diseases, water and nutrient deficits or soil physical traits. Breeders and agronomists work jointly to protect yield gains by incorporating resistance, or modifying the environment. As increasing production potential becomes more difficult due to reaching genetic plateaus, and as markets become more diverse or more sophisticated, incorporating value-added traits may gain prominence. In the case of cassava, this often involves root quality traits.

During the past few decades many breeders have begun to address the public’s increasing concern about the potential environmental degradation that improperly managed agriculture can cause or exacerbate. This is not exclusive to any one sector, region, or economic stratus of farming. It is a pervasive concern developing from increasing pressure on quality and quantity of land, water, nutrient and energy resources.

In traditional systems, scarcity of agriculturally productive land often forces a decrease in fallow periods as populations increase, resulting in erosion and declining soil fertility. In technology-intensive agriculture, concern centres on non-renewable energy inputs, agrichemicals and their direct or indirect effects on human health and the environment, genetic uniformity of varieties and perceived health or environmental risks of genes transferred from unrelated species.

Several major crops may be vulnerable to pests or pathogens, or to climatic variations, from use of a narrow germplasm base. Across the continuum of levels of technology adoption, there are concerns about how agriculture can keep pace with increased demand in the long term, without undue cost to the environment. Plant breeding is a key component in the comprehensive approach needed to sustain food production in the long-term future.

3. PROFILE OF A CASSAVA BREEDER

An attempt to describe the average cassava breeder and what he or she does will not completely fit any one individual. There are, however, generalized characterizations that can be useful in understanding this small group of specialists.

Many programmes have only one or two trained cassava breeders for the entire country, and several countries where cassava is a major crop, have none. In the early 2000s there were in the order of 120 full-time or near full-time cassava breeders/germplasm specialists worldwide. About half of these worked in Africa, about 40 in Asia and 20 in Latin America, roughly proportional to the area of the crop in each region. However, to give some additional perspective on this number, more than 500 scientists work on maize breeding in the United States alone, with more than 90 percent in the private sector (Lamkey and Staub, 1998).

Many other specialists are involved directly or indirectly in breeding. Entomologists and pathologists dedicate part of their time to evaluating resistance and advising on breeding strategies; physiologists

and agronomists evaluate for diversity of specific adaptation/performance traits; extension personnel are involved in regional and on-farm trials and in varietal release; social scientists and economists evaluate the *ex ante* impact of different research options, and give feedback on economic benefits; and the growing network of biotechnology-related specialists contribute directly and indirectly to cassava genetic improvement.

Cassava breeders generally: (1) work in the tropics or subtropics; (2) have broad responsibilities outside what would normally be considered plant breeding activities; (3) work with crops other than just cassava, often other root crops; (4) work within a team of scientists, either inter- or intradisciplinary; (5) are public sector employees, in a research institute or university; (6) work in an institution with persistent funding difficulties; and (7) have considerable contact with other cassava scientists at the international level. None of these characteristics, however, is a fixed attribute; continual change in this description can be expected.

Cassava breeders, perhaps because of the long-term nature of genetic improvement of the crop, or perhaps because the work is fundamentally interesting and satisfying, often have made long-term careers in the crop. This is certainly advantageous in giving the continuity of effort that is required for steady incremental genetic improvement, the main output of most breeding programmes.

4. THE BREEDER'S TASKS

Plant breeding is more than designing a series of related experiments. It involves design and implementation of a comprehensive system, which will normally span at least 15–20 years. Individual scientists are usually responsible for defined segments within the long continuum of breeding-related activities, from basic genetic studies, to germplasm management, to varietal release, to impact studies. As cassava research teams tend to be small, and because private industry plays a relatively minor part in cassava improvement, the responsibilities of cassava breeders tend to be broader than those of breeders for many other crops. As a rule, less specialization is possible. As networks, both national and international, develop further, there should be more possibilities for appreciating the benefits of the broad sharing of specialized research. Where breeding is part of a university programme, the breeders typically have both teaching and research responsibilities.

Most breeders dedicate the majority of their research time to managing selection in preliminary through to advanced trials, and to the promotion of new varieties to farmers. Some are responsible for maintenance and evaluation of a small- or intermediate-sized germplasm collection. Probably not more than one-fourth of the world's cassava breeders make crosses as a means of obtaining new variability. Most receive seeds or vegetative material from international programmes, and a few rely solely on existing variation in local clonal material. Many breeders have a shared responsibility with extensionists for evaluating new experimental varieties in farmers' trials, and for subsequent release for commercial use.

5. RESOURCES AND TOOLS OF THE TRADE

The basic raw materials with which plant breeders work are genetic diversity and environments in which the characters of interest will be expressed. Each of these principal areas includes a set of tools which aid the breeder in accomplishing objectives. These are introduced here, and further detailed in subsequent chapters.

5.1 GENETIC DIVERSITY

For establishing and managing the base of genetic diversity, the breeder requires: (1) systems of creating or introducing genetic diversity; (2) a germplasm conservation system; and (3) an information management system.

A number of techniques is available to create variability, including hybridization, mutation, modification of ploidy levels, somaclonal variation and recombinant DNA techniques.

Cassava germplasm conservation is typically accomplished with field-grown plants, but is increasingly in the form of *in vitro* plantlets in controlled environments. Cryopreservation of meristem shoot tips or other tissues will likely be the conservation method of choice in the future. Pollen or seed conservation may be used to conserve genes, but not specific gene combinations because of the species' highly heterozygous nature.

Advancing computer and communications technologies have vastly broadened the options for information management in the past few decades. Nearly all cassava breeders have access to a computer, but at the same time, the design and deployment of integrated information management systems are often poorly developed.

5.2 SELECTION ENVIRONMENTS

This text uses the term *environment* in the broad sense, including laboratory, greenhouse, screenhouse and field environments. A breeder must pay close attention to defining the conditions to which the genetic diversity should be exposed in order to express its important inherent traits accurately. Various tools aid in characterizing and selecting appropriate environments (e.g. agro-ecological databases, remote sensing and variety trials), manipulating environments (e.g. increasing or suppressing pest populations, irrigation and fertilization), or better observing variation in plant response (e.g. photosynthetic rate, pest reactions, quality variations). With the tools of molecular biology, there will be a continually more refined ability to observe variations among genotypes at the DNA level, and to relate these differences to traits of interest.

6. EARLY SELECTION BY FARMER-BREEDERS

Plant breeding is part of the evolutionary continuum ranging from domestication of wild progenitors, to farmer selection, to the application of modern science. Cassava breeding began when women first consciously propagated a particular plant of ancestral cassava in preference to another of a different genotype. This type of selection apparently began 5 000 to 7 000 years ago, and continues to the present, especially in traditional systems.

Cassava was probably vegetatively propagated since the time of domestication. This is a genetically conservative strategy, since new variability is slow to arise, but with the advantage that any superior genotypes are fixed. Although asexually propagated in virtually all commercial plantings, cassava also produces true seeds. These may be either the result of a recombination between distinct genotypes, or sometimes of selfing. The extent of new variability that these seeds represent depends upon the cultivation system and genetic composition of materials from which they are derived. In a monoclonal situation (single, genetically uniform variety in a field), seeds result from selfing or its equivalent (crosses among genetically identical plants). In a diverse mixture of genotypes (more typical of traditional cassava culture) the diversity arising from naturally produced seed may be very broad.

There are apparently no documented examples of farmers anywhere, either at present or in the past, actually collecting cassava seeds and planting them in separate nurseries for the express purpose of selecting new varieties. There are, however, various reported observations of farmers recognizing cassava seedlings that arise spontaneously in their fields (e.g. Hahn, 1979; Hershey *et al*., 1979). Farmers may recognize the plants derived from these seeds as potential new varieties, and give them special care to compensate for their lower vigour at the initial stages. They then vegetatively propagate them for comparison directly with existing varieties. This process, while evidently the principal form for the creation of diversity within *Manihot esculenta* over thousands of years, was probably never a common practice, but rather occasional and regionally sporadic, as it continues to be to this day. On the other hand, many farmers recognize seedling plants but consider them weeds, plants that will be too weak to produce significant yield.

Cassava farmers everywhere have the custom of testing new varieties and replacing old ones over time. The frequency of this testing and the rate of replacement can vary widely. In general, farmers test many more varieties than they adopt for use. Experience indicates that some core clones may retain a significant role across several generations of farmers. It seems that, typically, the predominant clones in a traditional cassava-growing region change every 10 to 20 years. Change can be the result of a decline in performance, or of a new and better introduction replacing stable, older varieties. The Collaborative Studies of Cassava in Africa (COSCA) quantified reasons farmers gave for abandoning cassava varieties. Although yield-related traits headed the list, about two-thirds of varieties were abandoned for other reasons (Table 1.1).

Table 1.1 Reasons given by farmers for abandoning cassava varieties in Africa

Reason	Percent
Low bulking of roots	20
Low yield	16
Weed competition	11
Poor in-ground storability	10
Susceptible to pests and diseases	8
Poor processing quality	7
Undesirable branching habit	5
High cyanide content	5
Poor cooking quality	2
Poor yield of planting material	1
Introduction of better varieties	1
Susceptible to drought	1
Low leaf yield	1
Others	12
Source: COSCA, and Nweke (1994)	

Indigenous farmers in the Americas may maintain more than 100 distinct, identifiable cassava clones in cultivation. Typically, however, only a relatively few varieties account for most of the area planted. The question arises as to why, if a few clones have been identified as superior, the less desirable ones are maintained at all. In fact, farmers themselves may be at a loss to explain why they maintain the less desirable types (Boster, 1984). Whether consciously or unconsciously on the farmer's part, adaptive factors may be some of the most important influences on cassava variety selection in traditional systems. Farmers are aware that environments (both biological and physical) may be highly variable throughout the years. Diversity is a means of assuring that some proportion of the varieties grown will tolerate most combinations of stress that may arise in a given season. Diversity is not a strategy for maximizing yields in any given environment, but is an insurance against failure, especially in unpredictable environments. If conditions change over time, for example, from changes in cultural practices or pest and disease evolution, the multiple-variety system has enough plasticity to adapt. With a large number of cultivated genotypes, the genetic plasticity may approach that of open-pollinated species, where each individual is genetically distinct.

The influences of biological and physical conditions on cassava's evolution appear to have been somewhat localized. Due to wide early dispersal of the crop and relatively low levels of later genetic interchange among regions, many distinct, locally adapted gene pools evolved. Later chapters elaborate on the significance of this for breeding strategies.

7. THE MODERN ERA OF SELECTION BY SCIENTIST-BREEDERS

Involvement by trained plant breeders in cassava improvement began early in the twentieth century. Although records are often lost or held in obscure places, it appears that the earliest programmes were in Brazil, India, Indonesia, Madagascar, Nigeria and the United Republic of Tanzania . Some of these were small, isolated programmes that were discontinued before or during World War II. Table 1.2 lists some of the major landmarks in cassava breeding history and accomplishments.

Table 1.2 Historical perspective on cassava genetic improvement [a]

1930s
• Variety introduction and crossing in Indonesia and Malaysia to support starch market • Unsuccessful attempt to find resistance to cassava mosaic disease (CMD) in local varieties in East Africa • Resistance to CMD discovered in interspecific crosses with *Manihot glaziovii*
1940s
• World's major cassava producer, Brazil, established breeding programme in Campinas, Sao Paulo • Basic genetic and cytogenetic studies • Cassava breeding work in Asia disrupted due to World War II
1950s
• Low level efforts continue in breeding in national programmes of Africa and Latin America • Multiple-generation backcrosses of CMD-resistant hybrids to *Manihot esculenta* result in resistant varieties with good agronomic type • East Africa breeding programme on CMD discontinued
1960s
• Monograph on *Manihot* published (Rogers and Appan) • Major international collection initiated in centre of origin for cassava (Latin America)
1970s
• Two international centres (CIAT and IITA) develop cassava breeding programmes with global coverage • Comprehensive breeding methodologies established • Root quality methodologies, especially cyanogenic potential and starch content • Priorities and methodologies for host plant resistance to key pests • Physiological criteria defined for plant type • Demonstration of potential for large advances in yield potential • Training of large contingent of breeders for national programmes in Africa, Asia and Latin America • Development of *in vitro* techniques for germplasm conservation, cleaning and interchange • Thailand develops comprehensive breeding programme to respond to rapid growth in export market for dried chips
1980s
• Definition of agro-ecosystem based breeding strategy in CIAT, IITA, and several national programmes • Establishment of Cassava Biotechnology Network for application of advanced tools

- Advances in virus detection techniques and *in vitro* methods greatly increase level of security for germplasm exchange
- Causal agent of CMD determined to be a potexvirus
- Initiation of efforts in semi-arid tropics, with focus on Northeast Brazil and sub-Sahel Africa
- Development and incorporation of methods for more effective farmer participation in variety development
- Global economic downturn forces reduction in efforts of many programmes

1990s

- Adjustments to meet industrial demands in Latin America for chips, starch and flour
- Widespread adoption of new varieties in Asia, especially in Thailand
- Steady adoption of new varieties in Asia and Latin America
- Cassava genetic map
- First transformed cassava
- Outbreaks of variant biotypes of CMD in East Africa controlled with introduction of resistant varieties

2000s

- Continued rapid adoption of new varieties in Africa, Asia and the Americas
- Application of marker-assisted selection for host plant resistance and root quality traits
- Biofortification, especially for vitamin A, zinc and iron, become key breeding objectives for most vulnerable populations
- Haploid techniques investigated
- Molecular markers allow definition of potential heterotic gene pools

[a] *Nearly all highlights and accomplishments are the result of interinstitutional collaboration*

Warburg reported the CMD already in 1891 in East Africa (cited in Nweke *et al*., 2002). It continued to spread into West and Central Africa, and the severity motivated the British colonial government to launch a breeding programme at the Amani Research Station of the United Republic of Tanzania in the mid-1930s. Other stations taking up research soon afterwards were the Coast Experiment Station in Kibarani in Kenya, the Morogoro Experiment Station in the United Republic of Tanzania, the Agriculture Department in Zanzibar, and the Serere Experiment Station in Uganda (Nichols, 1947).

The Belgians and French established cassava breeding activities in francophone Africa, in Bambey, Senegal and in Yangambi in the Congo. The Institut National pour l'Étude Agronomique du Congo Belge (INEAC) established almost 40 research stations, and many included cassava trials. Scientists at the INEAC raised large numbers of seedlings, including some from crosses of parents from Brazil and Côte d'Ivoire.

One of the best-organized early breeding programmes was located at the Station agronomique de Lac Alaotra, Malagasy Republic (Madagascar). This programme was based on modern, high input conditions and involved a wide range of germplasm with resistance to CMD, large numbers of seedlings tested each year, selection through various stages and testing through an island-wide network. The programme terminated before major impact through adoption could be achieved.

The most successful early breeding programme in Africa was at the Amani Research Station in the United Republic of Tanzania, under the guidance of H.H. Storey and R.F.W. Nichols. They searched for resistance to the mosaic disease among a wide germplasm base, but found only moderate levels,

especially in some varieties from Java (Indonesia). Their breakthrough, however, came after crossing cassava with the wild species, *Manihot glaziovii.* World War II caused a scale-back in the breeding. Then in 1951 D.L. Jennings took over breeding, continued selection in the cassava x wild species populations, and achieved higher and more stable resistance. In 1956, one year before the Amani Research Station closed, he distributed segregating populations to several African countries. From these seeds, B.D.A. Beck and M.J. Ekandem, at the Moor Plantation in Ibadan, Nigeria, selected the now-famous hybrid, 58308 in 1958. On Nigeria's independence, the breeding programme moved to Umudike in Eastern Nigeria. Unfortunately, most of the materials and records were lost during the Nigerian civil war (1967–1970). However, the original 58308 had still been retained at the Moor Plantation. It became the basis for mosaic resistance breeding in the newly formed International Institute for Tropical Agriculture (IITA) breeding programme in Ibadan, headed by S.K. Hahn.
In Asia, breeding focused on yield potential for supplying starch markets. India developed another of the early and long-running cassava improvement programmes. Travancore University in Trivandrum, Kerala (southern India), began cassava research in 1940. This work expanded under an initiative by the Indian Council of Agricultural Research, through the establishment of the Central Tuber Crops Research Institute (CTCRI), also at Trivandrum. Breeding work in the early years emphasized basic studies on flowering behaviour, genetics, cytogenetics and breeding methods. This programme pioneered work in chromosome morphology, male sterility and interspecific hybridization. CTCRI collected 960 accessions locally, a surprisingly large variability for a country where introduction of the species was comparatively recent. Magoon (1969) suggests that many of these types resulted from chance seedlings or bud mutations. If these are from a narrow genetic base of original introductions, the range of genetic variability is probably not nearly as broad as total numbers would suggest.

In 1937, E.S. Normanha at the Instituto Agronómico do São Paulo (IAC), Campinas, State of São Paulo, Brazil began what would become the oldest continuing cassava breeding effort (Normanha, 1970). It is appropriate that Brazil, where native genetic diversity is most extensive, should have pioneered in cassava breeding work.

The establishment of two international agricultural research centres (IARCs) in the late 1960s, with responsibility for integrated cassava research and for collaboration with national research programmes, was a major catalyst in the evolution of national programme capacity for cassava breeding. IITA was established near Ibadan, Nigeria. The cassava section of the Tuber and Root Improvement Programme of IITA collaborated with programmes in Africa and India (due to the presence of CMD in the latter). At this point the mosaic resistance was well established in the East Africa and Moor Plantation populations, and IITA focused on combining this with higher yield potential. This began an extensive breeding scheme to serve much of Africa in the coming decades. The programme is grounded in the series known as the Tropical Manioc Selection (TMS) varieties. By the early 1990s, TMS 30572 was the most popular variety grown in Nigeria (Nweke *et al*., 2002).

A sister centre, the Centro Internacional de Agricultura Tropical [International Centre for Tropical Agriculture] (CIAT), began operations in 1967 near Cali, Colombia. Although cassava research was initially part of a root and tuber crops programme, as at IITA, work rapidly focused exclusively on cassava. CIAT now has global commitments, with a regional office in Thailand that focuses on cassava. Work in Africa is carried out jointly with IITA. Up to 1996, an interdisciplinary programme focused exclusively on cassava. In 1996 CIAT organized itself in a project mode and work on cassava became more integrated with other commodities and research thrusts.

While IITA began its work focusing on disease resistance (CMD and bacterial blight), CIAT began by aiming to increase yield potential. This reflected the regional constraints at the time. Over the years both programmes considerably broadened their objectives; both became more attuned to the diversity of cassava environments, the range of traits required for different end uses, and the potential of new molecular approaches.

For two centres that evolved in the midst of the Green Revolution, a certain influence from the breeding successes in wheat and rice was probably inevitable. There was some expectation initially that cassava

would respond rapidly to the selection for an improved harvest index as a way to achieve high yields. While this did indeed prove to be an effective way to increase yield potential under favourable conditions, much of the cassava area was subject to a range of physical and biological yield constraints that could only be overcome by long-term breeding efforts that included host plant resistance and tolerance to soil and climatic constraints.

Due in part to the stimulus provided by the internal demands for new cassava technology, and in part by the outreach work of the IARCs, many national research programmes on all the cassava-growing continents began, or expanded, breeding work in cassava during the 1970s. These included programmes in Brazil (Centro Nacional de Pesquisa de Mandioca e Fruticultura [CNPMF], Cruz das Almas, Bahia); Mexico (Instituto Nacional de Investigaciones Agropecuarias [INIA]; currently Instituto Nacional de Investigaciones Forestales y Agropecuarias [INIFAP], Huimanguillo, Tabasco); Cuba (Centro de Mejoramiento de Semillas Agamicas [CEMSA]; currently Instituto Nacional de Investigaciones en Viandas Tropicales [INIVIT]), Santo Domingo, Villa Clara; Thailand (Field Crops Section of the Department of Agriculture, with principal cassava breeding research at Huai Pong and Khon Kaen); Philippines (VISCA and IPB/UPLB); Indonesia (University of Brawijaya in Java); China (SCATC in Hainan); the Democratic Republic of the Congo (PRONAM); Burundi, Cameroon, Ghana and Rwanda. Table 1.3Table 1 summarizes recent institutional efforts in cassava breeding in this century. Many, but not all, of these programmes still exist.

Most breeding programmes are involved in clonal evaluation of local and introduced materials. Several routinely evaluate populations derived from true seed, but only a few actually make hybridizations to develop segregating populations. In Latin America, only national programmes in Brazil and Cuba maintain crossing nurseries. In Asia, China, India and Thailand have ongoing hybridization programmes, while a few other countries (Indonesia, Malaysia and the Philippines), periodically produce crosses. The situation in Africa is similar. Most national programmes evaluate local or introduced clonal material, but rely on introductions from IITA for segregating populations. Programmes involved in seed production or introduction typically evaluate 4 000 to 6 000 seedlings per year, though some larger programmes, for example, Brazil and Thailand, process much higher numbers.

Table 1.3 Examples of national institutional efforts in cassava breeding in the twentieth century[a]

Country/institution/ initiation	Principal objectives/accomplishments in breeding
LATIN AMERICA	
Brazil Instituto Agronomico do Campinas, Sao Paulo. 1937	• Pioneering work in taxonomy, genetics and cytogenetics • Selection of high yielding, Cassava bacterial blight (CBB)-resistant clones for food, feed and industrial purposes for the subtropics of southern Brazil
Brazil CNPMF/EMBRAPA, Cruz das Almas, BA (outgrowth of Faculdad de Agronomia, Universidad Federal de Bahia). 1969	• Conservation and evaluation of a national germplasm collection • Coordination of regional and national programmes in major ecosystems • Coordination of first intensive global breeding effort for semi-arid tropics • Successful farmer participatory research programme in varietal development
Brazil EMPASC, Santa Catarina	• Intensive selection for bacterial blight resistance • Coordination of a global effort for the subtropics

Country/institution/ initiation	Principal objectives/accomplishments in breeding
Cuba INIVIT, Santo Domingo	• Development of a multiclonal system to extend cassava production throughout most of the year • Establishment of a managed programme for clean seed production
Colombia Instituto Colombiano Agropecuario, Palmira, Valle (later, CORPOICA). 1967	• Establishment of a diverse local germplasm collection • Selection of successful high-yielding hybrids and landrace varieties for the fresh market • Innovator in farmer participation in variety selection
Dominican Republic CEMSA	• Selection and promotion of local and introduced varieties
Ecuador Ministerio de Agricultura	• Selection and release of a high-starch variety for flour and feed industry
Mexico INIFAP	• Selection of high-yielding varieties resistant to bacterial blight and superelongation disease
Panama IDIAP	• Selection of high-yielding varieties resistant to bacterial blight and superelongation disease • Selection of high-yielding mite-resistant varieties
AFRICA	
Burundi Institut des sciences agronomiques du Burundi, Bujumbura. Late 1970s	• Comprehensive multisite evaluation system established • Extensive introduction of new variability from Nigeria and South America
Cameroon Cameroon National Root Crop Improvement Programme. 1977	• Local germplasm collection established and evaluated • Well-defined study of agro-ecosystems for defining breeding objectives • Breeding for stress tolerance based on physiological principles • Wide adaptation of released varieties
The Democratic Republic of the Congo Program Nacional Manioc, M'vuazi. 1974	• Germplasm collection and field evaluation for resistance to major diseases and pests, high yield, high starch, low cyanogenic potential • Identified resistance to cassava mealybug • Comprehensive breeding programme including selection from seedling stage through multiplication of improved varieties • Selection of varieties for leaf production for human food
Nigeria Moor Plantation; National Root Crops Research Institute	• Local germplasm collection established and evaluated • Resistance to CMD developed to high levels and widely commercialized

Country/institution/ initiation	Principal objectives/accomplishments in breeding
Rwanda Institut des sciences agronomiques de Rwanda. 1979	• Comprehensive multisite evaluation system established • Extensive introduction of variability from Nigeria and South America (mite resistance and highland adaptation from latter) • Selection of high yielding clones with resistance to mosaic and green mite
ASIA	
India Central Tuber Crops Research Institute (CTCRI), Trivandrum, Kerala. 1967	• Comprehensive cytogenetics work • Successful varieties for world's most productive cassava farmers • Early work on polyploidy, with first tripoid variety release in 1996 • Inbred development and use in breeding systems True cassava seed research for commercial applications Innovative *Lab-to-Land* concept for integrating research and extension
Indonesia Central Research Institute for Agriculture and Brawijaya University. 1969	• Highly successful varieties for starch industry • Innovative linkage with private sector in variety development • Selections aimed at meeting a highly diversified market for food and industrial uses
Malaysia. Malaysian Agricultural Research and Development Institute. 1975	• Establishment of one of Asia's first diverse germplasm collections • Comprehensive selection scheme established, including acid peat soils • Physiologically-based breeding for high yield and early harvest, with high starch • Contributions to understanding of G–E interactions
Thailand Department of Agriculture, Rayong. 1974; Kasetsart University, Bangkok	• Comprehensive selection scheme with focus on industrial market • Highly successful varieties for industrial markets; 100% of area planted to new hybrids • Highly effective linkages among public and private sectors to promote new varieties • Sharing germplasm with other Asian countries • Significant expansion of genetic diversity through introduction of basic germplasm from Latin America
Philippines Philippine Root Crop Research and Training Center, Leyte; Plant Breeding Institute, Manila	• Establishment of comprehensive breeding programme culminating in well-defined regional trial network with systematic procedures for variety release and recommendation • Broadened national germplasm base through extensive international introductions
China South China Academy for Tropical Root Crops, Hainan Island; Upland Crops Research Institute, Guangdong. 1982	• Establishment of a comprehensive breeding programme with all stages of evaluation • Introduction of broad new genetic diversity from international sources • Techniques to move rapidly and massively from *in vitro* culture to the field for introduced materials

Country/institution/ initiation	Principal objectives/accomplishments in breeding
Viet Nam Department of Agriculture, Dong Nai Province. 1983	• Establishment of a complete selection programme based on local and introduced material • Rapid advances in selection for yield and broad adaptation
[a] *Does not include every programme worldwide. Intended to provide representation from each region*	

Several countries maintain local germplasm collections, which may vary from just a few to several thousand, such as in Brazil. Germplasm conservation practices are almost universally less than adequate. Although *in vitro* conservation practices are well developed, nearly all programmes maintain their germplasm as field collections, with all the inherent difficulties resulting from physical and biological constraints.

Breeding goals across countries, as documented in regional workshops or symposia, appear to be remarkably similar. Nearly all programmes include among their goals: high yield, high dry matter or starch, early maturity, tolerance to local pests and diseases, and adaptation to local environmental conditions. High starch content is a nearly universal goal, but practically all other quality-related traits vary widely according to processing requirements and end use. With almost half of the world's cassava intercropped, varieties compatible with local systems are required. The widespread adoption of goals for stress tolerance and pest resistance reflect a recognition that most farmers apply few inputs to alleviate factors causing yield and quality variations. Specific growing, processing and marketing situations require other objectives for individual countries or regions.

The establishment of the Cassava Biotechnology Network in 1988 was the first step in a long-term strategy to bring the eventual benefits of biotechnology to the most relevant research areas. Several national research programmes are building the groundwork for the application of molecular techniques in cassava improvement, aided by the international research centres, universities and the private sector. Involvement of advanced laboratories in both developing and developed countries will contribute to the systematic accumulation of basic knowledge needed for long-term progress.

The 1990s were another turning point for cassava worldwide. While there are some exceptions, there was a broad weakening of the support for both national and international programmes. Nweke (2003) notes that in 1986, IITA's root and tuber programme had 15 core scientists, many of them working on cassava. In 2000 the cassava project had just one scientist, a breeder. CIAT restructured its research programmes in the mid-1990s, and there was no longer an interdisciplinary team dedicated solely to cassava. This came about for complex reasons, including a shift to biotechnology research, the so-called donor fatigue that resulted in reduced budgets for the international research centres, and budget crises in many developing countries.

At the same time, demand from industry for cassava and its products was increasing, especially in Asia and Latin America. In Africa, demand from population growth also put pressure on research institutes for improved technology. These situations brought more pressure on the private sector and advanced laboratories to become much more significant players in development and testing of technology. In Latin America, this was manifested in the formation of CLAYUCA (Spanish acronym for: Latin American and Caribbean Consortium for Support of Cassava Research and Development) in 1999, whose mandate is to form a sustainable regional mechanism of both private and public entities to aid in supporting, financially and technically, priority activities to help cassava achieve its potential as a vehicle for development.

8. WORKING TOGETHER: THE ROLE OF NETWORKS

Cassava breeding has become increasingly internationalized. Several formal and informal networks link scientists for interchange and problem solving. They provide the opportunity for programmes to specialize and to benefit from outside expertise or germplasm. CIAT in Colombia and IITA in Nigeria give priority to supporting and complementing national programmes in the networks. Not every programme can justify the costs of maintaining a large germplasm collection, developing expensive training materials, or producing their own hybrid seed populations. Larger national programmes and the international centres can effectively develop linkages to collaborate in these and other activities. This division of labour relies on good communication across international borders. In spite of the almost universal good will of cassava scientists to collaborate, economic constraints and the larger political environment often place limitations on easy interchange.

Few cassava research programmes are financially well-endowed. All can benefit by sharing expertise and germplasm. This sharing has evolved from highly informal to a trend for more interchange via organized networks. Regionally based networks bring together resources to resolve problems within defined geographic/political/cultural boundaries. Examples are the East African Root Crops Research Network (with offices in Kampala, Uganda), the Southern African Root Crops Research Network (with offices in Lilongwe, Malawi), the Latin American Cassava Breeders' Network, and the Asian Cassava Breeding and Agronomy Network. Several global networks focus on specialized areas of universal interest across regions. The Cassava Biotechnology Network and the *Manihot* Genetic Resources Network are the principal examples. The networks typically attempt to make the most efficient use of limited resources by coordinating activities across institutions, and using the added strength of multiple participants to leverage new funding. Most are guided by an elected steering committee. Current information on any of these is available from CIAT or IITA (Table 1.4).

Table 1.4 International networks related to cassava genetic improvement

Network	Year founded	Participation	Goals/activities related to breeding
Cassava Breeding and Agronomy Network for Asia	1987	All cassava-growing countries of Asia	• Germplasm exchange (all levels from basic to advanced hybrids) • Updating/exchange of breeding methodologies
Pan-American Cassava Breeders Network	1987	All cassava-growing countries of Latin America and the Caribbean	• Germplasm exchange (all levels from basic to advanced hybrids) • Updating/exchange of breeding methodologies
Cassava Biotechnology Network	1988	All cassava-growing countries, and other interested advanced institutions	• Linkages between grower and consumer needs, with advanced research • Application of advanced techniques, especially molecular, to cassava improvement
Eastern Africa Root Crops Research Network (EARRNET)	1993	Burundi, the Democratic Republic of the Congo, Kenya, Madagascar, Rwanda, Uganda	• Training, and collaboration with other R&D institutions • Local germplasm collection and evaluation • Improved quarantine facilities and procedures • Exchange of improved germplasm

Network	Year founded	Participation	Goals/activities related to breeding
			• Characterize cassava-growing environments • Facilitate use of biotechnology tools
Southern Africa Root Crops Research Network (SARRNET)	1993	Twelve countries of the Southern African Development Community (SADC) under umbrella of SACCAR	• Develop new varieties suitable for processing and commercial criteria • Evaluate improved introduced clones • Evaluate introduced seed populations • Release and promote new varieties
Latin American and Caribbean Consortium for Cassava Research and Development (CLAYUCA)	1999	Countries with contributing public and private sectors in Latin America and the Caribbean; CIAT	• R&D agenda developed by membership • In the past, has included a strong component of varietal testing and multiplication for commercial development
Global Cassava Development Strategy (GCDS)	2001	All cassava-growing countries	• Initiated by IFAD • Promotion of projects to support genetic improvement
Global Cassava Partnership for Genetic Improvement (GCP-GI)	2002	All cassava-growing countries	• Under auspices of GCDS • Promotes development and use of advanced molecular and genetic technologies

The International Society of Tropical Root Crops (ISTRC) was established in 1970 and meets triennially for scientific interchange and promotion of root crops in research and development agendas. The society has been crucial in improving the status of the root and tuber crops, through the creation of an interacting world community of scientists and other specialists. In more recent years, a number of networks has taken over some of the original functions of the ISTRC, but it must be recognized for its pivotal unifying role among workers in these neglected crops.

In 1978 IITA was instrumental in supporting African national programmes to create the Africa Branch of the society (ISTRC-AB), to stimulate research, production and utilization of root and tuber crops in Africa.

The most recent network to aid cassava genetic improvement is the Global Cassava Partnership for Genetic Improvement (GCP-GI) (Fauquet and Tohme, 2004). This partnership grew out of the Global Cassava Development Strategy (GCDS), which was initiated by the International Fund for Agricultural Development (IFAD) and had its largest activity in the 1990s. The GCP-GI was conceived at a meeting of leading experts in cassava research in Italy in 2002. GCP-GI works under the umbrella of GCDS, which in turn is hosted by FAO. Its objectives are: (1) to use advanced molecular and genetic technologies to create cassava that is higher yielding, more resistant to diseases and pests, more marketable, and more nutritious, for the benefit of the poor; and (2) to further develop these new tools and technologies to make them more useful for cassava improvement and freely available as public goods (Fauquet and Tohme, 2004).

At the outset, the founders recognized the need to work on production constraints (viral diseases, cassava bacterial blight [CBB], arthropod pests [e.g. green mites, stem borers, whiteflies and mealybugs], drought and added value traits [post-harvest deterioration, protein deficiency, biofortification, starch content and quality and cyanogenic compounds]). This work would be accomplished, in part, by developing and using advanced molecular technologies (micropropagation, doubled haploid breeding, genetic resources, genomics, transformation and biosafety). Founding organizations included national and international programmes in cassava-producing countries, and advanced laboratories in developed and developing countries.

9. OUTPUT OF BREEDING PROGRAMMES

The products of plant breeding programmes generally include both information and genetically improved germplasm. As a rule, most of the information produced by a breeding programme is intended for breeders and other scientists in the course of their continuing research. Improved germplasm, usually in the form of new varieties, provides measurable benefit to producers and consumers. Most breeding programmes consider development of improved varieties to be their principal output. There are no readily available registers of new varieties, even though virtually all programmes have released varieties. Nonetheless, the international centres (CIAT and IITA), have compiled much of the information available in their respective regions of collaboration. The majority of releases through the 1980s was in the form of recommending existing superior landrace varieties, after testing in multilocation trials. While these varieties are often already available to farmers, their official release may provide impetus for programmes aimed at certified seed production and distribution, and the promotion of improved agronomic practices.

Most programmes have now also released bred varieties (over 200 in Africa, alone), and this process has accelerated rapidly since the late twentieth century, as the efforts of programmes established 15 to 20 years earlier came to fruition. The large number of releases of new cassava varieties since the mid-1980s is essentially the result of work begun in the 1970s, and sometimes earlier. Where programmes have sustained their breeding research, a continuing steady stream of new varieties can be expected. Numbers of releases are increasing rapidly in all continents, and this should be followed by substantial impact at the national production level. Chapter 22 discusses further the release process as it applies to cassava and gives some detail on specific programmes and countries.

10. BENEFITS FROM BREEDING

Virtually all cassava breeding programmes have an over-arching goal of bringing economic or nutritional benefits to their clients. Often these clients are the growers who will adopt improved varieties, and thereby increase income or nutritional status. Target beneficiaries, however, may also be processors who will affect better efficiencies, or consumers who will see lower prices, better quality, or another desired feature. The economic benefits of new cassava varieties have been most evident in Africa and Asia, but are generalized across most cassava-growing countries. A breeder, and all who collaborate in the process, have the satisfaction of knowing that the benefits of this technology can be one of the most cost-effective ways to bring economic and social benefits to a target region.

In the private sector, positive benefits are generally measured by growers' willingness to return each year to buy new seed. Success for the breeder is measured in financial returns to the company for delivering new varieties. In areas where the for-profit sector flourishes, there are generally capabilities for assessing spread of varieties (area planted) and the economic benefits as compared with varieties previously grown. Currently the private sector has minimal direct involvement in cassava varietal development, and the public sector has been far less adept at evaluating its success in terms of varietal adoption and impact. Taxpayers and donors ultimately need to be convinced that their investments in public-sector breeding are giving expected results.

By the 1990s many of the breeding programmes begun, or reinvigorated, in the 1970s and 1980s, had released new varieties. Few, however, had the resources to follow up with diffusion and impact studies. Fortunately, some additional resources were made available and there was a number of efforts, in all the continents, to quantify the economic and social benefits of cassava breeding research.

Cassava is an inherently difficult crop in which to study adoption. Most seed (stem pieces) is grown on-farm, or traded, and very little enters commercial markets. There are few indicators from commercial sources, of area planted to new varieties. Secondly, the typical small-holder will adopt new varieties progressively, i.e. try them first on a small, experimental area, and gradually increase the area planted as they continue to perform well. This system creates a more complicated situation for determining adoption.

Impact studies for cassava varieties are still very inadequate, but the information on successes on all continents has been accumulating for over a decade. Chapter 23 gives further details on the status of adoption and impact of new varieties. Manyang *et al.* (2000) estimated an average yield advantage of 49 percent for new varieties planted on some nine million hectares in 20 countries of Africa. In Asia, new varieties cover nearly all of Thailand's cassava-growing area of almost one million hectares. China and Viet Nam each plant over 30 000 ha to new hybrids. In Latin America, adoption has been somewhat slower, because growers have had access to a much wider range of local variability. Nonetheless, Brazil, Colombia, Cuba, Haiti and others have extended new varieties throughout the region, for an estimated annual increase in value of production exceeding US$12 million (CIAT, 2003a).

New varieties very often have a multiplier effect, in stimulating farmers to adopt other technology components. Since varieties are often available at no cost (to the farmer), it is an easy and convenient technology to try out, and to adopt, so it is often the first stage for farmers wanting to increase their food production or profitability. If this technology succeeds, it can be an opening to trying purchased inputs like fertilizer, or investing in land improvements like live erosion control barriers.

Chapter 2. The cassava plant and its products

1. TAXONOMY, MORPHOLOGY AND ANATOMY

Cassava is a member of the genus *Manihot*, and the family Euphorbiaceae, which is characterized by latex-producing species. In 1910 F. Pax recognized 128 species in 11 sections of *Manihot*. With later additions by Pax and others, in 1968 about 154 species had been recognized. Through the first half of the twentieth century, scientists commonly separated cassava into two species based on bitter (*Manihot utilissima*) and sweet (*Manihot dulcis*) root characteristics. This is now recognized as a highly artificial species division and is rarely used.

Rogers and Appan (1973) authored the most recent comprehensive taxonomic treatment in their monograph: *Manihot*, Manihotoides (Euphorbiaceae), based on extensive field work throughout the Neotropics. They recognized 98 species, and eliminated several synonyms with cassava including: *multifida*, *flexuosa*, *janiphoides*, *diffusa*, *flabellifolia*, *melanobasis*, *aipí*, and *utilissima*. Mr Antonio Allem, of Brazil's Genetic Resources and Biotechnology Center (Brasilia) working mainly with Brazilian species, is responsible for much of the classical taxonomic work in recent years, and this is reviewed in Allem (2002). Since the 1990s, molecular taxonomy has contributed substantially to our understanding of *Manihot* genetic diversity. Chapter 4 gives further details of this work and our understanding of cassava genetic diversity.

For many years there was considerable debate about the origins of cassava, whether within the Meso-America/Northern South America centre of species diversity, or in the Brazilian centre of diversity, or neither. Allem (2002) presents evidence, later supported by a number of authors working on molecular evolution, that wild progenitors of cassava still exist, and that the cultivated species probably originated on the southern edge of the Brazilian Amazon.

Cassava is a woody shrub, generally from 1-3 m in height. The principal economic product is starchy roots. The plant may be propagated either vegetatively (stem cuttings) or sexually (true seeds). While all commercial plantings are from cuttings, propagation from seed is important for breeding programmes. Lignified stem pieces from mature plants may be planted directly after they are cut or after storage of up to several months. Storage conditions strongly influence sprouting ability and subsequent plant vigour and yield.

Upon sprouting, one or more axillary buds on the stem piece develop and form, in sequence, nodal units consisting of a node, a bud, a palmate leaf blade subtended by a long petiole, and an internode whose length and mass depend on the genotype, age of the plant and environment. The shoot shows marked apical dominance and new leaves are produced in sequence on the main stem. Once the apex becomes reproductive, from one to six of the axillary buds (usually three or four) develop and produce the forking (or branching) characteristic of cassava. Little is known about regulation of branching in cassava. Some clones will branch early and continue branching while others have never been known to branch. Under constant environmental conditions, the interval between the formation of successive branches tends to be constant (CIAT, 1979; Tan and Cock, 1979).

A long petiole, subtending the palmate leaf blade, plays an important role in orienting the leaf to intercept the maximum amount of light. At high temperatures (>24°C), the time from appearance to full expansion of a leaf is about two weeks. The size of fully expanded leaves increases with the age of the plant up to about four months and then declines. At low temperatures the maximum size is smaller and the largest leaves are produced later. There are large varietal and environmental effects on the leaf area. Drought stress (Conner and Cock, 1981) or a limited supply of nutrients (CIAT, 1979) can greatly reduce leaf size.

Thickened roots are the main carbohydrate storage organs of cassava. As early as 28 days after planting, the plant deposits large numbers of starch granules in the xylem parenchyma of the fibrous roots. Anatomically it is not possible to distinguish at this stage between roots that will later thicken and those that will continue as fibrous roots (Lopez, 1976; Keating, 1981). From about six weeks after planting, some of the fibrous roots begin to thicken rapidly, laying down large quantities of xylem parenchyma

that are packed with starch granules. The number of roots that will eventually thicken is determined early in growth, with little change from two to three months after planting. There does not appear to be any specific trigger, such as photoperiod, to root thickening. Cock *et al*. (1979) and Tan and Cock (1979) suggested that starch deposition begins when the supply of photosynthate exceeds the requirements for growth of stems and leaves.

The cassava plant has simultaneous development of leaf area and storage roots. This contrasts with the cereal crops, where there is phasic development in which leaves develop first, followed by grain filling. In phasic development there is little competition for the substrates used for growth of the photosynthetic and the storage organs. However, in cassava the current supply of assimilate is partitioned between growth of leaves and roots, the latter being the principal commercial product. This means that there is an optimum leaf area index for root growth: if partitioning unduly favours leaf growth, then there is less assimilate available for root growth. Conversely, too little leaf growth will limit photosynthesis and crop growth rate, and this in turn will limit yield. Genetically manipulating this balance should be one route to obtaining high yields.

2. ADAPTATION AND RESPONSE TO THE ENVIRONMENT

As cassava is so widely cultivated throughout the tropics, and often in environments with minimal modification through fertilizer, irrigation, or other inputs, the crop is subject to a wide variation of environmental factors. Among the most important of these are temperature, photoperiod, light intensity, water, relative humidity and soil characteristics. Variations are greatest across geographical areas, but can also be substantial across time within a given site.

Cassava grows in the tropics from sea level to about 2 200 m elevation, in areas receiving more than 400 mm average annual rainfall. In the subtropics, maximum elevation is somewhat lower. The species has not succeeded much beyond the Tropics of Cancer or Capricorn, both because of the need for a long growing season, and also the difficulty of storing planting material for extended periods (during a cold winter, for example).

The long growing period, like that of most non-cereal energy crops in tropical agriculture, lends it to adaptation in a wide range of production systems. Cassava may be an important component of cropping systems ranging from shifting cultivation with a long fallow phase, to intensive, continuous annual cropping (for review, see Toro and Atlee [1980], Fresco [1986] and Ospina and Ceballos [2002]). Small farmers are the principal producers, although large plantations are becoming more common, especially in Asia and Latin America, as the crop is industrialized.

Cassava production expanded broadly throughout the lowland tropics in the twentieth century, mainly on the less-fertile, poor-quality agricultural lands. In traditional, low-input cropping systems, cassava is often an end-of-cropping-phase species, the last crop before returning land to fallow. In Africa the capacity of cassava to grow and yield well on low-fertility soils, its ability to withstand locust attacks and drought, and its low cost of production, motivated farmers to replace other traditional root crops such as yams. In areas where population growth has caused a reduction of the rotation pattern in shifting culture and a commensurate decline in soil fertility, cassava is one of the few crops that can thrive without purchased inputs, provided that some form of rotation remains. Similarly, in much of tropical Asia, cassava is relegated to lower-quality land not suited for rice production. In one of the most notable agricultural success stories of recent years, the area planted with cassava in Thailand increased fivefold in the 1970s to meet an export opportunity in Europe. Most of the production continues to be on underexploited land of the Northeast, and by small landholders.

2.1 TEMPERATURE

Cassava is sensitive to frost and as it has a growing season of nearly one year or more, this trait essentially limits its range to the tropics and subtropics. When grown in frost-prone areas such as the winter season in the subtropics, it is generally harvested or cut back before winter. Minimum mean temperature for growth is about 17°C, where the absolute minimum does not fall below about 10°C. Below these temperatures, stake sprouting is extremely delayed and may fail completely; growth and yield drop off markedly. The upper limits for temperature adaptation appear to be within the range of most tropical environments, though data are sketchy.

2.2 PHOTOPERIOD

Although results are not consistent, under long days (greater than 12 or 13 hours), total plant weight tends to remain the same or decrease, and the proportion of root weight to total plant weight (harvest index) generally decreases (Bolhuis, 1966; CIAT, 1982). Photoperiod also influences reproductive development and, while not directly associated with yield formation, has implications for canopy development (e.g. branching habit) as well as seed production in a breeding programme. Long photoperiod induces flower initiation and consequently branching, in many genotypes (da Cunha and da Conceiçao, 1975; de Bruijn, 1977; and Keating *et al*., 1982).

2.3 LIGHT INTENSITY

Intensity of light received by individual leaves can vary as a result of cloud cover, competition for light by an intercropped species, or intraplant/intraspecific shading. Intercropping effects can be especially pronounced when cassava is completely shaded. For example, farmers commonly grow cassava under coconuts in parts of Asia. Like its wild relatives, cassava appears to be highly sensitive to reduced light intensity. Shading generally affects root yield more than top growth (Fukai *et al*., 1984). As shading also reduces canopy temperatures, it is often difficult to determine which of these variables exert the predominant effect on crop growth rate (Keating *et al*., 1982).

2.4 SOIL WATER AND AMBIENT RELATIVE HUMIDITY

The literature is replete with suggestions that cassava is highly tolerant of drought. There are regions in all continents where farmers grow cassava with less than 500 mm of rain per year. Even in higher rainfall areas, the crop may have to withstand sustained dry periods. The species combines several mechanisms that allow it to withstand both short and prolonged water stress periods. The principal response to moderate water shortage is a reduction in leaf area. The plant maintains normal root growth, and may even increase it (Conner *et al*., 1981; Conner and Cock, 1981). The combination of smaller leaf size and slower leaf formation rate, rather than shorter leaf life, accounts for reduced leaf area. This reliance on reduced leaf formation rather than leaf fall is important to cassava's ability to maintain high root yield under stress.

The ability of cassava to regulate its stomata to maintain high midday leaf water potentials and prevent water loss is a key mechanism for tolerance to prolonged drought (El-Sharkawy and Cock, 1984). Although partial closure of stomata also restricts the CO_2 supply to the leaf, it leads to a stable leaf water potential during stress. By this mechanism, stressed leaves are capable of maintaining photosynthetic rates around 40–60 percent of non-stressed leaves during a period of at least three months (El-Sharkawy and Cock, 1984). The crop can rapidly recover once released from stress by forming a new leaf canopy; and these leaves may show even higher photosynthetic rates than those of the non-stressed crop (CIAT, 1990; El-Sharkawy *et al*., 1992).

The semiarid regions of northeast Brazil are heavily dependent on cassava’s ability to produce a crop in these harsh conditions. Cassava is recognized for its potential role in food security in the semiarid zone of West and Central Africa, between the latitudes of 10ºN to 14ºN, and longitudes 5ºW to 20ºE. While still a minor crop, it is crucial for many small-scale subsistence farmers (Tshiunza *et al*., 1999).
At the other end of the spectrum, farmers cultivate cassava in regions of very high rainfall, such as Colombia's west coast (>6 000 mm/year). The main problems in these conditions appear to be root rot

and a slow growth rate due to persistent cloud cover. Cassava is generally intolerant of water logging. In most areas where periodic flooding occurs, farmers harvest soon before or soon after flooding. Scattered reports of varieties that tolerate standing water have never been experimentally confirmed.

2.5 SOIL CHEMICAL PROPERTIES

A high percentage of cassava is produced on soils with low pH (often resulting from high aluminium saturation in highly leached soils, or in the organic tropical peat soils), and generally low levels of major nutrients, especially phosphorus. Cock and Howeler (1978) compared several major crop species for growth at different liming levels in savannah soils of high Al concentration. Cassava was the most tolerant species, giving about 55 percent of maximum yield at zero lime, while maize and beans produced nothing. On the other hand, cassava is generally intolerant of high pH and saline soils.

Cassava has a reputation as a crop adapted to low fertility conditions, although it has rather high P and K requirements in solution (Edwards *et al*., 1977). The ability to grow well on low-P soils is almost completely dependent upon an efficient association with mycorrhiza. Potassium is crucial because cassava extracts large amounts from the soil. As much of this is in the roots, which are marketed, there is limited opportunity for recycling (Howeler, 1985).

Most landrace varieties, when grown under high fertility conditions, increase foliage yield proportionally more than root yield. This is a normal response in primitive varieties of many crop species that have not been genetically improved for response to more luxurious conditions. As has already been amply shown, it is quite possible to breed cassava both for responsiveness to good soil fertility, and tolerance to poorer conditions.

2.6 PESTS AND DISEASES

Both growers and scientists have historically considered cassava a rustic crop with few serious pest or disease problems (Purseglove, 1968). More recent evidence, however, shows that this belief is based primarily on observations of regionally evolved and selected varieties, grown under traditional cultural practices (Lozano *et al*., 1980). Within these systems, the pest populations are often in balance with their natural enemies and varieties have evolved with moderate resistance to local pests. Plantings may be widely separated in space, thus limiting the rapid plant-to-plant spread of micro-organisms or arthropod pests.

Cassava is still widely grown as a small-farmer crop in systems with few external inputs, but farmers are increasingly adopting new varieties and new production systems. In addition, the crop is expanding into new areas as population pressures move agriculture into more marginal lands. Such changes, either in cultural practices or in variety, can result in pest outbreaks due to an imbalance in the established equilibrium. As cassava is a long-season crop, insecticides or fungicides often must be applied over a long period to provide satisfactory protection. Normally, this is neither economically nor environmentally sound. For many pest problems the best control strategy is through host plant resistance and/or biological control.

3. PRODUCTION

Cassava is the fourth most important supplier of food calories in the tropics. World production has risen rapidly in the past few decades, principally accounted for by increases in the area planted in Africa and Asia (Table 2.1). World production (early 2000s) of about 190 million tonnes is the energy equivalent of 60 to 70 million tonnes of cereal grains. From 1970 to 2000, world production doubled, and overall has kept pace with population growth in developing countries. Nonetheless, there are large imbalances among regions.

Table 2.1 Regional cassava production (fresh roots), yield per hectare, and human consumption

Regions and principal producer countries	Annual production (million tonnes)				Root yield (tonnes/ha)	Consumption (cal/cap/day)
	1969/71	1979/81	1989/91	1999/03	1999/03	2000
Africa	**38.6**	**48.8**	**67.1**	**100.7**	**8.9**	**209**
Democratic Republic of the Congo	10.2	12.9	17.7	15.6	8.1	870
Ghana	1.5	1.9	3.2	8.9	12.2	649
Madagascar	1.2	1.6	2.3	2.5	7.1	381
Mozambique	2.5	3.1	3.9	5.4	5.8	635
Nigeria	9.5	10.8	18.2	33.2	10.1	264
Uganda	1.9	2.1	3.4	5.1	13.2	299
The United Republic of Tanzania	3.4	5.5	7.0	7.0	10.2	307
Other	8.4	10.9	11.4	23.0		
Latin America & Caribbean	**35.2**	**30.6**	**32.3**	**32.3**	**12.5**	**58**
Brazil	29.9	21.3	24.2	22.4	13.5	108
Colombia	1.4	3.1	1.8	1.8	10.2	83
Paraguay	1.4	2.0	3.8	3.8	15.5	311
Other	2.5	4.2	2.5	4.3		
Asia	**22.9**	**44.7**	**52.4**	**54.5**	**14.9**	**18**
China	1.9	3.4	3.3	3.8	16.0	4
India	1.5	5.9	5.4	6.9	25.6	15
Indonesia	10.7	13.6	16.4	16.8	13.0	157
Philippines	0.4	2.2	1.9	1.7	8.1	62
Thailand	3.2	15.1	21.8	17.9	16.9	33
Viet Nam	0.9	3.3	2.7	3.3	11.4	44
Other	4.3	1.2	0.9	4.1		
World total	**96.7**	**124.0**	**151.7**	**187.7**	**10.7**	**43**

Sources: Summarized from Trends in CIAT Commodities 1993. Working Document No. 128. CIAT, Cali, Colombia, and FAOSTAT data (2004)

The usual yield of about 10 tonnes/ha is far below maximum experimental yields of over 60 tonnes/ha in a 12-month growing season. However, these yields (about 3 tonnes/ha dry root) compare favourably with those of other basic energy crops such as cereals. With traditional management, in environments where only one crop per year is possible, cereal yields are only 1−2 tonnes/ha/year.

In Africa, the Democratic Republic of the Congo, Ghana and Nigeria alone account for almost two-thirds of total production on the continent. Average yields in these countries range from 12.2 tonnes/ha in Nigeria to less than 7.1 tonnes/ha in the Democratic Republic of the Congo. For Africa as a whole, production increased dramatically in the period 1970–2000 at an average rate of two million tonnes per year, or 3.4 percent per year (FAOSTAT). About two-thirds of this increase was due to an increase in area, and one-third due to an increase in yield.

Mean yields in Asia and Latin America were nearly identical until the early 1990s. In the past decade, Asia has improved yields significantly, mainly owing to widespread adoption of improved varieties in the largest-producing country, namely, Thailand. Mean yields in Asia are now almost 15 tonnes/ha. Yields vary widely, from 8 tonnes/ha in the Philippines to 25 tonnes/ha in India, having the world's highest yields. Major Asian producers are Thailand and Indonesia, with 33 and 31 percent, respectively, of the continent's total production. The increase in Asian cassava production since 1970 is primarily the result of expansion in Thailand, which grew from less than one million tonnes in 1957 to four million tonnes in 1972, and peaked at 24 million tonnes in 1989. Production stabilized after the late1980s, due to quotas in the European market for cassava pellets. Expanding markets for starch and regional markets for animal feed have partially offset declining demand for pellets in Europe, and take an ever-increasing share of production.

In Latin America, Brazil dominates production, with 75 percent of the area. Cassava is cultivated throughout the country – in the hot, humid jungle of the Amazon basin, in the dry areas of the Northeast, and in the subtropical South. Brazilians grow cassava for food, animal feed and industrial uses. In the 1970s, yields declined from 14 tonnes/ha to less than 12 tonnes/ha, and total production decreased slightly to 25 million tonnes a year. The drop-in yield is related to the expansion of soybean production and movement of coffee production to areas further north. These two high-value crops tend to displace cassava from more fertile soils. Import policies and government subsidies for crop production also play major roles. Other large Latin American producers are Colombia, with 1.8 million tonnes and Paraguay with 3.8 million tonnes. Although Brazil, Colombia and Paraguay produce almost 90 percent of the cassava in the Americas, the crop is regionally vital in many other countries.

Cassava is typically a labour-intensive crop to manage: bulky planting material (stem pieces); a growth habit that complicates mid- to late-season field machine operations; the harvest that so far can only be partially mechanized; and processing that, for most uses, involves considerable manual labour. For example, a study in southern Brazil (Pará State) showed a total of 249 person–days per hectare required, from land preparation through storage of cassava meal (Ximenes, 2001). Howeler (In press) summarized similar information for Asia, where there is a low of 52 person–days per year in Thailand, to 327 days in India. In Africa, among the major cassava producing countries, growers utilized an average of 195 person–days per hectare (from a low of 175 in Côte d'Ivoire to 222 in Nigeria) (Dunstan Spencer and Associates, 1997). Weeding and harvest combined generally constitute well over half of the labour requirements. This can be an advantage in providing employment in regions where employment opportunities are scarce. It is disadvantageous in keeping the price of cassava competitive with feed and food products from other crops with better labour efficiencies. Compared with just a few person–days per hectare per year input for grain crops in industrialized systems, there seems to be considerable opportunity to increase producer efficiency in cassava production, probably including varietal traits. While there has been some recent emphasis on developing cassava traits for amenability to mechanization, this has not yet become a widespread goal.

4. UTILIZATION

Cassava owes part of its popularity to the wide diversity of uses for the roots: fresh or processed for human food and animal feed, and in various industrial products including starch and starch-derived products, alcohol and high fructose-glucose syrups. Processing seems to have been an integral part of cassava culture for as long as the crop has been cultivated. For food use, for example, the roots may be simply cooked, or they may be converted to roasted or steamed granules, flours, dry chunks, fermented pastes, drinks and many other variations.

The main features of cassava that impact its form of utilization are its starch content, nutritional value, post-harvest storage characteristics and toxicity. Cassava utilization typically performs five main roles: (1) famine reserve; (2) rural food staple; (3) urban food staple; (4) livestock feed and industrial raw material; and (5) earner of foreign exchange.

While every continent shows considerable variation among countries, there are some broad trends to be noted (Table 2.2). Nearly all cassava in Africa is destined for human consumption. However, this is undergoing a transformation, a shift from production for home consumption to commercial production for urban consumers, and in some cases, livestock feed and industrial uses (Nweke *et al*., 2002). This transformation is still in the early stages, and by far, most cassava is still used as a basic food crop (Table 2.3).

Table 2.2 World utilization patterns of cassava (percentage of total production)

	Human food		Animal				
Region	**Fresh**	**Processed**	**feed**	**Starch**	**Export**	**Waste**	**Stock**
World	31	34	11	5	7	10	1
Africa	38	51	1	<1	<1	9	<1
America	18	24	33	10	<1	14	<1
Asia	34	22	3	9	23	6	4
Asia (without Thailand)	46	28	4	12	2	9	<1

Source: Cock (1985)

Table 2.3 Global cassava utilization growth rates (past and projected) and shares by continent, 1983/93 and 1993/05 (percent)

	World	**Africa**	**Asia**	**LAC[a]**	**Share of total**
Total use					
1983-1993	2.4	4.3	1.6	0.7	100
1993-2005	1.8	2.4	2.5	1.5	100
Food					
1983-1993	2.4	3.9	0.1	0.7	59
1993-2005	2.2	2.5	2.0	0.8	58
Feed					
1983-1993	1.1	7.6	4.7	0.6	24
1993-2005	-0.2	1.8	2.5	1.3	22
Other use					
1983-1993	4.7	5.3	6.8	1.1	17
1993-2005	3.1	2.3	4.2	3.4	20

[a] *Latin America and the Caribbean*
Source: Henry and Hershey (2001)

Asia has been a largely industrially-oriented region. Indonesia and Malaysia were major industrial starch producers since before World War II, although these industries declined after the war. Thailand re-energized the cassava sector when it capitalized on European market opportunities for dried chips, beginning in the 1970s. In more recent years, China, India, Indonesia and Viet Nam have been moving aggressively into industrialized, value-added cassava products although cassava is still important as a basic food or feed crop of the urban and rural poor in most of these countries.

With Latin America being the region of origin of cassava, it is to be expected that some areas will continue to cultivate the crop for traditional food uses that have been nearly unchanged for centuries. This is mainly true of the Amazon and Orinoco basins, but also in large areas of northeast Brazil and northwest South America. In Latin America as a whole, the main driving forces for new forms of cassava utilization are the demand for energy sources for balanced feed rations in animal diets, and for industrial starch.

Post-harvest management of the cassava crop usually involves either cooking or processing. The exceptions are the feeding of raw roots to animals, and its occasional use as a fresh snack food, especially in parts of Africa. Processing and cooking serve the main functions of lowering levels of cyanogenic glucosides, dealing with the high perishability of harvested fresh roots, improving palatability, and/or lowering water content to reduce transportation costs.

While roots are by far the most commonly used part of cassava, leaves are very high in protein, vitamin C, iron and calcium, and they are used both as human food and in animal feeds. When used for human consumption, leaves are generally cooked for a long period of time. Leaves are an important vegetable in the Democratic Republic of the Congo and in the United Republic of Tanzania, but are little used in Uganda and West Africa (Nweke *et al*., 2003). In Latin America, Brazil is the principal country to make use of leaves, but even in Brazil it is not a major product.

4.1 NUTRITIONAL VALUE

Cassava is primarily an energy source (Table 2.4). Roots have an average dry matter content of about 35 percent, high in comparison to most other roots and tubers. Dry matter can vary from about 20 to 45 percent, and depends on variety, age of the root at harvest, growing conditions (especially temperature and soil moisture) and health of the plant.

Table 2.4 Composition of cassava roots (peeled) and leaves (fresh weight basis)

Component	Roots	Leaves
Water (%)	62.8	74.8
Energy (kJ/100g)	580	5.1
Protein (%)	0.53	5.1
Fat (%)	0.17	2.0
Starch (%)	31.0	-
Sugar (%)	0.83	-
Dietary fibre (%)	1.48	5.1
Ash (%)	0.84	2.7
Minerals (mg/100g)		
Calcium	20	350
Potassium	302	56
Phosphate	46	-
Magnesium	30	-
Iron	0.23	-
Source: Westby (2001)		

Starch and sugar comprise about 90 percent of the dry matter, with starch by far being the most important. The metabolizable energy of dry cassava is 3 500 to 4 000 kcal/g, similar to that of maize flour.

Vitamin C, thiamine, riboflavin and niacin are present in significant amounts in the roots. In CIAT's core collection (a subset of the 6 000+ germplasm accessions), ascorbate ranged from 1 to 40 mg/100 g fresh weight of roots, with a mean of 9.5 mg (Chavez *et al*., 2000). Although 50 percent or more of the vitamin C is destroyed in boiling or processing, minimum daily requirements can be satisfied in areas where consumption is high.

Content of beta-carotenes, precursors of vitamin A, is closely related to root flesh colour, with yellow-rooted varieties having significantly higher contents than white-fleshed types. Vitamin A deficiency, which can cause blindness, is one of the world's principal nutritional challenges. CIAT evaluated the core collection and found a range from 0.1 to 1.0 mg/100g fresh weight, with a mean of 0.23 mg. White, deep yellow and orange roots had means of 0.13, 0.85 and 1.26 mg carotene/100g fresh root, respectively (Chavez *et al*., 2000).

Crude protein content is only about 1-2 percent, dry weight basis, although there are reports of levels as high as 6 or 7 percent (CIAT, 2003b). As levels are so low, protein quality is not highly important in most situations. Nonetheless, due to the very high per capita consumption of cassava in sub-Saharan Africa, it is the third most important supplier of dietary protein, after maize and groundnut (Dixon *et al*., 2003). In critical food shortage situations, the importance of the protein content is even further elevated. The balance of amino acids is reasonably good, with the exception of the deficiencies of the sulphur-containing acids. About 50 percent of the crude protein (as measured by N x 6.25) corresponds to true protein. The remainder consists of free amino acids, nitrites and cyanogens (Buitrago, 1990).

Cassava leaves have protein contents of about 20–22 percent, dry weight basis. Ascorbate averages about 20 times higher in leaves than in roots, while carotene is 100-200 times higher in leaves (Chavez *et al*., 2000). Further studies are required to understand bio-availability after different forms of post-harvest management and processing.

Key trace minerals in cassava are iron and zinc. Root contents average 9.6 and 6.4 mg/kg dry weight, respectively. Content of leaves is about ten-fold greater than that of roots (Chavez *et al*., 2000).

4.2 POST-HARVEST PERISHABILITY

Cassava has no well-defined period of physiological maturity; roots can be stored in the ground for months as part of intact plants. However, harvest triggers a complex series of biochemical reactions, which begins with vascular discoloration and leads eventually to complete root decomposition. This rapid post-harvest deterioration has played a major role in the evolution of post-harvest management practices. Although deterioration is still poorly understood at the biochemical level, there appears to be two essentially independent sets of processes: physiological and microbial deterioration. Most of the work relevant to possible genetic improvement has concentrated on physiological deterioration.

Physiological deterioration is the result of processes initiated by wounding at harvest, with the main wound usually being the cut surface at root detachment. It can be visible as vascular darkening as early as 24 hours after harvest. An average duration prior to onset is four or five days. The process shows many of the common characteristics of plant wound responses. The symptoms are brown–black vascular streaking in areas below the peel. Deterioration is probably a wound response that does not remain localized but spreads rapidly through the root (Rickard, 1982; Beeching *et al*., 1995). The process involves an increase in enzymatic activities (phenylalanine lyase [PAL], peroxidase, and polyphenol oxidase) and the production of compounds from the phenylpropanoid pathway, including four hydroxycoumarins (esculin, esculetin, scopolin and scopoletin). There is evidence for the metabolism of scopoletin to an insoluble coloured product, which may be the cause of vascular discoloration during storage (Buschmann *et al*., 2000). Roots resistant to physiological deterioration accumulate less scopoletin than susceptible ones (Wheatley, 1982).

PAL and scopoletin are part of the phenylpropanoid pathway, probably involved in many aspects of plant wound response, including pathogen challenges. Given the decidedly beneficial roles of PAL, suppressing its activity either before or after harvest would probably not be a viable approach to controlling deterioration (CIAT, 1995). Scopoletin probably has an anti-pathogen role in the root pre-harvest, but might be suppressed post-harvest without detrimental effects. A prerequisite to pursuing genetic engineering approaches is to understand better the biochemical processes involved (see Chapter 17).

There is substantial influence of variety and environmental factors both in time of initiation and rate of progress of deterioration. Many types of preharvest stress are known to prolong the period before initiation of deterioration, especially those that result in a decrease in leaf area. One of the most drastic of these is complete pruning prior to harvest, shown to be highly effective in increasing storability. However, quality declines substantially, and this practice has not been widely adopted.

Microbial deterioration often starts within a week after harvest, and is caused by a complex of micro-organisms. Spoilage is fastest in roots that are badly damaged or are kept in a humid environment.

In traditional production and utilization systems, rapid deterioration is usually not a constraint. Producers, marketers and consumers have all developed techniques to deal with this trait. However, as cassava increasingly becomes part of market economies, with greater needs for storing and shipping roots to urban markets or centralized processing centres, increased storage time becomes imperative. Considerable success at the pilot scale level has been achieved by treating roots with a low-toxicity fungicide (thiobenzadole), followed by storage in plastic bags. This is a low-cost, simple method that extends shelf life to about two weeks. There has been preliminary research on genetic approaches, either through conventional breeding or genetic engineering. Moderate genetic variability exists for post-harvest deterioration, but no concerted effort has been made to explore the limits of progress by breeding.

4.3 TOXICITY

Of many thousands of landrace varieties and experimental genotypes tested, all produce some level of hydrocyanic, or prussic, acid (HCN), poisonous especially to warm-blooded animals. Intact cassava tissues do not contain HCN. The cyanoglycosides linamarin and lotaustralin, and the enzyme linamarase, are compartmentalized within the cell. If cells rupture, the enzyme comes into contact with the glycoside and HCN is released. In damaged plant tissues, one can find traces of hydrogen cyanide as well as the two non-hydrolyzed cyanogenic glycosides. Most literature of the past refers to cassava's HCN content. It is more accurate to define these compounds as cyanogens, and their concentration as the cyanogenic potential of cassava (CNP). Roots high in CNP are typically classified as bitter by farmers and consumers, while those low in CNP are called sweet, or sometimes (in parts of Africa) cool.

Cooking, drying and most other traditional processing methods for roots destined for human consumption, reduce CNP to very low levels. Boiling reduces root CNP by about half, and many other processing methods reduce it by more than 90 percent. Undoubtedly this has been one factor in the development of many of the complex processing procedures that have evolved for cassava. Leaves have cyanogen levels that are often 5-20 times greater than roots, but after boiling 15 to 30 minutes, CNP can be reduced to nearly zero.

The reasons for the evolution of a range of toxicity levels in cassava have been the subject of many years of debate. Cassava appears to be one of the few crops in the world in which there is conscious selection favouring the more toxic varieties over the less toxic ones (Wilson, 2003). Studies in Malawi showed that bitter types reduce theft. Since most of the potential thieves are young males, they are deterred by the need for lengthy processing, which is traditionally carried out only by women (Mkumbira *et al.,* 2003). In many places farmers report higher yields from high CNP types, but this is typically not corroborated by controlled experimental results. Farmers commonly report that bitter cassava repels wild or domestic animals that uproot and feed on sweet types. The prevalence of these reports gives some weight to the theory that this was a significant factor in cassava's evolution. On the other hand, studies in Amazonian villages where 80 percent of the calorie intake is from high-CNP cassava, were not definitive in associating cassava toxicity and lower predation. Among many species surveyed, only the black agouti was said to prefer sweet varieties (Wilson, 2003). Farmers/processors also often associate the degree of bitterness of fresh roots with the quality of processed products, such as farinha (course flour) in Brazil. Researchers are just beginning to explore the biochemical evidence to explain this. Other possible explanations of cyanide's role seem to be less compelling. Evidence of insect or mite resistance, drought tolerance or nitrogen storage mechanisms from cyanogens is largely anecdotal, or at

best, empirical. Extensive and systematic research is needed to clarify the roles of cyanogenesis in cassava.

Farmers often grow high and low cyanogen types as though they were two distinct crops, used for distinct purposes.

Humans have at least two mechanisms to cope with cyanide in the circulatory system. The first is a binding reaction between HCN and serum albumen. The second is a conversion of HCN to the far less toxic thiocyanate by the enzyme rhodanase and 3-mercaptopyruvate sulphur transferase. Acute toxicity in humans appears to be rare. Almost always, where high-CNP varieties are grown, there is an accompanying tradition of adequate processing. If normal procedures are used, acute cyanide toxicity does not occur. However, scientists need to be extremely cautious about introducing high-CNP varieties to areas where a tradition of processing does not exist. They also should monitor HCN levels in products from new processing technology.

Long-term ingestion of low levels of HCN is a more common problem than acute toxicity. Chronic toxicity can occur where consumption is high (up to the equivalent of 1 kg or more of fresh roots per day over a long period), and where the consumption of iodine and protein, particularly animal protein, or other sources of sulphur amino acids, is very low. In the Democratic Republic of the Congo and Nigeria, ataxic neuropathy (nervous degeneration) and goiter (which leads to cretinism in severe cases) have been associated with high levels of cassava consumption. Konzo, a neurological disorder identified in the Democratic Republic of the Congo, Mozambique and the United Republic of Tanzania, appears to be associated with long-term, high levels of consumption of cassava with high cyanogenic potential, but occurrences are rare. Chronic cyanide toxicity has not been reported in areas of high cassava consumption in Latin America or Asia, which reinforces the hypothesis that goiter and ataxic neuropathy are caused by a complex interaction of several factors. The problem can often be solved with supplementary iodine in the diet, such as iodized cooking oil.

4.4 HUMAN CONSUMPTION

Cassava is overwhelmingly more important for human consumption in Africa than in other continents. Nonetheless, there is also significant consumption in many other countries, especially Brazil, Colombia, Indonesia and Paraguay.

Peeling and boiling is the simplest form of preparation, but this is appropriate only for sweet varieties. Boiling drives off low levels of hydrocyanic acid, but the linamarin itself is not destroyed. Most varieties selected for cooking become soft in 10- 20 minutes. Growing conditions can have substantial effects on root quality for fresh consumption, for reasons still poorly understood.

An enormous array of different products are made from cassava. Jones (1959); Albuquerque and Ramos (1980); Lancaster *et al*. (1982); Cock (1985); and Balagopalan (2002) reviewed many of these. Drying cassava is the easiest way to extend shelf life, reduce potential toxicity and reduce cost or energy for transportation. Most forms of processing for human food have a dry meal stage, either as a final or intermediate product. Variations in the form of preparation can be broadly divided into unfermented and fermented. An unfermented meal is made by grinding or slicing peeled roots, followed by pressing out excess liquid, drying and milling to form a meal. Fermentation may be achieved either by soaking whole roots, or allowing the ground mash to ferment. The end product may be a dried meal (farinha in Brazil, gari or fufu in West Africa, and kakonte in Ghana); a paste or dough (chickwangue in the Democratic Republic of the Congo); or baked or fried products (tapioca in Brazil, casabe in the Caribbean and gaplek in Indonesia).

4.5 ANIMAL FEED

Cassava has become an important commercial energy source in animal feeds since the 1970s. With the addition of protein, methionine and adequate levels of minerals and vitamins, low levels of cassava can replace maize in pig, poultry and ruminant diets with no decrease in performance. At higher levels,

problems may be encountered in getting animals to consume adequate quantities. Leaves, with about 22 percent protein on a dry weight basis, can provide a good protein source; however, the harvest and processing of leaves on a large scale is still a constraint because of high labour costs, or lack of efficient mechanization.

Cassava can be fed fresh after chipping, or even as whole roots in low-intensity systems. Although the crop is suited to continual harvest over a long period of time, fresh-fed cassava is not possible in many areas due to the potential of cyanide toxicity. It also may be too labour-intensive and costly for larger-scale operations, as compared with feed forms that are more efficiently managed. Dried chips or pellets are easily incorporated into balanced diets, and can be handled as any other dry feed. Both roots and leaves can be ensiled. This is an effective means to prolong storability of a perishable product, and to reduce cyanide toxicity potential. As with any ensiled crop, management is critical to assure proper fermentation and minimize growth of non-lactic acid bacteria.

As a rule of thumb, dried cassava can be incorporated economically into balanced rations when the market price is less than 70–75 percent of the price of maize. This differential is due to the need to incorporate additional protein into the cassava-based diets. In most tropical countries, cassava can compete under these economic conditions, unless the grain alternatives (generally maize or sorghum) are subsidized differentially as compared with cassava.

Buitrago (1990) provides an excellent and comprehensive overview of cassava in animal feeding.

4.6 STARCH

A wide array of food and industrial products use cassava starch, the main growth area for cassava products on a global basis. World trade of cassava starch grew from less than 10 000 tonnes in 1970 to more than 800 000 tonnes in 2000 (FAOSTAT). Much of the growth is the result of intense efforts to diversify markets for cassava products as the European market for dried cassava diminished in the 1990s.

Starch may be used directly or as a raw material for further processing. Starch-based products fall into three main categories: (1) native, or unmodified starch; (2) starch modified by physical, chemical or biological means, usually for industrial purposes; and (3) sweeteners, including high fructose syrup and glucose.

Cassava starch has uses in the food industry, for paper making, as a lubricant in oil wells, in the textile industry and as the substrate for the production of dextrins for glue manufacture. In between the two World Wars, distillers produced alcohol from cassava in Australia and Brazil. This use declined with the availability of cheap supplies of petroleum products, but there has been occasional renewed interest in producing alcohol from cassava during periods when the price of crude oil rose. A major question concerning the production of alcohol from cassava is the energy balance of the system. The energy used in distillation is considerable, but technological advances could reduce this. Often sugar cane has a better energy balance because cane stems can be easily used as fuel, whereas cassava stems are needed for propagation of the next year’s crop.

In general, the modified starches compete directly with starches of other origins, on the basis of cost, quality and convenience. On the other hand, the native starch has value for the specific properties of cassava.

Nearly all kinds of starch of vegetable origin contain two types of glucose polymers: an essentially linear molecule (amylose) and a highly branched polymer (amylopectin). In an analysis of CIAT's core collection, Wheatley *et al*. (1992) found a range of 17-26 percent amylose (as compared with about 28 percent amylose for maize and wheat starch).

Most starch is extracted from fresh roots. Roots are rasped or macerated to release starch granules from the cells. The starch solution is then sedimented, centrifuged or vacuum-filtered to separate the starch. Drying is carried out under the sun or artificially.

Cassava starch has characteristics that are especially appreciated by certain sectors of the industry (CSTRU, 2005) (Table 2.5). Unlike the starches of cereal grains and tubers, cassava roots contain low levels of impurities, including proteins and lipids. This high purity extends the range of its applications. Other important characteristics are:

- odourless*:* The absence of unpleasant odours enables this product to be conveniently blended with other flavouring ingredients;
- paste clarity: Cooked cassava starch (1 percent) has a light transmittance of 40–70 percent at 650 nm. Corn and wheat starch are in the range of 60 percent transmittance. This tendency for higher clarity of cassava starch makes it suitable for combining with colouring agents;
- the high ratio of amylase to amylopectin (80:20) gives cassava starch a high peak viscosity but low potential for retrogradation, resulting in a gel with good freeze-thaw stability.

Table 2.5 Properties of cassava starch

Property	Value
Chemical composition (percent dry basis)	
- Protein	0.15–0.30
- Fat	0–0.01
- Ash	0.10–0.15
Granule size (μm by image analysis)	3–4
Amylose content (percent by chromatography)	17–23
Amylose size (DP_n, by chromatography)	2040–4640
Swelling power at 85ºC (0.1 g starch in 15 ml water)	40–62
Solubility (percent) at 85ºC (0.1 g starch in 15 ml water)	22–42
Paste viscosity (3 g starch at 14 percent moisture in 25 ml water)	
- Pasting temperature (ºC)	67.0–74.0
- Peak viscosity (RVU)	350–490
- Trough viscosity (RVU)	110–210
- Final viscosity (RVU)	180–290
- Breakdown (RVU)	160–340
- Setback (RVU)	50–110
Thermal analysis (Differential Scanning Colorimeter, 30 percent starch)	
- Onset temperature (ºC)	60.0–65.0
- Peak temperature (ºC)	67.0–74.0
- Conclusion temperature (ºC)	79.0–87.0
- Enthalpy (J/g)	14.0–17.0
Retrogradation (percent by thermal analysis of starch gel kept at 4ºC for 7 days)	28.0
Degree of hydrolysis (percent using 1 percent each of α-amylase and glucoamylase at 37ºC, 48 hrs)	25–60
Source: CSTRU (2005)	

5. TRADE

Cassava trade as a percentage of total production is small. Fresh cassava trade has always been constrained by the high cost of shipping a relatively low-value product. Some developed countries, especially those with large populations of immigrants accustomed to eating cassava, import for the fresh market, mainly from Central America (to the United States and Canada) and from Africa (to Europe). Prior to World War II, producing countries, especially in Asia, exported cassava starch to the colonial

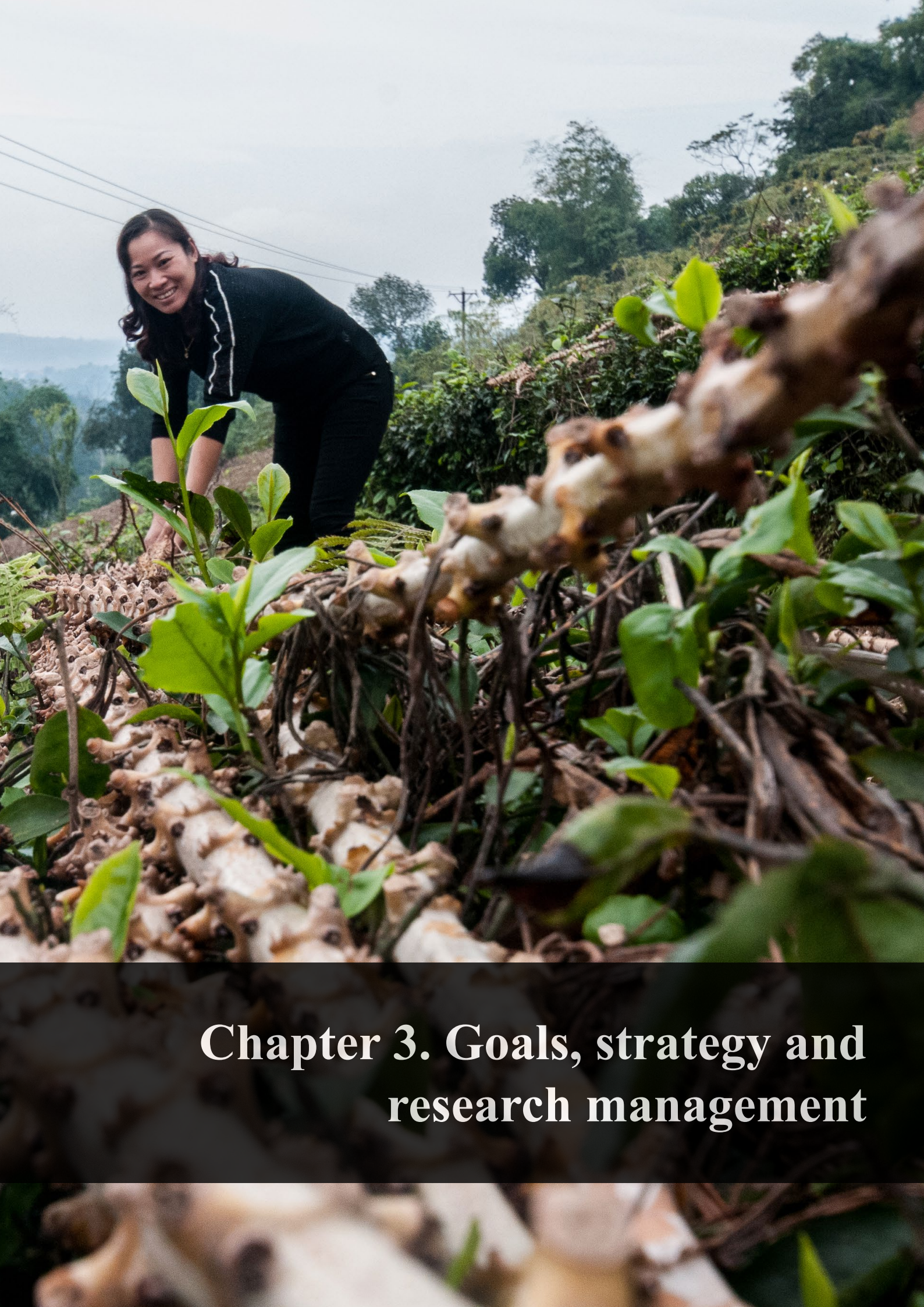
powers. This industry declined after the war, as battered economies tried to rebuild. As maize production surged, especially in the United States, this became the principal source of starch in global markets.

In the 1950s and 1960s, the main export product from cassava was flour, and was nearly all from Indonesia and Thailand. The 1970s brought the boom in demand for dried cassava in balanced ratios, and Thailand built a thriving industry to supply that market. Although this market declined considerably from its peak in 1990, trade has shifted to include a significant increase in cassava starch trade. Thailand leads the world in export of many cassava products. Africa grows cassava almost entirely for internal consumption, neither buying nor selling significant quantities (Table 2.6).

Table 2.6 Exports of cassava products (tonnes dry equivalent) by region

	1970	1990
World production	32 863 400	61 835 976
Dried chips and pellets		
World	1 603 626	3 617 009
Africa	64 702	630
Americas	4 684	79 774
Asia	1 506 511	3 305 698
Flour		
World	203 990	87 477
Africa	372	2 453
Americas	34 248	1 438
Asia	167 767	83 074
Starch		
World	4 579	867 405
Africa	4 452	424
Americas	8	29 592
Asia	35	828 300
Tapioca		
World	29 857	55 230
Africa	6 849	623
Americas	1 2008	1 134
Asia	21 740	53 137
Source: FAOSTAT data (2004)		

The growing demand for industrial cassava products will be a primary driver for new production technology in this century, demands that will translate to new opportunities for breeders to create better performing varieties. At the same time, traditional markets will also benefit from the breeders' ever-increasing ability to target specific traits for improvement. Cassava will, like other major crops, evolve from a commodity to a diverse array of traded products that depend on distinct genetics.



Chapter 3. Goals, strategy and research management

Setting goals may be the single most important step in research planning for a breeding programme. It is virtually impossible to develop appropriate and successful varieties without a foundation of clearly defined goals, and a strategy for meeting them. It is also impossible to set these goals in a vacuum. The interactive ingenuity of scientists, farmers, consumers and others, brings strength and legitimacy to research planning. Goals and strategy normally evolve over time, but the better they are elaborated at the outset, the more likely that costly, time-consuming modifications in strategy can be avoided.

Examples from the history of crop breeding amply illustrate the general principle that new varieties must usually go hand in hand with adjustments in other parts of the whole system of production, processing and marketing to achieve significant success. Farmers have selected over many generations for system optimization, not focusing only on high yields, but a balance between input costs, crop yield and quality, risk, income and others. Adjustments in the non-genetic components of the system are usually required for overall improvement in economic yield. An interdisciplinary research approach is basic. The more complete the information base before goals are set, the more likely these goals will be on track.

1. DEFINITIONS

The terms goals and objectives have very similar meanings and their use can be confusing. Usually goals are taken to mean the broader purpose of a breeding project or programme, such as to increase farmer income, to improve consumer nutrition or to decrease pesticide use. Objectives usually relate to more direct research results and may represent steps on the pathway to achieving goals. Examples of objectives in breeding might be to improve yield potential while maintaining high eating quality, or to improve host plant resistance to green spider mite. Nonetheless, there will often be grey areas where either the term objective or goal may be equally appropriate, or may be used interchangeably.

2. METHODOLOGY

It is usually helpful to first define goals: what will be accomplished through research? and then determine the research results that will be required to lead to those accomplishments. Institutional administrators normally provide broad goals, within which research leaders define specific objectives. A breeder's input into establishing goals and specific objectives depends on the particular institutional environment. It is imperative to understand the overall organization and management of research, how priorities are defined, and who are the relevant decision-makers. Table 3.1 is a hypothetical example of the levels of organization that research planners may need to consider, and how support of cassava genetic improvement might fit into the larger institutional and political environment.

A breeder may take the approach of setting goals based on resolving production constraints, on the basis of exploiting new opportunities or of some combination of these. If there are obvious constraints to production, such as a seriously limiting disease, this will likely take priority before other opportunities can be pursued, such as improved yield potential. Constraints resolution, while certainly a valid and often necessary approach to defining goals, is essentially a conservative approach that aims at full expression of existing genetic potential, e.g. adding insect resistance to a variety so that it can attain its full yield potential. On the other hand, creative thinking may open new possibilities for breeding that look towards novel goals for production, processing and marketing.

Table 3.1 Examples of administrative organization and research planning

1. Minister for Agriculture
- National goals for food production
- Trade policy for agricultural products
- R&D support for agriculture
- Policies on production support

2. Institutional director
- Institutional policies
- Allocation of priorities among research areas
- Hiring and evaluation of senior staff
- Securing funding
- Coordination among departments

3. Department leader
- Coordination of research among scientists
- Allocation of funding among projects and scientists

4. Plant breeder
- Hiring and evaluation of field/laboratory personnel
- Breeding programme design
- Allocation of funding among breeding projects
- Grant proposal development

The breeder should not set goals that can be more appropriately reached by other means. For example, a problem of protein deficiency in a cassava-dependent region does not automatically justify breeding for improved protein content of the roots. The same funds may be more effective if applied to promoting diet diversification. Some pests may be controlled by simple and inexpensive stake treatment, or by biological control, and would not justify a resistance breeding approach.

Some research environments encourage or accept risk-taking, and others expect scientists to pursue only those avenues with a high likelihood of success. The riskier research approaches (those with greater difficulty to achieve stated goals) will often be those with the highest payoff when they do succeed. Aiming for a 10 percent yield increase in a five-year period is a reasonable and relatively safe goal. Attempting to modify basic starch structure for new market opportunities could be much more difficult, but with a decidedly greater payoff in many cases. A practical approach for many breeders may be to define a few or several goals, with a range of risk and benefit. This should assure at least some degree of success and at the same time open opportunities for more difficult, high-impact successes. While *ex ante* cost–benefit analyses of breeding programmes are inherently fraught with uncertainty, they are a necessary exercise in the real world of tight research budgets and high expectations from the end user of new varieties.

Goals and objectives are built upon a foundation of information and assumptions about the proposed universe (biological, physical, socio-economic) for adoption of new technology. While a comprehensive study is certainly preferable, few programmes find the resources for this. Given the difficulty of obtaining the ideal level of background information, common sense, personal experience and intuition have often had to play as important a role as precise survey data or economic analysis. Too few programmes pay sufficient attention to studying specific target area characteristics and needs. They often base objectives only on experiences with other crops, or in other regions. There is not a right or a wrong interpretation of baseline information. Private industry will normally act upon indicators of the potential for profitability of new technology. The public sector may place breeding goals in a broader context of regional development.

A balance between short-, medium- and long-term goals is appropriate for most programmes. Often, research administrators and funding agencies need to see some relatively rapid payoff to research, to justify continued investment, but the progressive, long-term genetic improvement that most breeders aim for, also needs to be part of early planning.

A baseline study is necessary, but not sufficient, background to goal setting. Goals need to be relevant several years into the future, rather than at the time they are formulated. The breeder needs to project growers' and consumers' needs 10, 15 or more years into the future. Even if breeding involves only the introduction and testing of existing varieties, at least five years are generally needed for varietal release and significant adoption.

A problem-solving approach is usually an appropriate beginning point for defining critical goals. A useful procedure is actually to list production or utilization problems to be solved, assign priority on a 1-3 scale, and assess the viability/probability of resolving each problem by breeding, or, alternatively, by change of cultural practices (including improved agronomy, chemical or biological control of pests and diseases). Further examination of the means to resolve problems within the generalized realm of cultural practices will of course be necessary, but at the planning stage, all the breeder needs to decide is whether or not to include a particular problem as a breeding goal. Problems that might be nearly equally well resolved through breeding or through change of cultural practices might receive some input from both sides. Other problems will require simultaneous, complementary changes in cultural practices as plant genotype is changed, or vice versa. More concrete examples of these situations are given in chapters dealing with specific breeding objectives. A generalized rule of thumb is to keep the number of breeding goals to a minimum, to assure significant progress in improvement of a few characters rather than achieve minute progress in many characters.

In the early 1990s CIAT attempted to quantify and rank the importance of all significant yield constraints on a continent-wide and global basis (Table 3.2).

Table 3.2 Summary of principal categories of yield constraints in cassava at a continental level and expected yield gains (percent) resulting from their alleviation

Constraint	Americas	Asia	Africa
Soil factors	21	30	16
Management	22	18	20
Intrinsic varietal	15	20	14
Climatic	6	9	10
Pests	9	3	12
Diseases	13	2	17
Post-harvest	15	18	11
Current yield (tonnes/ha)	11.2	12.3	9.0
Potential yield (tonnes/ha) [a]	23.8	24.1	22.6

[a]Potential yield is calculated by adding the percentage, and multiplying by current yield. However, in reality not all of the constraint factors would behave in an additive fashion.
Source: Economics Section, CIAT Cassava Programme Annual Report (1995)

These data require continued updating as improved information becomes available, but can be a useful beginning point. Overall, the greatest diversity of problems occurs in the Americas. The diversity of biological constraints derives from the fact that cassava originated and evolved here, along with the pests and pathogens associated with the crop. Some of these were introduced to Africa and Asia, but many were not. The Americas also have the ecogeographical extremes for cassava adaptation and growth, and therefore provided diverse environments in which the crop would evolve. On the other hand, some of the pests and diseases became devastating in Africa, because the natural enemies that

helped to limit populations did not exist. Asia has, in general, avoided many of the more serious yield-constraining organisms. Most of those that are present cause only moderate damage.

Table 3.3 shows, through hypothetical examples, the process by which a breeder might combine quantitative and qualitative information to set breeding priorities. These examples also illustrate the importance of evaluating for each objective, whether a breeding or management approach is a more appropriate solution.

Table 3.3 Hypothetical exercise in establishing breeding priorities

Constraint	Income loss (%)	Strategy	Technology components	Projected yield gains (%)[a]	Breeding or crop management. approach	Costs of technology development	Costs of technology implementation
Cassava bacterial blight	22	Reduce inoculum pressure	- Introduce rotation - Change planting date - Improve phytosanitary status of planting material	6 8 7	Manage-ment	Low Low Medium	Low Low Medium
		Reduce plant damage	- Host plant resistance	15	Breeding	High	Low
Low soil fertility	20	Increase soil fertility	- Inorganic fertilizer - Green manure	15 12	Manage-ment	Low Medium	High High
		Better-adapted variety	- Increase nutrient uptake efficiency	8	Breeding	High	Low
Variable market quality	15	Improve harvest management	- Redesign harvester for less root damage	10	Manage-ment	Medium	Medium
		Improve stability of quality	- Longer shelf life	7	Breeding	High	Low

[a]As solutions are not independent of each other, estimated yield gains from applying various approaches will not be equal to the sum of their individual contributions

3. BREEDING FOR THE FUTURE

Success in plant breeding (for any crop) is often associated with previous or parallel events or processes that make new varieties more necessary or acceptable. Probably the most common historic example is the introduction of chemical fertilizers to improve production potential, especially with grain crops. The remarkable progress in yield improvement of maize, rice and wheat in the second half of the twentieth century was based largely on responsiveness to better soil fertility, as compared with lack of responsiveness of traditional varieties. Responsiveness to irrigation and tolerance to higher plant density

were also key factors in some crops and in some regions new markets brought similar but somewhat less widespread effects.

Pest or disease outbreaks can bring high and rapid pressure on breeders to resolve the problem with new varieties. While the cassava mosaic disease has been a long-term problem and breeding goal in Africa, the outbreak of a devastating new variant of the disease in Uganda in the early 1990s made the development and adoption of new varieties even more urgent.

Cassava breeding has until recent years focused on the traditional production systems and markets. While progress has been steady, the major impact in the future is going to be from the breeding programmes that have goals linked to future changes in some part of the production/processing/marketing system. These changes will vary regionally, but the common denominator is the continuing move from a crop grown for home use and local markets, to one that enters broader markets and is subject to an array of value-adding processes.

4. A CLIENT-ORIENTED APPROACH

Keeping in touch with producer and consumer needs, as opposed to the research station situation, at all stages of the breeding process, can minimize surprises and failures when developing new varieties. This is not to say that selection at all stages needs to be done on farmers' fields, nor that farmers themselves need to be directly involved in the selection process. Breeders, however, should be intimately aware of, and have frequent contact with, their principal clients, the farmers. In the past decade, breeders and social scientists have made significant progress in defining methodologies for incorporating farmers' perspectives into the variety development process. Many factors influence the character of farmer participation in research: the cultural milieu, the breeder's personal style, institutional policy, and programme objectives, for example. Chapter 20 covers farmers' roles in varietal development in some detail.

5. SPECIFIC OBJECTIVES

During various international meetings in recent decades, breeders across countries and continents have shown remarkable consistency in the general areas of priority for breeding. Frequently mentioned objectives are: high yield, early harvestability, high root dry matter, other quality traits required for local markets, resistance to principal local pests and diseases, tolerance to adverse soil and climatic conditions, good plant type and good stake quality. The following descriptions encompass many of the specific objectives of current breeding programmes. Later chapters discuss each of these in further detail.

5.1 ADAPTATION TO THE AGRO-ECOSYSTEM

A fundamental requirement of any crop variety is that it should have a general adaptation to the soil and climate of the target region. For cassava, temperature and rainfall patterns are especially important. Distinct genotypes may be needed for optimum adaptation to different combinations of temperature and rainfall. Without this basic physiological adaptation, there is no opportunity for other traits to be expressed.

In contrast to the leading cereals, adapted mainly to good soil and well-watered conditions, the Euphorbiaceae are distinctive in their rugged ecological adaptations. In most parts of the world, cassava's competitive advantage is in the more marginal areas, and especially with soil fertility/acidity and drought stresses. In fertile, well-watered areas, higher value crops can generally produce more income for the farmer than cassava, and may be easier to manage in more mechanized systems. This generalization may change if specific new value-added traits are incorporated into cassava, if productivity is greatly increased, or if the marketplace begins to place higher value on current traits. Most cassava breeders will be dealing with moderate or high stress, rainfed production situations. Generally, cassava breeders and agronomists should reinforce those qualities that promote the productive use of agricultural niches presently unsuitable to cereals and other more demanding crops.

Cassava's adaptation to difficult environments sometimes poses a conundrum for breeders and policy-makers. On the one hand, cassava is often one of very few options for farmers on marginal lands. Improving the crop's adaptation and productivity in these environments should improve the economic status of farmers. On the other hand, a strategy to protect the environment for the long term might be more appropriate. A goal might be to reforest these fragile areas, or combine perennial crops with high-value, high-input annual crops. For example, Limsila *et al*. (1994) proposed that cassava should retreat from erosion-threatened soils in Thailand, and that research should then focus on more intensive culture with higher inputs on less problematic soils. The policy environment has crucial implications for breeding programme objectives for agro-ecosystem adaptation.

Cultural practices are another key facet of agro-ecosystem adaptation. Cassava is intercropped on about half of the production area worldwide, although that percentage seems to be declining slowly. Selection for the appropriate cropping system and management conditions is essential. This may be relatively simple when a single cropping system predominates in the target region, but tropical cropping systems are typically highly variable and complex. To complicate matters further, new technology in farming systems and cultural practices is likely to be developed simultaneously with new varieties. Therefore, varieties must be selected not for the present systems, but for some uncertain future situations.

Although improved cultural practices are likely to be part of a new production package, these practices need not necessarily be costly or involve purchased inputs. A breeding goal should be to make efficient use of these inputs, especially those that have a higher cost–benefit ratio, are difficult to obtain or pose particular health or environmental hazards. This may apply especially to fertilizer and pesticides, but could also mean responsiveness to labour- or energy-intensive inputs such as land preparation.

5.2 YIELD POTENTIAL AND YIELD STABILITY

Yield potential of farmer-selected varieties is often low. Many other objectives often hinge upon the premise of an improvement of this yield potential. As with virtually any crop, one must expect only limited possibilities for yield improvement solely through genetic means. An accompanying package of improved cultural practices is usually essential. As a rule of thumb, historical yield gains for most crops have been equally divided between improved genetics and improved cultural practices.

The term yield potential as used by cassava breeders often differs conceptually from the commonly understood definition. It is best described as: yield under management conditions similar to those used by the better farmers of the target area, and without pest or disease constraints. Pests and diseases can be managed separately from physiological yield potential, through a range of control options. In most cases, even the better farmers will not be growing cassava in luxurious conditions of fertility or water management. In some regions, this will mean that yield potential should be measured in very stressful conditions of low fertility and water shortages. When there is little prospect of applying purchased inputs to cassava, experimental yields under non-limiting conditions will be of academic interest only. On the other hand, there will certainly also be situations where farmers grow cassava with optimum inputs, and these may need to be supported by an appropriately targeted breeding programme.

Yield potential for cassava should also include a time factor, that is, yield per unit of time, to distinguish early, medium and late maturing varieties. Maturity in cassava has a distinctly different meaning than in grain crops and is less clearly demarcated. Nevertheless, it is often a concept of considerable importance to farmers. Early maturity is usually defined as more rapid root bulking, rather than any type of physiological maturity. Typically, farmers want to have the best of both worlds – a variety with rapid bulking, but one that also retains high yield and quality when kept unharvested for long periods.

Low-income and otherwise at-risk farmers are often more concerned about stability of yield (or more precisely, stability of income or food security) from one year to another, than about achieving maximum yields. Other types of stability may also be important for particular situations, such as stability across different cropping patterns or soil types.

5.3 ARCHITECTURE

Plant architecture may impact yield potential, adaptation to different cultural practices and production of vegetative planting material for the next season. High early vigour may be desirable for weed control, or may be undesirable in a situation of intercropping with a species of low competitiveness. An upright plant type may be preferred in some areas because of ease of management; in others, a highly branched type may be useful for early canopy cover for weed control and reduced soil erosion. There is broad genetic diversity for plant type among landrace varieties, indicating an historically wide range of needs by farmers according to specific situations.

5.4 PEST AND DISEASE RESISTANCE

Pest and disease resistance presents some of the more difficult objectives for a breeder to develop, given the unpredictability of the pathways of pest and pathogen evolution over time. At the same time, it is often one of the more important objectives, given the generally low effectiveness and high cost of alternative measures. Pest populations are likely to change when new varieties or cultural practices are introduced, but many different elements can interact to complicate the direction of change. Certainly, a strategy of breeding for high resistance to all potential pests and diseases is impractical if not impossible. An obvious starting point is to examine yield constraints in the current varieties and production systems. The possible control methods, including host plant resistance, should be compared with established priorities. For the longer-term goals, entomologists and pathologists should provide input on probable patterns of change according to different scenarios of cropping system evolution.

5.5 QUALITY

For many breeders, concern about root quality crystallized in recent years, after recognizing the increasing emphasis that processors and consumers assign to quality in most markets. Texture, taste, starch content, starch quality and cyanogenic potential are among the fundamental traits to consider in establishing objectives. Markets determine the specific criteria. Often these criteria are very subtle and require detailed surveys of farmers, processors and consumers. Many landrace varieties have been carefully selected for root quality, and it may not be necessary to improve that quality. Maintaining a given level of quality during the process of modifying other traits can be a sufficiently challenging objective. In reality, there is often a trade-off, at least in the first phases of varietal improvement, where there is some decrease in quality owing to overriding emphasis given to improvement of yield potential, resistance or some other priority. This may be acceptable as an interim situation, while in the long term, emphasis needs to be targeted simultaneously at improving both quality and other priority traits.

Traditionally, cassava breeders did not heavily weigh nutritional considerations, as the main nutritional component is energy (or calories). This is inherently associated with starch production, which in turn is essentially yield itself. Other nutritional components may, for practical purposes, be ignored in some breeding programmes. Nonetheless, given the high dependence of certain regions on cassava, especially in Africa, as a calorie source, other macro- and micronutrient components should be considered. The principal micronutrient trait identified for improvement in cassava is β-carotene, precursor of vitamin A. The widespread health effects of vitamin A deficiency justify an all-out effort at a multipronged solution that includes breeding. Biotechnology will eventually open additional opportunities for practical modification of nutritional characters.

5.6 INTEGRATED IMPROVEMENT

Transforming cassava from a traditional, low input, medium productivity crop to a high productivity crop, efficient at utilizing moderate input levels, involves manipulating a number of characters. Acceptance by growers and consumers is commonly determined by a wide range of traits. Focusing on any one of them, to the exclusion of others, will be unlikely to meet with acceptance. It is nearly a maxim that no matter how strong the gains made in a particular character, success of a variety will be limited by its weakest traits. A very high yielding variety is likely to find little acceptance if quality does not meet certain minimum standards, and vice versa. In Latin America, where cassava evolved along with

a wide range of biological constraints, growers often require multiple pest resistance. In Africa and Asia the breeder may consider fewer objectives, but they will still need to be multifaceted.

One of the basic rules of breeding is that the difficulty increases and rate of progress decreases geometrically, with an increase in the number of genes being manipulated. Given that most traits of agronomic importance in cassava appear to be multigenically controlled, the formidable challenge of multiple trait selection becomes obvious. Hoopes and Plaisted (1987) gave the following example for potato breeding: "It is easy to name at least 20 traits that would be desirable in a new cultivar. If all these traits theoretically could be combined in a cross, and if one-fourth of the offspring had each of the traits at the desired level, only about one individual in a trillion would have all 20 traits. Even if a selection level of 50 percent could be used for each trait, only one genotype in a million would combine all 20 characteristics. In practice, selection levels are often closer to 5-10 percent for many traits, further reducing the odds of finding the desired genotype." The breeder has to prioritize carefully and limit the number of traits considered, in order to expect noteworthy overall progress within a practical time frame.

6. RESEARCH ORGANIZATION

Scientists normally pursue breeding goals within the framework of an established research structure. Research management is the process of obtaining and utilizing research resources (personnel, infrastructure, genetic resources, land, supplies and transportation) to achieve defined goals. This management involves various levels of decision-making, from the researcher through the national political leaders. Each level within the management structure will have different perspectives that need to be reconciled in the overall variety improvement scheme.

In a broad sense, research is often organized either in a project mode, or in a system mode, or may be some combination of the two. From a purely scientific point of view, a system mode is perhaps more appropriate, where the researcher can plan for the long term; balance short-, medium-, and long-term goals; and integrate all the necessary disciplines to reach objectives. Large private seed companies of major crops often operate in this mode. However, foundations and funding agencies that support public research rarely allow this luxury. They generally require research to be packaged in more defined units, with clear starting and ending points. Project-based research may encourage greater accountability by scientists, while system-based research tends to be more efficient for reaching long-term goals. The most common situation is probably where broad, long-term goals are sought through a series of focused and coordinated shorter-term projects, but where there are often gaps due to unfunded or inadequately funded projects.

6.1 BALANCING OBJECTIVES AND RESOURCES

6.1.1 Funding

Plant breeding is a science requiring considerable long-range planning. The benefits of research are usually separated by several years from the actual start-up time for a breeding programme; the lead-time is longer than for many other crop science disciplines. Due to many long-term research commitments, the breeder requires a high level of year-to-year stability of funding in order that gains are not compromised.

Planning of funding is probably the one area most prone to conflict among the different levels of research management. At each successively higher level, managers are trying to balance an increasingly larger number of entities requiring funds. It is only reasonable to expect that at each level, research managers will attempt to obtain what is considered by the next higher level to be a disproportionate share of resources available, for their own domain of responsibility.

Breeders may have little control over the funding they have available, or they may be fully responsible for writing grant proposals to outside donors. Probably any researcher, however, will have at least some influence over the way in which those resources made available are managed. Often the best way to attract more funding is through demonstrating actual and potential economic impact for the new varieties

being developed. In a research world where few scientists obtain funding easily, most cassava breeders also need to allocate a significant segment of their time to assuring adequate funding.

6.1.2 Personnel

Qualified and motivated support personnel are an invaluable asset to a breeding programme. Cost-cutting that reduces quality of employee output is rarely cost effective. On the other hand, many institutions, especially in the public sector, make it difficult to optimize personnel selection, because of policies that prioritize criteria other than worker productivity.

Funding for personnel versus other research resources may or may not be closely linked, depending upon the institutional structure. Historically, many research institutions set personnel levels at a relatively fixed number, and over time the personnel costs take an increasingly larger proportion of the budget, owing to increasing salary and benefits costs. This can become so extreme in some cases that little money remains for operations. Obviously this type of situation calls either for obtaining additional funding or institutional reform, of which neither is easily achieved; however, institutional reform seems to be the more difficult of the two.

6.1.3 Balancing resources

A useful concept in long-range planning is to view plant breeding output like the flow through a pipeline. In an established programme there is normally a continual flow of germplasm entering the various evaluation steps, with new varieties produced at the final stages. However, when a breeding programme first begins, the flow of germplasm through the pipeline is in disequilibrium, only one or a few steps of evaluation may be represented. The breeder must, however, from the outset, plan for a balance in emphasis among the different stages, based on resources available. Although the breeder may have very specific objectives and hope to accomplish these with existing genetic diversity, it is far more common that the objectives will lead to a long-term programme of continual upgrading of germplasm, requiring an ongoing germplasm flow through all stages in the pipeline. Planning for the appropriate distribution of resources among different phases of breeding is essential to keeping a balance of priorities.

As an example, it may appear in the first years that the breeder can handle large numbers of seed introductions, because nearly all his or her efforts go into these evaluations. Within a few years, however (assuming continued annual introductions), there will be the full range of evaluation stages, and a new balance of emphasis must be attained. This suggests that initially some restraint may be required to avoid bulges in the pipeline that take up a disproportionate share of resources.

6.1.4 Sharing resources

Generally, the breeder works within a team of scientists, organized either along disciplinary or interdisciplinary lines. Many resources can be shared by mutual agreement of team members. The various team members normally require labour, transportation and supplies on a somewhat different schedule, allowing for considerable sharing. For this to work with minimum conflict, there must be some commonly understood ground rules about priorities for distribution of resources and a generally cooperative spirit among team members. Any member viewed as usurping a disproportionate share will soon find a lack of willingness on the part of other team members to share resources.

6.1.5 Optimizing use of general services

Some institutions provide services on an institution-wide basis, which are not charged against the budget of any particular programme. These may include a labour or secretarial pool, laboratory services such as soil and tissue analyses, statistical analyses and others. In recent years of increasingly tight budgets, uncharged services are rather rare. Obviously these services involve an institutional cost that administrators will monitor, but it is to the advantage of individual research projects to make effective use of these services, within the goals of the research programme.

6.1.6 A research mind-set

Continually ask yourself if there is a better way of doing things. Observe how researchers in other programmes manage resources and learn from their experiences. Do not get stuck in a rut with inefficient

or ineffective practices. Taking risks to improve a programme will probably mean that some ideas fail, but those that succeed will more than compensate in their payoff.

6.1.7 Labour-saving strategies

Field research in cassava is almost unavoidably a labour-intensive proposition. Costs of labour vary widely from one country to another, as does availability of mechanization. Thus, the urgency of searching for labour-saving strategies will be determined in part by cost effectiveness of different options, and in part by any government or institutional policies regarding employment of labour versus minimizing labour costs.

Mechanization is more difficult for many aspects of cassava management than for other crops. Planting and harvesting are difficult to mechanize for small plots, even though possible on a commercial scale. Land preparation and weed control are more easily mechanized. Certainly, the growing demand for mechanization at the commercial level will also drive the introduction of innovations that can be adapted at the level of breeding nurseries.

Rental or leasing of goods and services may be more economical than purchase, especially where only short-term use is required.

6.2 ADDITIONAL FUNDING

When there is not enough money to complete the job, somebody in the research structure needs to take responsibility to look for more. This normally is the job of administrators, at least in the research institution environment. In universities, it is more common for a faculty to find its own funds. Increasingly, scientists in many types of research organizations are being requested to find at least part of their research support. However, even when finding and distributing funding is the sole responsibility of administrators, the breeder may influence these individuals by presenting convincing evidence of the need for more resources.

Donors generally prefer to fund activities they perceive as new or unique, at the forefront of new trends in research, and something with demonstrable results in the short term. They are looking for results that will accomplish certain scientific and/or social objectives, but they also usually hope to gain some status and recognition for creative thinking within their institution. A donor's objectives may not always be fully compatible with the breeder's priorities, but sometimes negotiation and compromise are necessary. Administrators are usually somewhat removed from the actual research details and may evaluate research proposals not solely on their scientific merit but also on other criteria. Sometimes it is helpful to describe projects in less conventional terms or concepts that will catch the attention of donors/administrators, e.g. germplasm enhancement, applied biotechnology, or socio-economically sensitive research. This has to be done in a way that is genuine rather than gimmicky. Catchy terms have a way of quickly becoming overused and passé, and then may cause a negative reaction from donors and administrators. A breeder wanting to attract money may have to use some of the new terms to describe traditional research. The breeder should be able to phrase research proposals to attract the attention of administrators and donors, while still keeping the merit of the research as a primary focus.

In attempting to obtain more research money, working through proper channels is crucial. Most institutions have established channels through which funding may be sought. A search for additional funding might follow this sequence:

(1) Gather and organize ideas from personal research experience, from within your institution and from outside organizations.
(2) Research potential donors' funding priorities and requirements for proposal submission.
(3) Submit basic ideas informally to immediate supervisor and research team members, for comments and suggestions.
(4) Write a draft version of the proposal.
(5) Submit proposal for informal peer review and criticism (e.g. team members).
(6) Revise proposal.

(7) Submit preliminary proposal to immediate supervisor and/or other administrative levels as appropriate, for comments and suggestions.
(8) Write final proposal.
(9) Present to appropriate funding agencies.

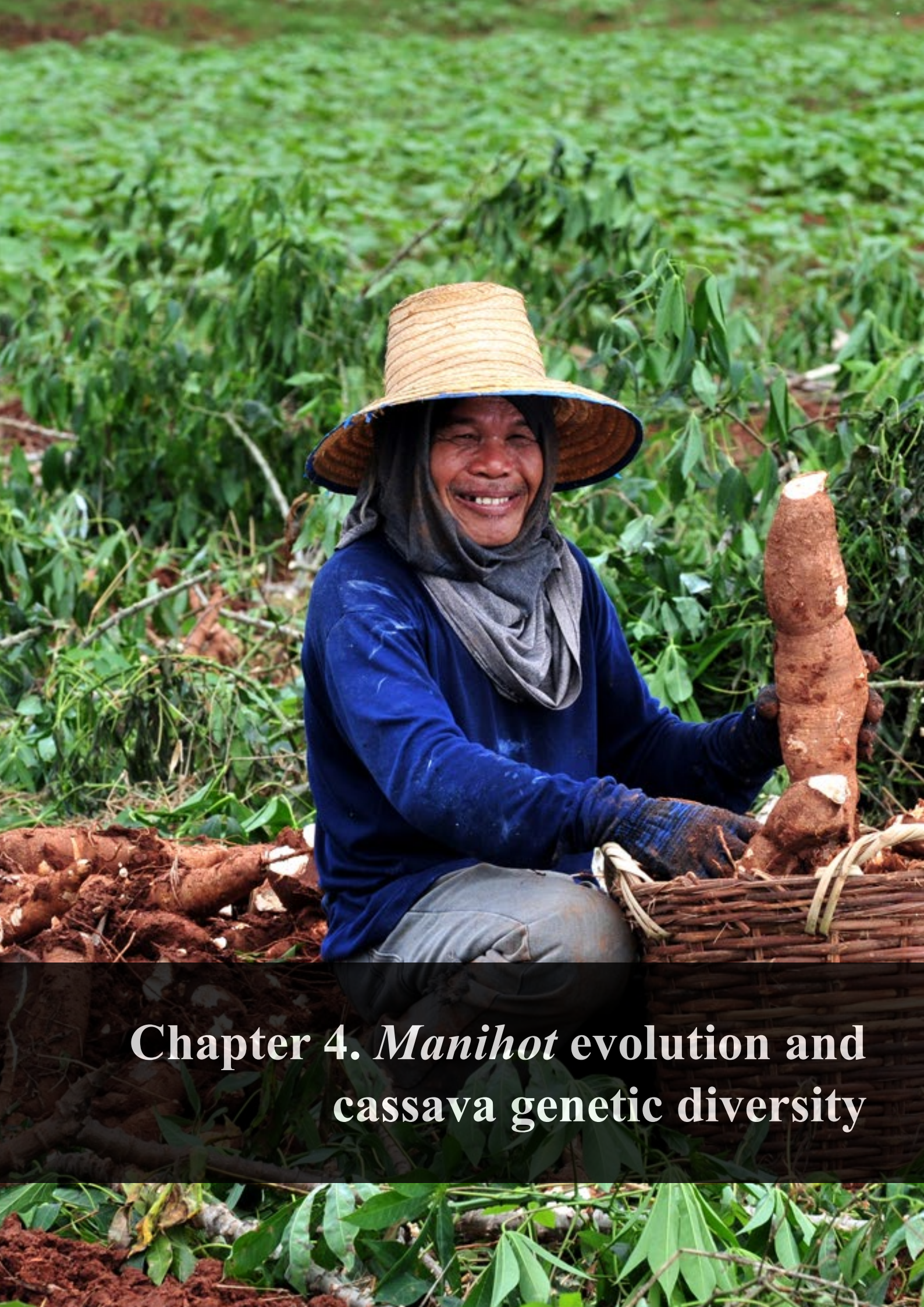
Many donor organizations have regional offices for the purpose of contacts with national programmes. The IARCs are not donor agencies; they rely themselves almost totally on donor contributions. They do however, have a range of contacts that can be a useful resource for national research programmes, and in many cases can provide collaboration in the process of developing project funding. There are many successful examples of national programmes and international centres combining expertise to obtain project funding.

It is an unpleasant reality that resources for research are sometimes scarce, or are at least perceived to be inadequate by the affected scientists. One should always first pursue the possibilities of obtaining more funding before starting to think about where to make cuts in the research programme. The reality is, however, that cuts or adjustments may have to be made at some point in one's research programme, and it is useful to have some guidelines to make cuts wisely. Ideally, the research institution will make cuts that do not compromise long-term goals. Budget cuts affecting projects that can be stopped and started up quickly are often more logical than projects that require long-term continuity. On this criterion, variety improvement programmes should be among the last to be cut, because they are long-term in nature, and cannot be easily stopped and restarted.

If cutbacks are imposed on the breeding programme, however, the same general guidelines should be applied within the programme. That is, projects within the programme that are only indirectly related to long-term goals should be the first to be reduced or eliminated. Generally, those long-term activities directly related to production of new varieties should be continued.

There may be an apparent conflict in following these suggestions, in that short-term results may be necessary to justify to administrators and donors the productivity of the research programme, and thereby to assure continued funding. Politics may dictate that some compromise be made in meeting long-term goals by giving a disproportionate share of emphasis showing more immediate results.

Experience and interaction with other breeding programmes outside the local assigned target region are often a substantial benefit both for the scientific development of researchers, and ultimately for the institutions for which they work. Many possibilities exist for this type of experience. The international centres frequently offer training, consulting and conferences, and these are some of the best possibilities for outside experience for cassava breeders. Recommendation and approval by the scientist's supervisor is always a prerequisite. The scientist or supervisor may contact the international centre (in the case of cassava, either CIAT or IITA) to learn about training possibilities in a given subject area. There are possibilities for courses, short- or medium-term specialized training, or degree work.



Chapter 4. *Manihot* evolution and cassava genetic diversity

Crops are the basis of sustenance of most humans, either consumed directly, or indirectly as animal feed. Agriculture's success is fundamentally dependent on genetic diversity. Existing diversity not only supports current food production, but also provides the genetic building blocks with which breeders construct new varieties. The ability of the breeder to mould a crop to the demanding specifications of growers, processors and consumers depends on having available a sufficient quantity of genetic building blocks with appropriate characteristics. As a rule, broadening the choices improves the breeder's effectiveness in assembling a new variety.

Ironically, the success of breeders in exploiting diversity to develop widely accepted varieties can threaten the existence of the genetically rich landrace varieties that underpin further crop improvement. Additionally, the expansion of agriculture and of population centres can threaten the habitats of related wild species. It is not enough just to study and understand genetic diversity; there is also a need to design comprehensive long-term strategies for its management as a permanent resource. Germplasm and other natural resources will not remain accessible for the future by default, but only by planning and financial commitment. Unmanaged resources tend to disappear. The Keystone Centre estimated resource needs for crop germplasm management to be in the order of US$300 million per annum, about twice what was being spent in 1990 (Keystone Centre, 1991).

Frankel and Brown (1984) described three phases of genetic resource activities, as they evolved for several major crops: (1) with the impulse derived from Vavilov's discovery of geographical centres of diversity, the first phase emphasized biogeography, taxonomy and evolution; (2) the second phase followed on the rapid displacement of landraces by the success of the Green Revolution, and emphasized conservation; and (3) with substantial collections in hand, work should emphasize their evaluation for use in genetic improvement programmes.

This chapter focuses on descriptive aspects of *Manihot* genetic diversity. The discussion assumes that the breeders' primary interest in genetic diversity is for the eventual contribution this knowledge and these physical resources make to the improvement of cassava as a crop. This is not, however, the only possible objective. There may well be other priorities for genetic resources research that are unrelated to cassava improvement (e.g. medicinal uses, ethnobotanical and archaeological research). These alternatives are not discussed here, but should not be ignored in a comprehensive management strategy.

1. INFLUENCES ON DIVERSITY

Most crops have been profoundly shaped by both the pre-domestication evolutionary forces on the wild species progenitors, and by post-domestication influences of natural and human selection. Influences on genetic diversity can assume either of two directions: broadening by the creation or introduction of new diversity through mutation, germplasm dispersal or intercrossing; and restriction by differential selection, genetic drift, or habitat destruction.

1.1 EVOLUTION AND DOMESTICATION

New variations arising from naturally occurring mutations or genome reorganization must have been a key part of the long-term evolutionary processes for *Manihot*, just as for any other genus. The pathways of this variation will only become clearer with the broad application of DNA analysis. The *Manihot* gene pool appears to be quite fluid and dynamic. There is a broad cross compatibility among species, which undoubtedly contributes to considerable natural intercrossing, with new diversity continually arising. It can probably be assumed that during cassava's domestication, a broad spectrum of diversity was sampled from the wild progenitor(s) to form the base of a cultivated crop. For cassava, almost exclusively a cultivated species, the continuing creation of new diversity comes about both from plant breeders and farmers. The latter is currently a relatively small contribution from intercrossing that occurs naturally in cassava fields, and eventually yields surviving seedlings selected by farmers.
The genus is clearly of New World origin, but further details of its evolution and distribution within the New World have been poorly understood. Only since the 1990s has there been better progress in defining

an evolutionary history, with the discovery of wild cassava, and the aid of molecular analyses to examine relationships between the crop and the wild species.

Centres of crop diversity are not synonymous with centres of origin. These centres of diversity may also be regions of relictual genetic diversity (museums), zones of relatively recent adaptive radiation, hybrid contact zones, or any combination of these. Likewise, wild relatives are not necessarily crop progenitors. They may also be feral escapes, hybrid derivatives of the crop and other wild relatives, or weedy companions (Bretting, 1990).

Archaeological evidence of cassava in northern South America indicates considerable antiquity for its cultivation. Radiocarbon dates are much earlier than those from the Brazilian/Paraguayan region. Nonetheless, as cassava was broadly cultivated in the New World several thousands of years ago, it is difficult to associate the sparse archaeological remains with crop origins.

The fact that most crops evolved in seasonal environments, where there is a tendency for plants to store food, could suggest that *M. esculenta* probably arose in a seasonally dry environment. However, there has never been sufficient molecular or archaeological evidence to support this. While both morphological and molecular data support the hypothesis that *M. aesculifolia* and *M. carthginensis* are some of cassava's closest relatives, neither is suggested as cassava's likely progenitor (Bertram, 1993).

There are few reliable phenotypic characters in the genus *Manihot* to indicate evolutionary relationships. Most of the species (including *M. esculenta*) show high intraspecific morphological variability. As it was not possible to confidently narrow origins with the use of morphology or archaeological evidence, a theory of multiple origins arose, but this was based less on positive evidence than on lack of evidence for alternative hypotheses.

In what was to become the first insight into an entirely new perspective on cassava's origins, Mr Antonio Costa Allem of CENARGEN in Brazil, discovered a putative wild population of cassava in Goias State in 1982, described as *Manihot esculenta* ssp. *flabellifolia*. Continued explorations showed that this subspecies was distributed in a zone of transitional forest between the Amazon basin and the drier savanna to the south and east, including areas of the states of Acre, Rondônia, Mato Grosso, Goiás and Tocantíns (Allem 1987; 1992; 1994).

M. esculenta ssp. *flabellifolia* is similar to cassava morphologically, but cassava has greater root thickening, swollen leaf scars and a stem morphology that is adapted to vegetative propagation (shortened internodes and thicker stems for more carbohydrate reserves). As with most *Manihot* species, *M. esculenta* ssp. *flabellifolia* is sporadic in its distribution; most populations typically comprise fewer than 15 individuals.

Early work with molecular markers to explore evolutionary patterns of *Manihot* indicated that South American and Central American species form two distinct lineages, and cassava is more closely related to the South American group. This work included RFLPs (Bertram, 1993; Fregene *et al*., 1994), AFLPs (Roa *et al*., 1997) and DNA sequences (B. Schaal, cited in Olsen, 2004).

At the next level of molecular evolutionary studies, variations in SNPs (single nucleotide polymorphisms) and SSRs (simple sequence repeats) were used to explore cassava's relationship to *M. esculenta* ssp. *flabellifolia* (Olsen, 2004). These studies compared a presumed wide genetic diversity of cassava clones selected from CIAT's core collection, and samples from a range of *M. esculenta* ssp. *flabellifolia* genetic populations. The results appear to definitively place cassava within the range of genetic variation of the subspecies. Across the eight loci examined, the cassava clones contain an average of 18.8 percent of the total variation of the wild species. *M. esculenta* ssp. *flabellifolia* genetic variation is sufficient to account for cassava's genetic diversity, without any need to involve a hybrid origin (Olsen, 2004). The composite of evidence from molecular studies gives strong support to *M. esculenta* ssp. *flabellifolia* as the progenitor of cassava (Table 4.1). Allem (2002) now proposes that there are three subspecies within *M. esculenta*: subspecies *esculenta, flabellifolia* and *peruviana*.

Table 4.1 Areas suggested by authors as the likely cradle of the domestication of cassava

Brazil	Pohl (1827)
Brazil	Mueller (1874)
Eastern tropical Brazil	de Candolle (1884)
Brazil	Pax (1910)
Peru	Cook (1925)
Brazil	Lanjouw (1932)
Northern Amazonia	Schmidt (1951)
Brazil – Central Paraguay	Vavilov (1951)
Venezuelan savannahs	Sauer (1952)
South America	Anderson (1954)
Peru or Mexico	Rogers (1963)
Brazil	Jennings (1963)
Southern Mexico, Guatemala, Honduras	Rogers (1965), Rogers and Appan (1970)
Eastern Venezuela	Reichel-Dolmatoff (1965)
Peru	Lanning (1967)
Mexico and Central America	Schwerin (1970)
Northern Amazonia	Lathrop (1970)
North America	Rogers (1972)
Amazonia	Spath (1973)
Central America and north-eastern Brazil	Purseglove (1976)
Amazonia	Schultes (1979)
Mesoamerica	Jennings (1979)
Goiás, Mato Grosso, Rondônia states, Brazil	Allem (1997)
Mato Grosso and Rondônia states, Brazil	Olsen and Schaal (1998, 1999)
Source: Summary by Allem (2001)	

Allem (2002) also provides interesting anecdotal evidence on the possibility that domestication of cassava from wild species is not that difficult and is in fact still taking place today in parts of Brazil. He also proposes a transitional link between cultivated cassava and its wild ancestor, in the form of a landrace called *manipeba* in northeast Brazil. This landrace (it is unknown how many distinct genotypes make up this landrace) appears to be botanically and agronomically intermediate between wild and cultivated cassava, and as such gives a possible snapshot of the route to cassava's domestication.

What does the current understanding of cassava's origins mean for the cassava breeder? It is still too early to know for sure. While the evidence for a single species as ancestor of cassava is growing, further confirmation is required. Hypotheses need to be developed and tested regarding cassava's movement from its region of origin and the potential influences of other species (apart from *M. esculenta* ssp. *flabellifolia*) in other regions, especially Mesoamerica. Also, molecular studies need to be applied to clarify evolutionary patterns of the other, nearly 100 *Manihot* species. The preliminary finding that less than 20 percent of the genetic variability of *M. esculenta* ssp. *flabellifolia* apparently exists in cultivated cassava (Olsen, 2004) may or may not indicate untapped useful variation in the progenitor species. Certainly, the possibilities are worth exploring, but at the same time, breeders should not be too surprised if they learn that the wild species do not have vast potential for improving a modern crop. Nor should breeders be too quick to assume that a character expressed in a wild population can make any contribution under the very unnatural conditions of agriculture.

1.2 DISTRIBUTION

None of the species existed outside the New World until the arrival of Europeans in the late 15th century. It was one of the early crops exported to Africa, brought first from the east coast of Brazil to the west coast of Africa in the late 16th century. It quickly became established as an important famine reserve crop. Due to the importance of the crop and the range of habitats into which it dispersed, apparently

wide new genetic diversity evolved in this relatively short period of cultivation in Africa (Allem and Hahn, 1991). Near the end of the 16th century, the Portuguese took cassava to Goa (India). The Spanish apparently made introductions from Mexico to the Philippines in the 17th century. In 1735, the French took the plant from Brazil to Cape Verde, Mauritius and Reunion. Around 1800 it was taken from these islands to Madagascar. From Mauritius it was imported to Indonesia and Ceylon (Sri Lanka) in 1740, and to Calcutta, India in 1790.

It is not known to what extent wild species were distributed by humans in ancient times. The introduction of a few species to other continents in modern times, however, is documented. Bringing new species into contact through human migrations could have contributed to the rise of new diversity, especially in the species-rich regions of Brazil and Mexico. *M. glaziovii* may have had an important impact on Africa through natural intercrossing with cultivated types to create the arborescent cassava (S.K. Hahn, personal communication).

1.3 FARMER SELECTION

Cassava appears to have evolved under highly localized biological and physical influences. This is probably not very different from the situation of many crop species. Due to early and wide dispersal of the crop and relatively low levels of genetic interchange among regions, many distinct and locally adapted gene pools evolved. Although normally vegetatively propagated, cassava produces seeds that can give rise to new variability in traditional farming systems. The plants derived from these seeds may be recognized by farmers as potential new varieties and given special care to compensate for their lower vigour at the initial stages. Thus, the farmer-breeder contributes to crop evolution by creating and selecting new genetic diversity.

1.4 BREEDING

Modern plant breeding is typically considered to have negative influences on a species' genetic diversity. Breeders usually draw on a relatively narrow range of the total germplasm base, and these narrowly based genotypes may eventually displace landrace varieties over large areas. This, however, is not the only possible scenario. With current widespread consciousness of the risks of loss of genetic diversity in agriculture, many breeders are considering alternative models.

The effects of modern breeding on the cassava gene pool have been modest compared with other major crops; there has, as of yet, been no large-scale loss of landraces from replacement by bred varieties. In Thailand, the widespread new varieties replaced essentially only a single traditional variety, Rayong 1. In other countries, there is more risk of loss of diversity as the pace accelerates for releasing new varieties from national and international centres. For the most part, however, change is unlikely to be rapid, given the characteristics of many cassava-growing regions (diverse; requiring a range of genotypes) and of cassava farmers (poor; limited access to new technology). Nonetheless, it is the breeders' responsibility, along with other germplasm experts, to plan strategies not only for the conservation of genetic diversity *in situ* and *ex situ*, but for the development and deployment of new varieties in a manner that minimizes risk of loss of diversity.

It is evident that wild species can contribute to increased crop genetic diversity, but a breeder’s interest in this diversity is conditional. Williams (1991) stated, "Breeders will never use wild species if they can find the genetic diversity they need in the cultivated germplasm." This is perhaps not so much an indictment of the conservatism of breeders as it is an acceptance of their realism and practicality. However, two trends are moving breeders towards greater interest in wild species as an expanded base of genetic diversity: a broader range of objectives and techniques that allow better identification and transfer of genes among species.

There has been little publicized or recognized positive impact that breeders have had on crop genetic diversity, and cassava illustrates this case well. Early introductions from Latin America to Africa and Asia were certainly from a narrow genetic base, thereby limiting the diversity available to farmers for selection of new varieties. An extreme example of this is Thailand, where until the 1990s, a single clone

covered all but a small percentage of a cultivated area exceeding one million hectares. Breeders' success was instrumental in increasing diversity, now with five or six new varieties planted in most of the area. CIAT has almost certainly introduced more diversity into both Africa and Asia in the past 25 years than had ever been introduced in previous history (Table 4.2). Clearly, this diversity has not all been incorporated into the national gene pools; only a small proportion makes its way through the breeders' selection process and an even smaller proportion to farmers' fields. In any case, it is illustrative of the positive impact that breeders can have on enhancing genetic diversity in crops, especially outside the centre of origin.

Table 4.2 Summary of international cassava germplasm shipments from CIAT, 1972-1992

	Seeds		
Region	**No. of crosses**	**No. of seeds[a]**	**No. of clones[b]**
Americas			
South America	1 644	107 434	1 578
Meso-America	1 201	65 313	698
Caribbean	793	48 074	767
North America	221	23 392	698
Subtotal	859	244 213	3 741
Africa	2 608	302 515	0
Asia & South Pacific	5 487	331 828	572
Australia	52	1 405	48
Europe	146	50 149	189
Middle East	0	0	12
Subtotal – outside Americas	8 293	685 897	821
Total	12 152	930 110	4 562

[a]Each individual seed is a genetically distinct entity.
[b]Clones may be repeated for different countries within and across regions

Choice of breeding strategy and methods also influences the impact of new varieties on a crop's overall genetic diversity. Methods that draw upon a broad genetic base to develop individual varieties and strategies for variety deployment that stress multiple releases, can have a positive impact.

1.5 HABITAT MODIFICATION

Human influence on evolution and genetic diversity of *Manihot* has been principally in the form of habitat modification, and introduction into new habitats. The encroachment of agriculture into natural

habitats is a major threat to genetic diversity of many species, not excluding those of *Manihot*. The extent of these influences is still poorly quantified, especially in historical terms.

Patiño and Hershey (1983) noted especially the cerrados of Brazil as an area facing risk of genetic erosion. Nassar (1979) documented genetic erosion in the state of Goias in central Brazil, based on reports of wild *Manihot* species and his own visits, spanning a twenty-year period. In several cases, all populations of a given species had disappeared by 1977-78.

Howeler *et al*. (2001) noted that the main threats to *Manihot* from land clearing and development occur in Brazil's Amazon, southern (subtropical), eastern (cerrado or savanna) and northeastern (caatinga) regions, and in Mexico. The southern and eastern regions of Brazil have been dramatically deforested since the middle of the twentieth century, for the seeding of pastures and the cultivation of export crops like soybeans, wheat, oranges and coffee. From an area that was largely untouched 50 years ago, almost half of the cerrados are now farmland (Howeler *et al*., 2001). The caatinga vegetation covers about 11 percent of Brazil's land area. The native caatinga vegetation decreased from a 64 percent cover in 1984 to 41 percent in 1997 (Allem, 1997). Land clearing has been most prevalent in areas inhabited by seven wild *Manihot* species known as the *maniçobas (M. caerulescens, M. diamantinensis, M. dichotoma, M. glaziovii, M. jacobinensis, M. janiphoides* and *M. maracasensis* [Howeler *et al*., 2001]). These species are adapted to a region with some of agriculture's harshest, drought-plagued conditions, and as such, may be a valuable resource for future breeding of cassava for semiarid environments.

Deforestation of the Amazon, though far less extensive (estimated at about 15 percent) has also threatened *Manihot* species (seven forest species of *Manihot* are known).

Although cassava is only locally important in Mexico, this is a secondary centre for wild species diversity. Expansion of cassava production presents little risk to the diversity of species in the region, at least into the medium-term future. On the other hand, development and expansion of agriculture are a clear threat to the fifteen or so *Manihot* species native to Meso-America.

2. APPROACHES TO STUDYING GENETIC DIVERSITY

2.1 TYPES OF VARIATION OBSERVED

Genetic variation may manifest itself at various levels of biological organization or expression: ecological adaptation, agronomic and consumer-related traits, morphological traits, chromosome morphology and behaviour, biosynthetic pathways and plant systems, and molecular variation. Traditionally, only traits expressed at the whole plant level were used to assess diversity, and until recently were still the most widely used. The descriptors recommended by International Plant Genetic Resources Institute (IPGRI – formerly IBPGR) for characterization and preliminary evaluation (Gulick *et al*., 1983) are mainly of this type. The utility and importance of plant traits for assessing genetic diversity cannot be minimized. Even with current molecular techniques, visible trait expression is often a practical means of evaluating genetic diversity for large numbers of traits and for large numbers of genotypes or accessions. It is a means of associating genetic diversity with traits of interest to breeders. As the expression of plant traits can be far removed from variation at the DNA level, there is a substantial possibility for complex interactions and influence of environmental effects. Sometimes these effects completely mask genetic variations.

The following discussion concentrates on present levels of knowledge and recommendations, with only cursory descriptions of techniques, because these are widely used and not specific to *Manihot.* Table 4.3 gives examples of types of information used to study genetic diversity and their status in *Manihot*.

Table 4.3 Principal sources of variation used to study crop genetic diversity

Types of variation observed	Methodology	Level of discrimination	Proximity to DNA-level variations	Examples of applications	Level of development in *Manihot*
Ecological adaptation; distribution; morphology	Classical taxonomy	Subspecies	Distant	Systematics and evolution; species distribution; breeding	Intermediate
Agronomic and consumer-related traits (most are quantitatively inherited)	Field and market evaluation	Genotype groups	Distant	Variety recommendations; genetic improvement	High for *M. esculenta*; low for wild species
Stable morphological traits (often simply inherited)	Characterization	Genotype	Intermediate to distant	Genotype identification; genotype stability	High for *M. esculenta*; low for wild species
Chromosome morphology and behaviour; DNA contents	Classical cyto-genetics; flow cytometry	Species; ploidy changes within species	Close to intermediate	Systematics and evolution; breeding	Low
Biochemical pathways and plant systems	Measures of pathway or system products	Species; genotype groups	Intermediate	Genetic improvement	Low
Biochemical markers	I. Enzyme and seed storage protein electrophoresis	Genotype	Close	Genotype identification; genotype stability; systematics	Intermediate
Molecular markers	II. DNA analysis				
	A) Restriction techniques				
	i) Nuclear DNA	Genotype	Very close	Genotype identif.; genotype stability; gene tagging; systematics and evolution	Low
	ii) Chloroplast DNA	Species	Very close	Systematics and evolution	Low
	B) Nucleotide sequencing	Gene	Analogous	Gene structure and function; systematics and evolution	Incipient

2.2 ECOLOGICAL ADAPTATION, DISTRIBUTION AND MORPHOLOGY

The species of *Manihot* are perennial and vary in form from acaulescent shrubs to trees with trunks 25 cm in diameter and a height of 10–12 m. They are generally sporadic in their distribution and never become dominant members of the local vegetation. Most thrive in seasonally dry regions, with few in rainforest ecosystems. Those found in the rainforest are usually invaders after clearing the forest. Thus, the species of *Manihot* appear to be shade-intolerant, capable of survival only with plenty of sunlight. They are not good competitors with other species.

All the species are sensitive to frost, thus limiting their distribution to elevations below about 2 000 m. Only two wild species, *M. grahami* and *M. anisophylla*, thrive in regions of occasional, but predictable, frosts.

As many of the species grow where long dry periods are common, they have evolved mechanisms of drought tolerance or avoidance. One of the most notable of these mechanisms is the production of storage roots where large amounts of starch are accumulated. In all species studied, these storage roots also contain the glucoside linamarin, which breaks down after cell injury to release hydrocyanic (prussic) acid (HCN).

Cassava is the only species of the genus that is widely cultivated. A few other *Manihot* species have had minor commercial use, especially as alternative sources of latex for rubber production (*M. glaziovii* and *M. caerulescens*).

Rogers and Appan (1973) classified areas of the Americas where species were dense or not dense, on the basis of a number of species present. Nassar (1978) refined this classification to describe four centres of diversity, three in central and northeastern Brazil, and one in Mexico. Studies on intraspecific diversity of the wild species are only recently advancing, principally with molecular tools (Bertram, 1993). It is impossible at this time to know whether there are identifiable centres of diversity for individual species other than *M. esculenta*.

Rogers (1963) regarded several species as close to cassava on the basis of adaptation and morphology (Table 4.4).

Table 4.4 Species of *Manihot* regarded as close to cassava on the basis of morphology, ecology and geography

Species	Range
M. carthaginensis	All countries bordering the Caribbean
M. aesculifolia	Mexico, Central America
M. grahami	Argentina, Brazil, Paraguay, Uruguay
M. flabellifolia[a]	Argentina, Brazil, Paraguay, Uruguay
M. saxicola	Guyana, Suriname, Venezuela
[a]*Considered a synonym of M. esculenta* ssp. *flabellifolia by Allem (2001)* *Source: Rogers (1963)*	

2.3 ECONOMIC AND CONSUMER-RELATED TRAITS

These are mainly traits that have been manipulated in the cultivated species for their adaptive and commercial value. Farmer selection for distinct combinations of agronomic and consumer-related traits is a fundamental reason for landrace genetic diversity. Thus, number of landraces in a region can be a crude indicator of genetic diversity within the species. Although there are few countries with precise information, best estimates provide some revealing contrasts (Table 4.5). Meso-America and western South America appear to show highest diversity of varieties, and Asia, the lowest (Africa was not included in the analysis for lack of information). This may or may not relate to the crop's origins, but certainly has relevance in terms of where breeders or collectors can productively search for additional diversity.

Germplasm curators, breeders and others have extensively evaluated existing landrace varieties of the major cassava germplasm collections for agronomically important traits. Most of the data has been used for purposes of selection in breeding programmes rather than to analyse genetic diversity. Some of this adaptive and agronomic diversity is documented for the Colombian germplasm collection held at CIAT (Hershey, 1987; Figure 4.1a to 4.1d). Frequency of resistance to pests and diseases probably results from combined effects of both human and natural selection. In general, and not surprisingly, higher resistance (as illustrated by examples of superelongation disease, concentric ring leaf spot, and cassava green mite) is found in regions where the pests or diseases are endemic. Root dry matter content is largely an

influence of human preferences. In Colombia, high dry matter is more often associated with regions where cassava has traditionally been consumed directly; and lower dry matter where the roots were processed.

Table 4.5 Estimated relative diversity of landrace varieties of cassava in selected countries of Asia and the Americas

Region/country	Estimated total landrace varieties[a]	Estimated landrace varieties per 1 000 ha of cassava
South America		
Argentina	24	1.5
Bolivia	60	1.5
Brazil	3 110	1.7
Colombia	1 932	12.3
Ecuador	176	6.8
Paraguay	192	1.0
Peru	513	16.0
Venezuela	303	7.8
Region total:	**6 310**	**2.7**
Meso-America and Caribbean		
Costa Rica	59	11.8
Cuba	66	1.4
Dominican Republic	50	2.2
Guatemala	57	19.0
Mexico	89	44.5
Panama	45	9.0
Puerto Rico	8	8.0
Region total:	**374**	**4.4**
Asia and Oceania		
China	8	0.0
Fiji	6	0.7
Indonesia	120[b]	0.1
Malaysia	80	2.3
Philippines	40	0.1
Thailand	4	0.0
Region total:	**258**	**0.1**
Overall total:	**6 942**	**1.3**

[a]Based on Hershey (1994). Includes estimates of total representation in CIAT collection, level of duplication, and proportion of accessions which are not landrace varieties. A landrace is loosely defined here as a farmer-selected variety, even if introduced from another region or country. Not all countries with substantial collections are represented.

[b]Differs substantially from previous estimates of Hershey (1994)

Researchers have generated most of their own information relevant to the study of genetic diversity. Given the need for controlled experimental designs and standardized evaluation criteria, this is logical and necessary. There is, however, undoubtedly a wealth of underutilized empirical information that farmers have accumulated as a result of their long experience with varieties. This can include characteristics of adaptation, resistance, morphology, quality, or any number of traits that may not be

apparent even after standardized evaluations. For most collections, this type of information has not been gathered, or has rarely been used. There is a need to collect and integrate indigenous knowledge into the assessment of genetic diversity.

2.4 STABLE MORPHOLOGICAL TRAITS

IPGRI defined a set of relatively stable morphological traits useful for cassava characterization (Gulick *et al*., 1983). In 1992 an IPGRI working group on cassava genetic resources prioritized these descriptors and modified some of them (Table 4.6). Most of the world's major collections have applied these, or something similar, to describe variation among their accessions. Many of these traits have adaptive, agronomic or market importance (e.g. plant branching habit, root flesh colour, leaf pubescence), but some are probably evolutionarily neutral (e.g. stem periderm colour). These characters should provide an excellent starting point for a more detailed analysis of genetic diversity, especially when used in combination with molecular traits. CNPMF in Brazil modified the IPGRI descriptors and published an illustrated pamphlet for breeders and germplasm curators (Fukuda and Guevara, 1998).

2.5 CHROMOSOME MORPHOLOGY AND BEHAVIOUR

Cytogenetics have been basic to understanding the organization of genetic diversity in many genera. The literature often refers to the constant chromosome number (2n=36) within the genus *Manihot*, though apparently not all of the species have actually been examined. Meiotic studies, along with information on other genera in the family Euphorbiaceae, suggest that cassava may be an allopolyploid derived from two closely related species. If all species have the same number of chromosomes, possibly all present-day members of the genus are derived from common, now-extinct ancestors. Over time, the species have become effectively diploidized.

One fairly broad approach to comparing species cytologically is through a measure of DNA contents (whether this is a molecular or a cytogenetic approach might be argued). These contents tend to be fairly stable within a species, and the variations that occur across related species do not necessarily indicate genetic affinity or distance. These data must be taken with other evidence to draw any broad conclusions. CIAT analysed DNA contents of 14 wild species, along with 17 accessions of *M. esculenta* (Table 4.7). There are clear differences among species, but there is insufficient evidence for the level of intraspecific variation in the wild species. The several clones of *M. esculenta* show a high degree of uniformity, with the exception of MBra 534, which has a DNA content well above the others. This clone is also morphologically and biochemically unique, showing evidence of wild species introgression.

Table 4.6 Minimum descriptor list for cassava

Apical leaf colour	Shape of central leaf lobes
Light green	Linear
Dark green	Elliptic
Light purplish green	Lanceolate
Medium purplish green	**Apical pubescence**
Purple	Absent (glabrous)
Colour of the petiole	Slight
Light green	Medium
Dark green	High
Light purplish green	**Stem periderm colour**
Medium purplish green	Light green
Purple	Dark green
Red	Yellow
Stem epidermis colour	**Root surface colour**
Silver green	White
Light brown/orange	Light Brown
Dark brown	Dark brown
Root flesh colour	**Flowering**
White	Present
Light yellow	Absent
Deep yellow	**Root cortex colour**
Pink or purple	White
Storage root peduncle length	Yellow
Absent (sessile)	Light red or purple
Short (<5 cm)	Deep red or purple
Intermediate/long (= or >5 cm)	
Source: CATIE (1981); Ekanayake (1994)	

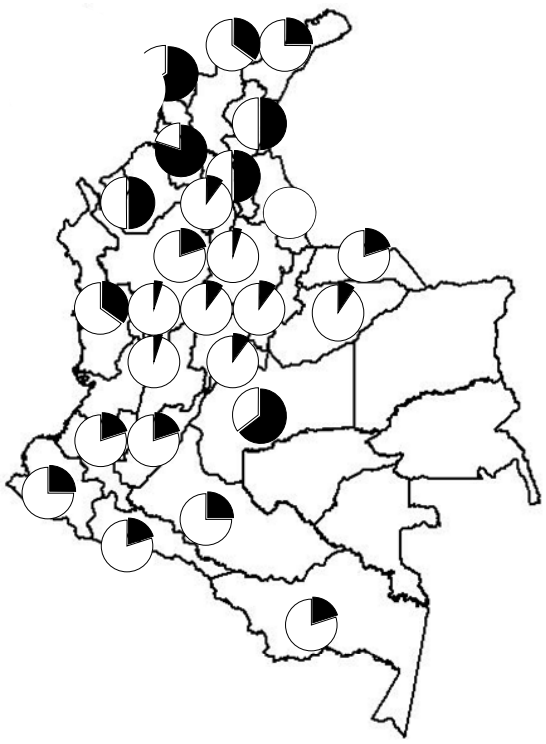
2.6 BIOCHEMICAL VARIATION

Primary gene products. The immediate products of gene transcription/translation are polypeptides, the components of proteins. Due to DNA structural variations, different alleles at the same locus can code for proteins that are qualitatively or quantitatively distinct. Differences in charge and size of proteins in buffer solutions allow physical separation by electrophoresis. In cassava, several isozyme systems demonstrate polymorphism, with the most polymorphic being αβ-esterase (Ramirez *et al*., 1987). Some preliminary work has also been carried out with seed storage proteins.

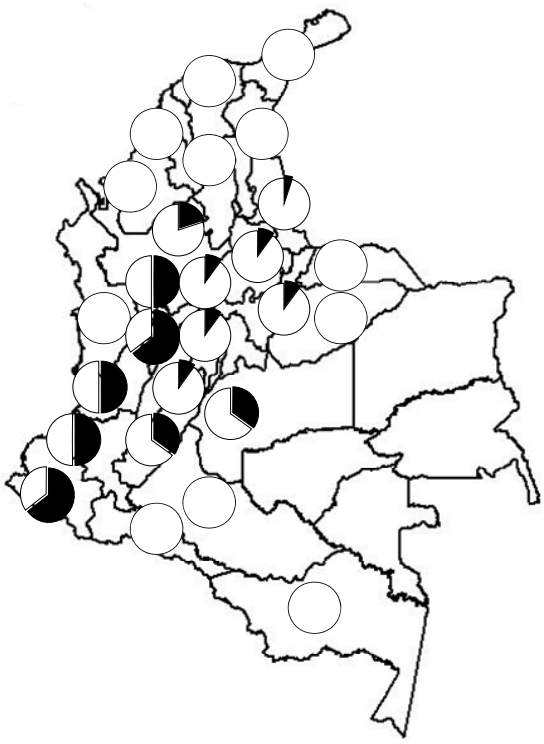
Protein synthesis depends on a particular gene being active, which can vary according to plant age, origin of tissue and environmental factors. Consequently, for results that can be reliably compared across genotypes, a high degree of standardization of procedures is required. The variations in results that can occur from environmental influences or lack of strict procedural controls are often not fully appreciated.

(1) **Isozymes**. Research on isozyme variations in several crops has allowed identification of centres of domestication, geographic patterns of genetic diversity, and dissemination routes of varieties. In the early 1990s, CIAT evaluated most of the cassava collection (over 4 000 accessions) for αβ-esterase patterns of root tip tissues. A large number of patterns (1 407) were represented by only one clone, while the rest had 2 to 39 clones with the same banding patterns.

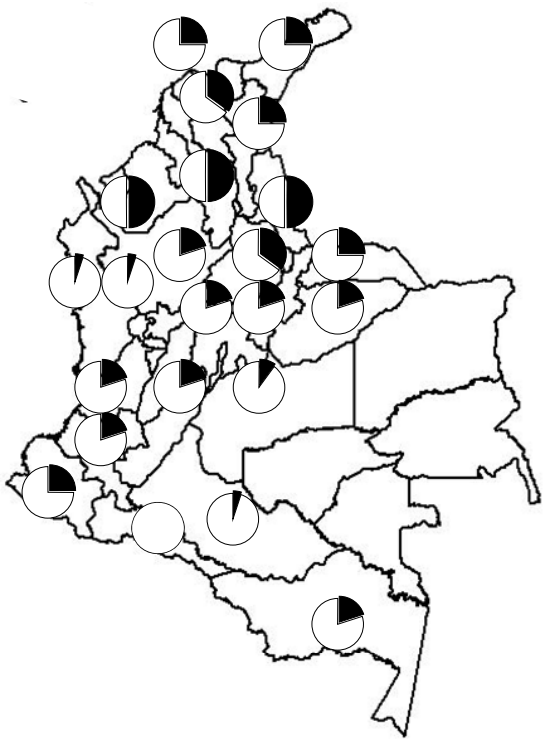
Figure 4.1 Frequency of trait expression in Colombian cassava germplasm, based on Department (State) where collected. Adapted from Hershey (1987)



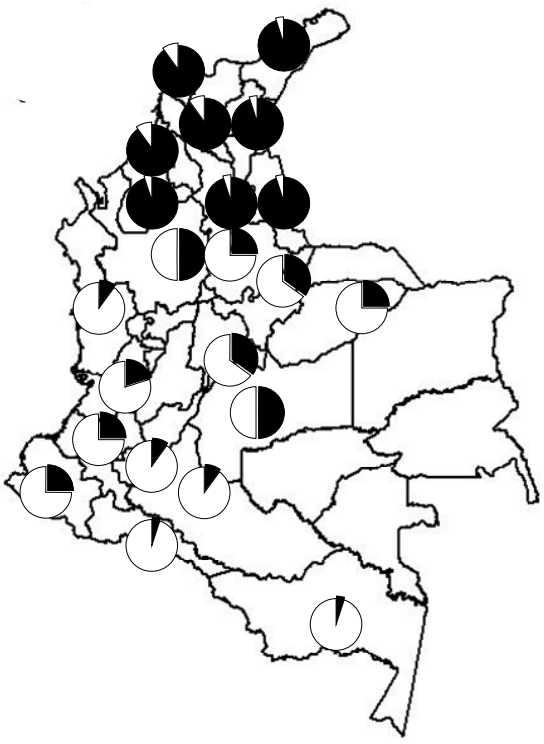
A. Resistance to superelongation disease



B. Resistance to concentric ring leaf spot



C. Resistance to cassava green mite



D. Root dry matter content

Low trait expression *High trait expression*

Table 4.7 Determination of DNA contents of *Manihot* species by flow cytometry

		pg DNA	
Species/accession	**Origin**	**Mean**	**STD**
M. esculenta			
MBra 383	Brazil	1.636	0.043
MBra 534[a]	Brazil	1.715	0.057
MCol 22	Colombia	1.571	0.030
MCol 638	Colombia	1.493	0.048
MCol 1505	Colombia	1.494	0.027
MCol 1522	Colombia	1.541	0.014
MPan 51	Panama	1.524	0.040
MPar 101	Paraguay	1.532	0.066
MPer 436	Peru	1.547	0.009
MDom 2	Dominican Rep.	1.568	0.013
MEcu 41	Ecuador	1.570	0.008
MInd 27	Indonesia	1.541	0.011
MMal 2	Malaysia	1.477	0.014
MMex 17	Mexico	1.514	0.011
MMex 59	Mexico	1.574	0.035
MNga 5	Nigeria	1.432	0.026
MVen 77	Venezuela	1.502	0.010
	Mean:	**1.543**	
M. aesculifolia[b]	Mexico	1.337	0.015
M. chlorosticta	Mexico	1.466	0.070
M. rubricaulus	Mexico	1.211	0.018
	Mean:	**1.338**	
M. anomala	Brazil	1.551	0.006
M. caerulescens	Brazil	1.544	0.035
M. epruinosa	Brazil	1.734	0.122
M. glaziovii	Brazil	1.687	0.008
M. hastatiloba	Brazil	1.399	0.003
M. longipetiolata	Brazil	1.396	0.008
M. pilosa	Brazil	1.738	0.005
M. pseudoglaziovii	Brazil	1.431	0.049
M. tristis	Brazil	1.571	0.022
	Mean:	**1.562**	
M. carthaginesis	Colombia	**1.764**	0.042
M. guaranitica	Paraguay	**1.756**	0.006

[a] *A cultivated landrace variety with morphological and biochemical evidence of wild species introgression*

[b] *Species considered taxonomically closest to M. esculenta by Rogers and Appan (1973)*

Source: C. Martinez, Rice Programme, CIAT. Unpublished data

In 1991 the Genetic Resources Unit of CIAT reported on an analysis of genetic diversity from an earlier sampling of 3 270 accessions from 21 countries and CIAT's breeding programme. There is no band with a clear relationship to region of origin. There are, however, sufficiently large differences in frequency of individual bands among countries to suggest non-uniform distribution of genetic diversity. Numerical taxonomy analysis indicated some grouping of countries. A group of Central American countries tended to group together, and most distant from these was a group of Asian countries. Curiously, the Fiji clones grouped together with Colombia and Venezuela.

For 12 country groups, the Nei index of genetic diversity was calculated from allelic frequency of the EST-1 locus, the only genetically defined locus among the various alleles controlling the esterase isozyme system (Table 4.8). Brazil, commonly considered a major centre of diversity of cassava, had one of the lower index values (42.0).

Table 4.8 The Nei index of genetic diversity calculated from allelic frequency of the EST-1 locus for collections originating from 12 Latin American countries

Origin	Sample size	Nei index
Brazil	624	42.0
Colombia	83	52.0
Cuba	67	26.0
Dominican Republic	5	00.0
Ecuador	94	65.0
Guatemala	80	52.0
Mexico	41	60.0
Panama	34	65.0
Paraguay	108	57.0
Peru	204	62.7
Puerto Rico	12	52.0
Venezuela	138	52.0
Source: CIAT, Genetic Resources Unit, Annual Report for 1991		

Highest values were for accessions from Meso-America and western South America. Overall, the variation observed in the esterase isozyme system does not allow easily defined grouping of materials from any given region, nor does it indicate marked differences in genetic diversity either within the Latin American centre of origin, or between Asia and Latin America. Again, these data need to be combined with analysis of other loci, and other parameters before any broad conclusions can be drawn.

Lefevre (1988) analysed genetic variability of 365 samples of diverse African origin, with isozyme polymorphisms. Cluster analysis identified several groups of related clones. Improved materials with resistance to cassava mosaic disease and cassava bacterial blight were similar at the molecular level. The isozyme techniques were able to detect intermediate genotypes between *M. esculenta* and *M. glaziovii,* which the author attributes to evidence of geneflow between the species.

Montarroyos *et al*. (2003) showed that cluster analysis of leaf tissue isozyme coincided well with clustering by morphological traits, indicating that the highly heritable visual traits can be simple and reliable cassava descriptors, for distinguishing among genotypes.

Some initial work with the wild *Manihot* species has shown a generally higher level of polymorphism for several isozyme systems than was observed for cassava (Table 4.9). This is not surprising, given the expected wide diversity of many traits across the species level.

Table 4.9 Description of isozyme systems for analysis of extracts from root tips of wild *Manihot* species after PAGE and starch electrophoretic separation

Isozyme	Polymorphism	Definition	Resolution	System
EST	Present	Good	Good	PAGE
ACP	Present	Good	Good	PAGE
GOT	Present	Good	Good	PAGE
DIAP	Present	Fair	Fair	PAGE
ME	Present	Good	Good	PAGE
PRX	Present	Fair	Fair	PAGE
SKDH	Present	Fair	Fair	PAGE
MDH	Present	Fair	Fair	PAGE
G6PDH	Present	Fair	Fair	PAGE
ME	Present	Good	Good	Starch
MDH	Present	Fair	Fair	Starch
PGI	Present	Good	Good	Starch
Source: CIAT, Genetic Resources Unit, Annual Report for 1991				

(2) **Seed storage proteins**. Grattapaglia *et al*. (1987) reported on what appears to be the only study of diversity for seed storage proteins in the genus *Manihot*. Soluble seed proteins from embryos of 19 *Manihot* species, all of Brazilian origin, were electrophoretically resolved by SDS-polyacrilamide electrophoresis. Using 15 reference bands, a similarity matrix was calculated in an attempt to quantify the affinity among species. The report cites a tendency for a higher similarity among species within the same section. These results suggest *M. pilosa* and *M. corymbiflora* are the species genetically closest to *M. esculenta*. The greatest distance (least similarity) was shown between *M. stipularis* and *M. caerulescens subsp. caerulescens*. These represent morphological extremes as well. *M. stipularis* is an acaulescent subshrub, and *M. caerulescens* is a tree species, up to 10 m in height.

While an important preliminary contribution to *Manihot* genetic diversity, these studies suffer from several deficiencies. There is little indication of variation 'within' as compared with 'among' species. Sampling methods are not sufficiently specified to know what type of populations were analysed. Of the two species where more than one sample was analysed, one showed high and the other showed low intraspecific variation. This shows the importance of understanding intraspecific variation before attempting to draw conclusions at higher levels.

2.7 DNA MARKER VARIATION

Differences in DNA sequences are not only the source of genetic diversity, but the essence of its definition. The closer one can get to a direct measure of variations at the DNA level, the more precise will be the estimate of genetic diversity. Two broad categories of DNA analysis are available: those methods based on variations in size of DNA fragments, and those based on actual nucleotide sequences. Given that no *Manihot* species to date have been sequenced, the more common techniques rely by far on analysis of characteristics of DNA segments rather than individual nucleotide sequences. This situation may change rapidly, however, as lower-cost and more rapid sequencing techniques are developed.

Various types of DNA can be analysed. Most commonly this involves digests of the complete nuclear genome, ribosomal DNA or chloroplast DNA. For ribosomal DNA (rDNA), the sources of data include the lengths of the repeating units in different taxa and changes in restriction sites. Digesting the rDNAs with enzymes with only one site in the repeating unit allows for ascertaining the length of the units. If sequencing is done, then the nucleotide sequences themselves represent the data. Sequencing of highly conserved regions encoding rDNA will likely be an area of increasing activity in the future. The results of such studies should be useful at the higher taxonomic levels.

Chloroplast DNA (cpDNA) occurs in the form of closed circles. Most plants have few repeated sequences. The cpDNA molecule is present in many copies per cell, making it easy to isolate in good quantities. Its small size means that, when digested, all the fragments can be visualized on a single agarose gel. cpDNA usually shows structural homogeneity within individuals, in different individuals of the same population, and in populations of the same species. This makes cpDNA especially useful for study of diversity at the species level, and it is relatively uncomplicated by subspecific variations, which are common in many of the other approaches described.

The analysis and mapping of restriction enzyme cleavage sites is the main approach to comparisons among samples. A second approach is to survey for structural mutations in the chloroplast molecules of different taxa. A third method involves sequencing different parts of the chloroplast genome and then comparing sequences from different taxa.

The methodology for analysis of DNA polymorphisms is evolving rapidly. Amplified Fragment Length Polymorphism (AFLP) is a PCR-based technique useful for genetic fingerprinting, and less laborious and time-consuming than earlier methods. About 70 bands are resolved per primer combination, with polymorphism depending on the relatedness of samples. In some of the early work at CIAT (CIAT, 1995) with a diverse group of cassava clones and four wild species, there were over 50 polymorphic markers per analysis.

PCR-based markers have been used in cassava and its wild relatives to answer a number of questions related to genetic diversity, for example: proximity of relationships among species (wild with wild, or wild with cultivated), or among varieties from different geographical regions; how well germplasm collections represent diversity at the farm level; utility of agronomic traits in representing genetic diversity; and tracing historical movement of germplasm among regions (Beeching, *et al*., 1993; Bertram, 1993; Marmey *et al*., 1994; Roa *et al*., 1997; Cabral *et al*., 2000; Carvalho *et al*., 2000a, 2000b; Carvalho and Schaal, 2001; Asante and Offei, 2003).

2.8 SAMPLING ALTERNATIVES

Estimating genetic diversity requires a sampling methodology that accurately estimates population parameters. If diversity is not accurately sampled, conclusions will be erroneous. The propagation system strongly influences the sampling strategy. *M. esculenta* is vegetatively propagated, and an individual plant can be the sampling unit. Each plant will be genetically identical to all other plants of the same clone. The wild species are outcrossing and seed-propagated; each plant is genetically distinct. A sufficient number of plants is needed in order to sample accurately the allele frequency of the population.

For cultivated cassava, many national programmes have a good representation of the country's total diversity in germplasm collections, and this diversity is readily accessible for study or use. Some traits are relatively easily measured and this can be done on the entire collection. For large collections and for traits that are difficult or expensive to measure, a subsample of the collection may be in order. An appropriate strategy in this case would be to define a core collection. Simply described, a core collection is a subsample of the whole collection that closely represents the total genetic diversity of a crop (and sometimes, of the crop's wild relatives). Such collections are usually in the order of 5–10 percent of the total collection. CIAT has defined a tentative core collection for cassava, and this has been used extensively for assessing genetic diversity of the crop, primarily for Latin America and Asia (Hershey *et al*., 1994). This concept is discussed further in Chapter 5.

The wild *Manihot* species present an altogether different challenge for defining a sampling strategy. Little of the naturally existing variability has been collected. Many wild species collections are represented by just a few genotypes for each species. Their evaluation is subject to substantial possibilities of false conclusions if the sample does not represent the population mean and variance for a given trait. Great care should be given to the interpretation of results of evaluations that do not take into account variation within species. Until more extensive wild *Manihot* collections are established, conclusions from studies on genetic diversity of these species must be considered preliminary.

3. A USER'S VIEW OF *MANIHOT* GENETIC DIVERSITY

Genetic diversity studies *per se* do not indicate potential for advance towards specific breeding objectives. A broad genetic diversity for molecular markers does not necessarily correspond to diversity for alleles controlling traits of interest, and vice versa. For example, several studies have demonstrated that including wild species in an analysis will greatly broaden the range of diversity of molecular markers, but practical experience shows that these species will rarely include agronomically valuable traits. Breeders generally have taken a pragmatic and user-oriented view of genetic diversity research, focusing on measures of genetic diversity that ultimately indicate something about value for breeding strategy.

How much genetic diversity is needed for breeders to achieve objectives? There is obviously no clear answer to this, but practical results of breeding programmes are revealing. The remarkable success of maize breeding in the United States is an example. For the past 50 years, maize yields have continued to increase in near linear fashion at a rate of about 70 kg/ha/year due to genetic gains, with no sign of levelling off. Much of the breeding is based on the derivatives of just two open-pollinated varieties (*Reid*: inbreds A632, B37, B73; and *Lancaster*: inbreds C103, Mo17 and Oh43). Just a tiny fraction of total available genetic diversity is incorporated into breeding populations.

The usual number of germplasm accessions that are used for intensive breeding work is almost always less than 5 percent, and usually less than 1 percent (Goodman, 1990). In crops with a long breeding history, common alleles have generally already been well-exploited. New alleles being sought are, by definition, rare. Probably in the order of 1 or 2 percent of existing cassava landrace varieties have been used in breeding on a global scale. This may appear small but it is probably greater than for most crops. At CIAT, just over 5 percent of the germplasm accessions was included in crossing blocks between 1985 and 1991 (Table 4.10). Since the initiation of CIAT's breeding programme in 1973 this may be closer to 10 percent. However, the number that actually contributes to elite clones for release to farmers is considerably narrower, perhaps about 1 percent.

Genetic resource specialists sometimes seem to exaggerate the usefulness to the breeder, of very broad genetic diversity, especially that found in wild species. Most of the diversity of wild species is manifested as traits that were perhaps useful to a non-domesticated plant, or in conditions very unlike those of modern crop production. Often the characters described as useful in wild species are also found in the cultivated gene pool, perhaps at a low frequency. A common argument is that it is impossible to predict which genes will be required in the future, and therefore all genetic resources should be preserved. This is a valid argument and perhaps sufficient to justify all genetic diversity work. However, the breeder relies not only on genetic material, but also on broad information about its behaviour. Even if only 1 percent of available genetic diversity is utilized in crop improvement, information on the remaining 99 percent is needed to understand fully the crop's evolution and potential for genetic modification.

Harlan (1976) pointed out six key questions that influence how frequently wild relatives of crops are used by breeders: How wild is the crop? How desperate is the situation? What are the pressures to turn out new cultivars? How available are the wild relatives? How difficult are the wild relatives to use? Is the breeder interested in using wild relatives? For cassava, the answers are far from uniform among

breeding programmes. In total, however, the result is a minimal use of wild species, perhaps mainly as a result of answering the question about how desperate the situation is.

Table 4.10 Utilization of germplasm in CIAT's cassava breeding programme, 1985-1991

Country	Total accessions in collection (1991)	Accessions planted in crossing nurseries	Percent used in crossing nurseries[a]
Argentina	16	3	18.7
Bolivia	3	0	0.0
Brazil	1 085	106	9.8
China	2	1	50.0
Colombia	2 010	63	3.1
Costa Rica	147	0	0.0
Cuba	74	12	16.2
Dominican Republic	5	0	0.0
Ecuador	117	4	3.4
Fiji	6	0	0.0
Guatemala	91	0	0.0
Indonesia	51	7	13.7
Malaysia	68	11	16.2
Mexico	100	2	2.0
Nigeria	19	15	78.9
Panama	42	2	4.8
Paraguay	192	17	8.9
Peru	405	3	0.7
Philippines	6	0	0.0
Puerto Rico	15	0	0.0
Thailand	8	4	50.0
United States	9	1	11.1
Venezuela	240	4	1.7
Total:	4 711	255	5.4

[a]*Excluding accessions used indirectly after being incorporated into hybrid combinations*
Source: Hershey (1994)

Manihot specialists commonly believe there has been frequent introgression among species. However, this hypothesis is based largely on empirical observations; molecular and genetic evidence is slight. One strong indication of likely introgression is the ease with which many species intercross, evidence that the genus is still rapidly evolving. In Africa, cassava scientists have observed tree cassava, and suggested these plants were a result of hybrids between cultivated cassava and *M. glaziovii*, introduced to Africa several times, first by Kew in 1887. The hybrids were intermediate between cultivated cassava and *M. glaziovii* in a range of morphological and biochemical parameters (Waynera *et al.*, 1994).

Isozyme and DNA restriction fragment studies should clarify the picture. The αβ-esterase isozyme studies at CIAT demonstrated what appeared to be one fairly definitive case of wild species introgression

into the cassava gene pool. MBra 534, known locally in Northeast Brazil as *Pornuncia*, is morphologically distinctive, with various wild type features such as leaf size and shape, and growth habit. This clone also demonstrates a unique band in its esterase banding profile, and has distinct DNA contents (see earlier section – Chromosome Morphology). To date, too few of the wild species have been analysed for isozyme patterns to draw any broader conclusions. However, if introgression was widespread, one might expect more cassava clones with bands derived from the wild species.

From a germplasm user's viewpoint, a relatively free flow of genetic information among species means that much of the genetic diversity which the wild species have to offer might already be within the *M. esculenta* gene pool. This would certainly be advantageous to the breeder, and would considerably reduce the need for complicated, long-term breeding procedures to extract genes from wild species. Even if this is the case, one would not expect all potentially useful genes from the wild species to have already been transferred to cassava under natural and farmer-selection conditions.

The long-term impact of biotechnology on crop genetic diversity is another subject of considerable current debate. There seems to be widespread perception that biotechnology is, overall, likely to reduce genetic diversity. This might occur if intellectual property protection restricts germplasm exchange, or if highly desirable genotypes are produced that displace present varieties. An alternative scenario, and a more likely one considering present directions of biotechnology research in cassava, is more optimistic. There is a high possibility of conserving diversity by permitting key traits to be incorporated into a broad range of existing, adapted landrace varieties. Conventional plant breeding, in the interest of reducing cost–benefit ratios, is obliged to develop a few varieties with relatively broad adaptation. Genetic engineering may provide more options to improve existing diverse genetic backgrounds.

4. BALANCING PRIORITIES

Few would argue against the general principle of protecting and conserving biological diversity. These activities have become more complex and costlier, and at the same time more urgent. The days are long past when the management of genetic resources encompassed only the making of a few collection expeditions, maintaining a field gene bank, and evaluating it for some basic agronomic traits. Choosing the best management options needs to be scientifically and economically sound, as well as legally and politically acceptable, to receive long-term support.

For cassava, there is little difficulty in identifying a broad range of areas of deficiency in research that could contribute to the crop's improvement. The justification seems self-evident for those close to the crop. However, experience and practicality suggest that unlimited resources will not be available, and priorities need to be established. This will be the task of each individual institution and of participants in any network that may be established. The following are not suggestions of guidelines, but point out some of the factors to consider in establishing those guidelines related to research on genetic diversity.

4.1 WILD VERSUS CULTIVATED

Clearly, far more has been done to understand genetic diversity of cultivated cassava as compared with wild species. Nevertheless, the understanding even of cultivated cassava is still rudimentary. The germplasm of cultivated cassava will be the main source of diversity for genetic improvement of the crop far into the future, if experiences from more highly developed crops are any indication. Transfer of genes for resistance to cassava mosaic disease from *M. glaziovii* demonstrated the value of wild species for expanding the germplasm base. Wild species studies will also contribute to understanding the evolution of cassava, and this will have an impact on the design of breeding programmes. While wild species conservation and evaluation are needed, it should not be carried out at the expense of progress in the understanding of *M. esculenta*. Allem (1994) proposed a practical approach to prioritizing research on wild *Manihot* species, based on gene pool definitions (Table 4.11).

The primary gene pool consists of those species that cross readily with *M. esculenta* (and therefore can be used directly and with relative ease in breeding programmes). These species are also most like

cassava in appearance, and most likely to allow rapid progress towards selecting for good agronomic types in the interspecific hybrids.

Table 4.11 Gene pools of cassava

The primary gene pool (GP1):[a]	
Cultivated materials:	*M. esculenta* ssp. *esculenta*
Wild progenitors:	*M. esculenta* ssp. *flabellifolia*
	M. esculenta ssp. *peruviana*
The closest wild relative:	*M. esculenta* ssp. *peruviana*
The secondary gene pool (GP1):[b]	
M. triphylla, M. pilosa, M. brachyloba, M. anomala, M. pruinosa, M. gracilis, M. tripartita, M. leptophylla, M. pohlii, M. glaziovii, M. dichotoma, M. aesculifolia, M. chorosticta	
[a] *Species that cross readily with M. esculenta* [b] *Species that cross with difficulty, but give some results* *Source: Allem (2001)*	

The secondary gene pool includes 13 species that can be crossed with *M. esculenta*, but with some difficulty. This gene pool, if used in conventional crossing, would require more time to recover segregants which combine the trait of interest in the wild species, with the yield and quality of cassava.

The tertiary gene pool can only be crossed with cassava with considerable difficulty (using conventional crossing techniques). Breeders are less likely to use these species as sources of genes. Clearly the classification of any species with regard to its genepool is subject to change if valuable new traits are identified or access to its genes is made easier.

4.2 *IN SITU* VERSUS *EX SITU* DIVERSITY

For purposes of this discussion, *in situ* will refer to wild species in their native habitat, and landrace varieties in the environment in which they evolved or have been cultivated for some time. *Ex situ* diversity is normally conserved in centralized, institutionally managed collections.

To date, *ex situ* conservation has played the leading role in preservation of *Manihot* genetic diversity, in the form of collections established by research centres and universities in cassava-producing countries. In terms of number of accessions, by far the most diversity maintained *ex situ* is of cultivated cassava. In the order of 16 000 accessions are maintained worldwide (Cohen *et al*., 1991). Only a handful of significant wild species collections exist and are found in Brazil, Colombia, Mexico and Nigeria.

Most *ex situ* collections were established to meet the needs of breeders seeking a base for genetic improvement. It is unclear to what extent, if any, this has skewed the selection of materials for inclusion in collections, or if any corrective measures are required. This has clearly meant a higher emphasis on cultivated as compared with wild species. Germplasm curators have rarely had in mind an equal emphasis across species within the genus.

There is a qualitative difference between management requirements for *in situ* conservation of domesticated and of non-domesticated biodiversity. Agricultural species are, by definition, already separated from their natural environment, and often can survive only in managed environments. Maintaining these species *in situ* may have less urgency than for species that would continue to evolve under complex natural selection forces in non-agricultural environments.

In situ conservation functions best when genetic diversity is concentrated in relatively small areas that are not immediately subject to high pressures of human activity that threaten their existence. Creating preserves and parks is most easily accomplished in limited areas, and before human encroachment has reached significant levels. *Manihot* might partially meet the latter of these criteria, but hardly the former. The *Manihot* species, as was mentioned earlier, are generally sporadic in their distribution. As they often inhabit disturbed areas, it is precisely the areas that may be most subject to encroaching influences of civilization, such as agriculture, road building or other constructions. In Brazil, their distribution coincides with some of the areas of rapid expansion of agriculture, especially the *cerrado* of central Brazil. While expansive natural reserves would have substantial positive impact on preservation of *Manihot* species in these areas, it is hardly a likely scenario. Significant *in situ* conservation will only be possible as part of a larger effort including a number of species occupying similar threatened habitats. Any efforts in this direction should not detract from the urgent need for collection for *ex situ* conservation as the more immediate and practical thrust.

5. MONITORING SYSTEMS

Part of the responsibility of the scientific community working on *Manihot* genetic resources is to design, establish and operate monitoring systems for genetic diversity. Some of these are already in place, especially with regard to *ex situ* conservation. Other areas where monitoring is needed are: (1) creating a periodically updated database on current collections, including numbers of accessions, evaluations carried out and conservation methods; (2) assessing impending risk of loss of natural habitat where wild *Manihot* populations are native; (3) defining contingency plans for rescue of collections at risk; and (4) using germplasm in enhancement and breeding programmes.

Bringing problems such as risk of germplasm loss to international public attention may not be easy. It may imply some institutional embarrassment, and therefore reticence. Goodman (1990) notes that for many crop species, it is not the landraces on the frontiers of development that are most at risk. Usually it is the collections in the hands of individual breeders. This may well be the case for cassava, but there are plans by the Global Crop Diversity Trust for a systematic international monitoring system to take effective action for collections at risk (personal communication).

Relatively few collections are sufficiently well documented to retrace original points of collection. Accessions having this information could be used to monitor varietal changes over time, i.e. an expansion or narrowing of diversity. For example, the world's most extensive collections come from Brazil and Colombia, and origin of most of these accessions is well-documented. The bulk of these collections was assembled in the 1970s. Sampling of a set of original collection sites throughout each country could provide considerable insight into changes in genetic diversity occurring over time.

Chapter 5. Germplasm management and exchange

The basic genetic diversity available to the breeder is normally the consequence of natural selection over millennia, added to more recent farmer selection and progress from breeding programmes. The breeder can gain time and increase probability of success by giving appropriate attention from the outset to management of the germplasm base. This chapter reviews principal activities of germplasm management: collection, conservation, evaluation, documentation, exchange and planning for utilization for variety improvement.

1. REGIONAL PERSPECTIVES

One can only speculate as to the total number of cassava clones cultivated worldwide. All current germplasm collections are subsamples of the total diversity, albeit some are more complete than others. In Latin America and the Caribbean, where the crop originated, there may be in the order of 10 000 clones, based on numbers in existing *ex situ* collections and on reports of collectors or other scientists from field observations. In Africa, diversification seems to have occurred rather quickly, probably in response to the broad array of growing environments and market uses, but was also enabled by continuing small-scale introductions since the original introductions in the 16th century. Due to the extent of cassava cultivation in Africa, the range of environments in which it is cultivated and the known diversification through natural intercrossing of landraces in farmers' fields, there has been a virtual explosion of genetically distinct genotypes cultivated in farmers' fields. It is unclear, however, whether there are any genes in African germplasm which do not exist in Latin American landraces. The COSCA identified some 1 200 local varieties in 281 villages in countries representing 70 percent of the continent's cassava. There are probably in the order of 5 000 distinct clones cultivated by farmers in Africa. In Asia, the number of landraces appears to be much more limited than in Latin America or Africa, perhaps in the order of 1 000 varieties. Indonesia seems to be the main repository of distinct landraces. Although India has a large germplasm collection, many accessions are the product of breeding programmes.

2. COLLECTION

For national programmes, local landrace varieties are usually the nucleus of a germplasm collection. Farmers often have selected these clones for many years, for adaptation to local soil, climatic and biological stresses, for compatibility with prevalent farming systems and cultural practices, and for quality traits required by local markets. These complexes of favourable genes are often the most appropriate background into which a breeder can introduce new traits. Even where fairly marked changes in genetic structure of a new variety are anticipated, the importance of an overall adaptation to local environmental conditions cannot be overemphasized, and because such adaptation is the result of complex physiological systems, controlled by many genes, the less the breeder has to do to re-establish adaptation characteristics in his or her breeding populations, the more rapid the progress will probably be.

In spite of this, local germplasm is often inadequately considered and underutilized, in part because of the mystique surrounding exotic (introduced) germplasm, and in part because of lack of appreciation of the positive attributes of local germplasm. These attributes are often not recognized until unadapted germplasm is introduced, and problems of adaptation and pest or disease susceptibility arise that were not previously seen as potential problems.

If a complete local collection is not already established, this is the first task of the breeder or of a germplasm specialist. To what degree should germplasm collection be tied to the needs of breeders as compared with more theoretical genetic resources management considerations? This is a question with no easy answer, and one that draws a range of opinion. As recently as the mid to late 1980s there was a prevailing attitude among germplasm specialists that priorities for collection and conservation should have little to do with breeding goals, but rather the preservation of the widest possible genetic diversity regardless of agronomic value. The argument for this approach is that germplasm management is for the very long term, and the genetic needs of the future are unpredictable. Later, there was more recognition

of the need for close linkages between breeders and germplasm specialists in defining priorities for collection and evaluation. The two viewpoints are in fact complementary. Giving priority to an approach that will yield practical, short-term progress in breeding, need not compromise longer-term objectives of comprehensive gene pool preservation.

In 1982 an IBPGR (now Bioversity International)-sponsored working group suggested international collection priorities (Gulick *et al*., 1983). These priorities were based first on the fact that cassava originated and completed a large part of its evolutionary history in Latin America. Thus, by far, the most extensive genetic diversity emerged here. Within Latin America, priority for collection was assigned on the basis of: (1) genetic diversity; (2) areas already collected; and (3) areas in danger of genetic erosion. As the data for all these criteria are sketchy, prioritization is based mainly on best guesses of experienced cassava collectors and breeders. Bolivia, Brazil, Honduras, Nicaragua, Paraguay, Peru and Venezuela had large regions designated as highest priority. In the years since establishing the priorities, considerable progress has been made in filling gaps, although several remain.

During the founding meeting in 1992 of the Manihot Genetic Resources Network, participants updated and revised collecting priorities. The group recommended that collection be prioritized to solve bottlenecks affecting existing breeding programmes. For wild *Manihot* there are still too many unknowns to define a detailed strategy, so Allem (1994) proposed using crossability with *M. esculenta* as an initial guideline (see description of gene pools in Chapter 4).

A logical stepwise planning for a national cassava collection may include the following:

(1) **Inventory of existing collections**, including at a minimum, locality of origin but preferably with a full complement of passport data. Major collections are normally reasonably well documented, but many countries have a number of minor breeder-managed collections that may have unique accessions.

(2) **Assessment of areas of cassava production** in the country and of probable genetic diversity in each area (subjective evaluation based principally on the number of clones cultivated and apparent diversity of observable traits. It is especially important to identify traditional and marginal areas of production where rare genes and specifically adapted germplasm may be found.

(3) E**stablishment of priorities for collection** within a country. These priorities should include, but not be based solely on, objectives of the breeding programme. Genetic resources conservation objectives should be included, usually as part of longer range goals. In areas where genetic erosion is considered to be a threat, collection would receive high priority whether or not the material was deemed useful to immediate breeding objectives.

(4) **Planning of a collection itinerary** and of resources required, including appropriate timing for collection of stakes and/or seeds, for logistical considerations, for personnel, transportation and equipment needs.

(5) **Securing funding**. The principal costs of collection are generally transportation and labour. Supplies required are usually minor – tags, twine, sacks, machetes and plant presses. Collections may often be organized to combine various objectives in a trip, e.g. including various crops or non-crop species. Often, plant collection can be a side activity during travel for other purposes. If something more ambitious is anticipated – for example, an extended, comprehensive collecting trip – a separate project proposal is likely to be required. Bioversity's regional offices can provide information on potential funding sources. Most international donors expect the initiative for such collections to come from a national institution (e.g. university or ministry of agriculture) and for financial and personnel support from that institution to the extent possible. Acceptance of outside support generally obliges the receiving institution to certain standards and procedures, including filling standard collection forms, depositing a duplicate collection at CIAT (Latin American collections), and making collected material available to other institutions. A component of training in germplasm management is usually expected during the course of the collection, as a longer-term contribution to developing germplasm resource management capability in a country.

(6) **Proceeding with collection**. Appendices I and II suggest field procedures for collecting cassava and wild *Manihot* species, respectively. IPGRI developed a standardized format for registering collections (with modifications by CIAT) (Appendix III). These data consist of accession identifiers

(passport data) and information recorded *in situ* by collectors. They are especially useful in identifying potential contributions of an accession based on its adaptation to a given environment and means of utilization.

When collections within a region are repeated, even after 20 or 30 years, there is likely to be a number of duplicate accessions. It is usually difficult to know whether or not a clone one sees in the field has been previously collected. It is preferable to collect extensively and eliminate duplicates at later stages by a standardized protocol. This process of duplicate identification can be made more efficient by incorporating appropriate criteria into the format for compiling information at the time of collection. In an expedition in Argentina in 1993, collectors included a preliminary description of each accession, based on stable morphological descriptors. This proved to be a solid basis for further testing by more precise techniques (CIAT, 1994).

The importance of comprehensive documentation of germplasm collections was not widely recognized until relatively recently. Only a few specialists had the foresight to take the range of data during collection expeditions that is now recognized as basic to understanding the organization of genetic variation in a germplasm collection. Few existing cassava collections have more than the date and location of collection, and local name, as background information; many accessions lack even these minimal data.

There is limited possibility of returning to the original sources and complementing existing data, so the bulk of collections is likely to remain with this deficiency. Given that many countries have already established collections containing most of the existing genetic diversity, there may remain a permanent information gap for a high percentage of the world's cassava germplasm.

Those programmes that anticipate further collection should consider recording, at a minimum, the passport data suggested by IPGRI. Information on the farmer's perception and description of the variety, including both its attributes and deficiencies, can be a valuable adjunct to later evaluations by breeders.

3. CONSERVATION

Conservation is currently limited almost exclusively to field, greenhouse/screenhouse and slow-growth *in vitro* collections. The future will see this expanded to cryopreservation, seeds, pollen and DNA fragments incorporated into micro-organisms.

Conservation of vegetatively propagated crops has always been laborious and costly relative to seed conservation. Nevertheless, it is often useful to maintain the specific gene combinations that have resulted from decades or even centuries of selection by farmers. As cassava is highly heterozygous, the only means of conserving these specific gene combinations is through vegetative propagation. Alternatively, if the interest is conservation of genes rather than genotypes, germplasm should be maintained as true seed. Germplasm maintained in seed form would ultimately be useful principally as a source of genes in a breeding programme and not directly as a source of varieties.

At least 43 countries and two international centres maintain local, regional or international collections. The largest of these are at CIAT, Colombia (5 400+ accessions), EMBRAPA, Brazil (4 000+ accessions), IITA, Nigeria (1 600+ accessions), and CTCRI, India (1 500+ accessions) (Table 5.1). INIFAP in Mexico and CENARGEN and the Universidade de Brasilia in Brazil have the largest available wild *Manihot* collections among national programmes. CIAT and IITA each have seed, *in vitro* or field collections of 30-40 species.

IPGRI recognizes two main categories of collections: base and working (Williams, 1984). A base collection of a vegetatively propagated crop, as visualized with present technology, can only be in cryopreserved form. Both field and *in vitro* collections are considered working collections. Although cryopreservation of meristem tips has been successful since the 1980s, the recovery rate is still too variable to apply confidently to large collections.

3.1 CONSERVATION OPTIONS

3.1.1 Field

Cassava collections have traditionally been maintained in field plots. Stem pieces are used as the propagules just as in commercial production. Theoretically, such a collection could be maintained for many years without regeneration. In practice, maintenance problems often increase after a year or two, making replanting at more frequent intervals necessary. Common problems include lodging from excessive growth and build-up of pests and diseases. Major advantages of field maintenance of collections are the technical simplicity and the availability of planting material for evaluations.

The following general recommendations apply to field conservation:

(1) The area where materials are maintained should be as free as possible of diseases and insect pests that could cause losses of clonal material or create difficulties in the transfer of clean planting material to other sites.
(2) A minimum of three to five plants is necessary for practical maintenance. If a plantation is also to be used as a source of production of stakes for planting of other trials, more plants may be required.
(3) Cassava can be maintained in field plantings as a perennial plant, but periodic renewal every one or two years is desirable to avoid problems of excessive vegetative growth, cumulative disease and insect problems and to facilitate maintenance generally.
(4) The distance between plots of different clones should be adequate to prevent undue competition among the plots.

3.1.2 Greenhouse/screenhouse

In order to combine the benefits of lower space requirements with continual availability of planting material for experimental use, CIAT devised a slow-growth system based on restricting the root development in small planting pots (bonsai effect). Plants occupy only a small fraction of the space they would occupy if allowed unlimited growth in the field.

Maintaining a cassava germplasm collection in containers has the potential advantages of space savings; better protection against pests, diseases and weather-related damage; and labour savings. Disadvantages can include difficulty in using plants as a source of planting material for field trials (generally small and weak stems), cost of infrastructure and cost of materials.

3.1.3 Slow growth in vitro

In the late 1970s, the University of Saskatoon (Canada) and CIAT developed techniques for routine *in vitro* conservation of rooted plantlets of cassava. These plantlets can be derived in a number of ways, but for phytosanitary reasons the recommended source is small meristem tips. These can easily be surface-sterilized against superficial organisms, and many systemic pathogens do not advance into the new tissue of a rapidly growing meristem. Extra precautions of chemo- or thermotherapy can also lower chances of contamination. Meristem tips are cultured in nutrient media in glass or plastic jars or test tubes, and maintained under controlled light and temperature conditions. Under minimum growth conditions, cultures can be maintained 12–18 months before renewal. Renewal can be by planting stem pieces or meristem tips from the *in vitro* plantlet into new sterile media, without the need for a field propagation phase. CIAT's facilities have the capacity to hold more than 6 000 accessions *in vitro* at 20°C (day)/15°C (night) temperatures, 12-hour photoperiod and 500 to 1 000 lux illumination.

CIAT monitored genetic stability of *in vitro* cultures using a combination of morphological and biochemical traits, and DNA markers. All results have so far been negative, indicating a high level of genetic stability after as many as 15 years of *in vitro* conservation and regeneration (CIAT, 1994).

3.1.4 Cryopreservation of meristem tips or somatic embryos

Liquid nitrogen storage of vegetative tissue tips should be the most secure and trouble-free system for conservation of clonal cassava germplasm. The major advantage is the virtual freedom from maintenance problems during storage, with the possible exception of low rates of mutation caused by

background ionizing radiation. Conservation could theoretically be carried out indefinitely with no need for renewal. Development of successful cryopreservation techniques has been somewhat slow and sporadic, due to limited funding, as well as what appears to be a somewhat recalcitrant species. CIAT and other laboratories developed basic cryopreservation techniques for meristem tips in the 1980s, using chemical dehydration and programmed freezing in liquid nitrogen. With later developments in encapsulation and quick freezing, more than 80 percent of accessions tested (mainly from the core collection), have recovery rates of greater than 30 percent, the minimum acceptable level for a long-term conservation strategy. Genetic stability could also be a concern, but preliminary observations have shown no noticeable changes in plant characters after cryopreservation. However, a cryopreservation strategy would need to include periodic monitoring of stability.

Somatic embryos already represent an efficient regeneration system for rapid propagation, and are a target for transformation. They have the potential to serve as the basis for germplasm conservation as well, especially if they can be adapted to and recovered from cryopreservation. Mycock *et al.* (1995) and Stewart *et al.* (2001) successfully preserved somatic embryos, with a 40–60 percent post-thaw viability. Danso and Ford-Lloyd (2004) introduced new cryoprotection and dehydration techniques and obtained 95 percent post-thaw viability (albeit, with a limited range of genotypes). Rate of plant recovery from the cryopreserved embryos was comparable to that of non-preserved ones. The optimal protocol involved induction of embryogenic calli on an induction medium (Murashige and Skoog medium supplemented with 2,4-D and sucrose), cryoprotection on 0.3 M sucrose for 21 days, followed by 16 h of dehydration and immersion in liquid nitrogen. Although plants recovered from somatic embryos appeared to be genetically stable this needs to be further tested and monitored. Current evidence suggests that sucrose cryoprotection followed by air dessication provides a viable solution for long-term conservation of cassava genetic resources via cryopreservation. This system will complement other *in vitro* methods.

Many wild species are notoriously difficult to maintain either as field collections or as *in vitro* plantlets. Several species have been recovered successfully from cryopreservation, including *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *peruviana* and *M. carthaginensis*.

Cryopreservation research for cassava has not received nearly enough support, given the serious risks to long-term conservation that this species faces.

3.1.5 Seed

Seed conservation in cassava has received limited attention. Varieties have been selected and propagated vegetatively to preserve specific gene combinations. After self- or cross-pollination, these genes are re-assorted into new combinations. Seed conservation can be a means of preserving genes, but not the specific combinations with which a breeder often wishes to work.

Cassava seeds are apparently orthodox in behaviour and therefore can be stored under conventional conditions of low humidity and low temperatures (Ellis *et al*., 1981). IITA (1979) reported storing seeds at 5°C and 60 percent relative humidity for up to seven years with no loss in germination ability. Seed can also be preserved in liquid nitrogen and recovered with high viability (Mumford and Grout, 1978; Marin *et al*., 1990).

Table 5.1 Cassava accessions maintained in national and international research centres

Region/country	No. accessions	Institute/programme
South America		
Argentina	177	INTA
Bolivia	18	IIA
Brazil	4 132	CNPMF/CENARGEN
Colombia	4 695	CIAT
Ecuador	101	INIAP
Paraguay	360	IAN
Central America		
Costa Rica	154	CATIE
Mexico	225	INIFAP
Nicaragua	37	UNA
Panama	50	IIA
Caribbean		
Cuba	495	INIVIT
Dominican Republic	46	
Eastern and Southern Africa		
Angola	13	
Botswana	11	
Kenya	250	RTCP
Malawi	170	RTCP
Mozambique	81	INIA
Rwanda	280	
South Africa	100	
Uganda	413	RTCP
United Republic of Tanzania	254	RTCP
Zambia	96	
Zimbabwe	6	
West and Central Africa		
Benin	340	SRCV
Burkina Faso	14	
Cameroon	250	
Côte d'Ivoire	300	
D.R. of the Congo	250	
Gabon	42	
Ghana	2 000	PGRC/CRI
Guinea	168	
Nigeria	435	NRCRI
	2 861	IITA
Senegal	57	ISRA/CDH
Sierra Leone	134	IAR
Togo	734	
Asia – Oceania		
China	86	SCATC/UCRI/GAAS
India	1507	CTCRI
Indonesia	251	CRIFC/MARIF
Malaysia	92	MARDI
Myanmar	21	ARI
Philippines	384	PRCRTC/IPB
Sri Lanka	112	CARI/PGRC
Thailand	250	RFCRC
Viet Nam	36	Hung Loc Agric. Centre
Source: Ng and Ng (2001)		

Although the mechanics of seed storage appear to be straightforward, further studies are needed to define appropriate methodologies from the standpoint of germplasm conservation theory. Various approaches are possible, including selfing, uncontrolled open pollination, or pollination among selected accessions. The long-term advantages of seed conservation warrant further work in these areas.

3.1.6 Pollen

One of the limitations to research on cassava pollen conservation remains the difficulty of viability testing. Neither staining nor *in vitro* germination are adequately reliable as indicators. Protocols for efficient, large-scale and rapid viability testing will be a necessary prerequisite to effective pollen conservation. Leyton (1993) resorted to *in vivo* pollination as a means of testing viability in a series of experiments on pollen cryopreservation. He obtained no seeds from any subzero pollen treatment (-4°, -12° or -70°C). Orrego and Hershey (1984) were unsuccessful in storing viable pollen after desiccation over silica gel.

3.2 CHOOSING A CONSERVATION STRATEGY

Advantages of *in vitro* conservation are the low space requirements and minimal possibility of loss of materials through diseases, pests, climate or soil factors. Disadvantages are the need for relatively sophisticated facilities for culturing sterile plantlets, and for maintaining reliable conservation conditions. Costs of field versus *in vitro* conservation are highly location-specific, depending upon local costs of labour, energy, supplies and infrastructure. Economies of scale are also a factor. For most small national collections, *in vitro* conservation may not be justified, unless the laboratory forms part of a conservation strategy involving other crops as well.

Epperson *et al*. (1994) carried out a comprehensive study comparing costs of maintaining field and *in vitro* collections at CIAT (Table 5.2). Total costs per accession were comparable (US$30 field and US$25 *in vitro*). Costs, security and convenience will dictate different strategies in different situations.

Table 5.2 Comparison of key costs (US$, annual basis) for the field and *in vitro* cassava germplasm collections at CIAT

Cost indicators	Field	*In vitro*
Variable cost	US$104 714 (53% labour)	US$11 077 (no labour)
Variable cost per accession	US$22 (4 695 accessions)	US$2 (5 992 accessions)
Added variable cost/year	US$1 472 (66 new acc./yr)	US$633 (342 new acc./year)
Total cost	US$141 462	US$147 996
Total cost/accession	US$30	US$25
Source: Epperson, J.E., D. Pachico and C.L. Guevara (1994). A cost analysis of maintaining plant genetic resources: the case of cassava. Unpublished CIAT document		

3.3 THE WILD SPECIES

In nature, all the wild species appear to be principally seed-propagated. As a strategy for genetic resources conservation, there is probably little need for conserving individual genotypes through vegetative propagation. In practical terms, a strategy that combines seed, field and *in vitro* conservation will increase probability of success for conservation of many difficult-to-propagate species. CIAT has embarked on a detailed characterization of natural habitats of the wild species to understand their adaptation requirements better, and ultimately to tailor a conservation strategy to groups of species with similar requirements.

Conservation of the wild species is difficult because many are not easily propagated either by seed or vegetatively. Work on *in vitro* culture shows that species vary widely in their media requirements for optimum conservation and regeneration (CIAT, 1984). Research at CIAT (CIAT, 1993) on methods to improve vegetative establishment of wild species compared leaf buds, shoots from rooted stakes, air layering, shoots from source plant, kinetin treatment and Hormonagro® treatment. Air layering was the most broadly successful method across species, but still resulted in a low rate of multiplication.

Wild species, seed-propagated in nature, may best be conserved in seed form, to represent the genetic diversity of heterogeneous source populations in the wild. Response to different treatments to improve seed germination varies by species (CIAT, 1993). *M. quinquipartita* responded to heat treatment and pre-germination at alternating temperatures. Several species benefit from embryo culture, but others do not. Microwave treatment and mechanical scarification were detrimental to most species. CIAT recommended using a sample for germination by direct seeding, and holding some seeds for reserve in case alternative methods are needed.

3.4 WHAT TO INCLUDE IN A COLLECTION

A germplasm collection obviously cannot contain a sample of every existing genetic variant. Worldwide, landrace varieties may be in the order of 10 000–15 000, but the number of new genetic combinations produced by breeders is in the millions. There is no justification to preserve permanently more than a small fraction of them. On the other hand, many gene bank curators and breeders will find that some types of germplasm other than local landraces should be preserved indefinitely. A convenient way to do this is to incorporate them into the same ongoing strategy for long-term germplasm conservation applied to landrace varieties.

Local landraces will be the nucleus of most collections. Planning for incorporation of non-landrace materials into a collection must be done carefully and systematically to avoid an explosion in the number of accessions and related management costs. Based on constitution of current collections, there is apparently considerable diversity of approaches. Generally, gene bank curators have considered incorporation of three principal types of materials: promising introduced materials, elite breeding lines and genetic stocks. Some possible guidelines follow for managing each of these groups.

Introductions will likely be more critical to forming the genetic base in Africa and Asia than in Latin America. If one makes strategic introductions to represent specific geographical diversity or fill certain gaps in local materials, it may well be recommendable to retain all these materials. Less focused introductions, especially large numbers of materials, should be pre-selected before permanent conservation. A reasonable approach to this is to characterize and evaluate introductions and incorporate only material with traits of interest. If exotic introductions are made in vegetative form, numbers are likely to be limited, but seed introductions quickly generate very large numbers of genotypes. It is rarely appropriate to conserve everything introduced as seed. Usually a systematic evaluation can identify useful new diversity.

Elite breeding lines may or may not become successful varieties. Those that do not reach farmers' fields may be lost unless specific steps are taken to preserve them. For those that are successful, there is even more of a need for systematic preservation so that a permanent, pure representation of the variety exists – the equivalent of breeder's seed in a seed-propagated crop. These stocks can be the basis of a seed programme, for clarifying any possible future problems related to varietal contamination and as a source for distribution to other gene banks or breeding programmes. The definition of elite is the key to a sensible conservation strategy. Only a very limited number of materials can be assigned elite status, or the costs involved in conservation quickly get out of hand. At CIAT, for example, a clone becomes elite only after passing through all preliminary stages of selection, and multisite selection in advanced yield trials for at least two years. On average, 10 to 15 clones a year enter the elite category. Even this relatively small number can eventually become burdensome for conservation, and this group may be given a lower management intensity for conservation.

Genetic stocks can be temporary or permanent parts of a collection, depending upon objectives. CIAT (1994) reported on incorporating a mapping population into the germplasm collection as a way to ensure that it would be widely available to participants in the Cassava Biotechnology Network. Stocks for a specific, limited study may not need to be preserved at all.

3.5 A TIERED CONSERVATION STRATEGY

For small collections, all accessions can normally be treated with an equally high priority for conservation. In larger collections, one may gain efficiencies by assigning levels of importance to different groups and managing their conservation distinctly. Local landraces are nearly always the top priority. Their conservation must be secured. This may be by two separate field locations, duplicate *in vitro* collections, or a field and an *in vitro* collection, for example. If a core collection (see later section) has been defined, this may get the highest of all priorities. Breeding lines and introductions, especially if retained in a collection in the country of origin, may be given a lower status for conservation.

4. DUPLICATE IDENTIFICATION

It is common during collection expeditions to sample inadvertently the same genotype more than once. Situations that increase the probability of collecting duplicates are: (1) different common name for the same clone; (2) a clone widely grown across a region; (3) collection expeditions to the same region at different time periods; (4) a clone sensitive to environmental variations and displaying variable phenotypes across microenvironments; and (5) inexperienced collectors.

Hershey (1994) estimated that CIAT's global collection could be reduced by 20-25 percent by eliminating duplicates. This has to be done with great care, however, and by relying on methods that will identify genetic duplicates with a high degree of confidence. CIAT established a four-step procedure (Hershey *et al*., 1991; CIAT, 1993): (1) identification of candidate duplicate genotypes by comparison of eight key morphological characteristics; (2) side-by-side field comparison of putative duplicates grown together in the same year; (3) re-characterization for morphological traits; and (4) characterization of putative duplicates with molecular markers. The efficacy of the molecular probe M-13 was demonstrated in that 20 percent of genotypes identified by other criteria as probable duplicates showed distinct fingerprints.

Ocampo *et al*. (1993) analysed 4 304 accessions from CIAT's germplasm collection, with the αβ-esterase isozyme system. From a total of 22 distinct bands, accessions grouped into 2 146 different banding patterns.

In similar work, Sumarani *et al*. (2004) analysed 70 sets of tentative duplicates (total of 139 accessions from 786 indigenous accessions in India's national collection). The esterase isozyme system produced a maximum of five bands per accession, and among the multiple sets, a total of 35 bands, proving to be a highly polymorphic system. Altogether, 62 out of 218 accessions were found to be duplicates. The authors suggest that duplicate identification should proceed in a logical manner from creating tentative groupings among a large number of genotypes, with rapid and inexpensive methods, to isozyme analysis with a reduced number of clones, and finally, confirmation by molecular probes. If facilities for molecular probes are readily available in a potential collaborating institution, an isozyme analysis might be eliminated altogether.

Duplicate accessions may either be eliminated or assigned to a lower level of conservation priority. As the number of molecular markers is now in the thousands, the level of confidence in identifying duplicate clones is becoming quite high. It is a question of whether the cost of identifying these duplicates is lower than the cost of maintaining them in the field or in the laboratory.

5. PRELIMINARY EVALUATION

A germplasm collection is useful as a resource to breeders only when accessions are well-described in terms of characteristics of interest. IPGRI recognizes two basic categories of evaluation for germplasm collections: (1) characterization – those characters that are highly heritable, clearly visible and are expressed in all environments; and (2) preliminary evaluation – a limited number of additional traits of lower heritability considered desirable by a consensus of users of the crop. Characterization (described in Chapter 4) is important basically as a tool for varietal description, identification of duplicates in a

collection, monitoring genotypic stability of clones stored *in vitro* or in other non-conventional forms, and varietal fingerprinting. Preliminary evaluation is often the starting point for breeders to identify an accession's potential value in a breeding programme.
Germplasm curators usually consider preliminary evaluation as complementary to characterization, a means of describing and cataloguing an accession. A breeder's general objective is typically to identify clones that can be used directly as recommended varieties, or as parents in a breeding programme. Many other crucial decisions hinge on this general objective, related to target production areas and their physical and biological characteristics, management practices to be employed, and processing and marketing characteristics.

Preliminary evaluation consists of six broad categories: (1) general adaptation; (2) resistance; (3) plant architecture; (4) yield; (5) root quality; and (6) other locally important traits. The procedures for evaluation of germplasm accessions may be very similar, or identical to evaluation of breeding lines. Much of the detail on evaluation and selection given in later chapters can be applied also to germplasm collections. There is, however, an important procedural difference: all germplasm accessions should be equally and fully evaluated. On the other hand, breeding lines may be pre-selected on the basis of a few key criteria, and only those passing this first step receive further evaluation. If large numbers of germplasm accessions need to be evaluated, some compromises may be made with regard to level of precision. With up to a few hundred accessions, multilocation evaluation in replicated trials may be possible. If accessions number in the thousands, the breeder or germplasm curator may only be able to manage unreplicated single row trials.

Many characters may appropriately be evaluated within a field-planted gene bank itself. Stresses that impose risks to the collection, and may result in accession losses if uncontrolled, should be evaluated in separate, specially designed trials. Serious pests and diseases or major soil problems are examples. The field collection often is not an appropriate place to evaluate yield or quality because of inappropriate plot design or the need to leave plants in the ground well beyond the normal harvest period.

Since the mid-1990s, the accession information and evaluations from CIAT's germplasm collection have been available on-line at www.singer.cgiar.org. This website is managed by the System-wide Information Network for Genetic Resources, the germplasm information exchange network of the CGIAR and its partners. While this is a reasonable first step to search for traits of interest, it is best done with additional consultation with breeders and germplasm curators who are familiar with the details of the evaluations and the germplasm itself. Clearly there is great value in the germplasm information database. At the same time, it will be most useful to a breeding programme if the evaluations are understood in the context of a complete picture that includes agroclimatic conditions, and the complete range of traits that are of importance to the breeder.

6. GERMPLASM EXCHANGE

Many breeding programmes obtain new genetic diversity through introduction from outside sources. The principles and methods associated with germplasm exchange are fundamental to the functioning of most breeding programmes. This discussion focuses on international cassava germplasm movement and those situations where similar principles apply to exchange between regions within a country.

6.1 BENEFITS AND RISKS

The potential benefits of germplasm introduction are essentially a function of the genetic variability available in local germplasm, and more specifically, of the strengths and weaknesses of that germplasm. Typically, the range of variability in local germplasm is regionally dependent, with the highest variability usually in the crop's centre of origin, the Americas. Even in areas of high variability, there can be large advantages to germplasm introductions or to capitalize upon advances made in breeding programmes elsewhere to introduce specific characters.

Two types of risks accompany germplasm introductions: phytosanitary and genetic. The phytosanitary risks – introducing new biotypes or species of pests or pathogens – are of paramount importance. Minimizing these risks should take very high priority in any germplasm exchange. The genetic risks are the risks of knowingly or unknowingly introducing undesirable genes along with the known desirable ones. Undesirable alleles may be those that confer susceptibility or non-tolerance to a particular environmental factor; alleles for poor quality; or in general, any that are considered less desirable than those controlling the same traits in local germplasm. These genetic risks are minimized by an appropriately designed evaluation and selection programme.

6.2 FORMS OF EXCHANGE

6.2.1 Vegetative

Vegetative exchange of cassava germplasm has two main advantages. First, clonal material normally has a background of information from prior evaluations, which helps the recipient predict its usefulness for specific purposes. Secondly, expertise in management of segregating populations is unnecessary. Propagation and management are quite straightforward with stake introduction. Recovery from *in vitro* cultures requires more time, but otherwise management for evaluation and selection is similar to stake-derived material.

The risk of introducing pests or pathogens along with stake material is high. In fact, most countries now have quarantine restrictions against receiving cassava germplasm in the form of stakes. Furthermore, CIAT and IITA, the main international suppliers of germplasm, generally restrict vegetative shipments to *in vitro* cultures. The principal advantage of *in vitro* introduction is phytosanitary. Insects, mites, bacteria and fungi are easily eliminated, and cultures can be indexed for several viruses to provide a high level of assurance of pest and pathogen-free material. From a standpoint of international quarantine, *in vitro* introductions are widely accepted within and among Asian and Latin American countries. Regulations on exchange within Africa, and between Africa and other continents, are more variable and generally more restrictive. No method is free of risk, but the technology for detecting pathogens is well-advanced.

Eliminating micro-organisms from material for exchange is normally beneficial, but there is some evidence that in the process of pathogen cleaning, beneficial micro-organisms are also eliminated, leaving plants derived from *in vitro* culture at some disadvantage (CIAT, 1989). In theory this might be overcome by inoculation either *in vitro* or after regeneration, but there has been no systematic development of a protocol. Presumably, after transplanting to the field in a cassava-growing region, these plants will be reinfested naturally with the beneficial organisms.

CIAT has made limited shipments of stakes derived from mother plants grown in protected screenhouse conditions and tested against known virus diseases. This method combines many of the phytosanitary advantages of *in vitro* culture, with the propagation advantages of stem pieces. This method is restricted to introductions within Latin America, but further refinement of indexing procedures could permit expanded use. The advantage of this system is the more rapid recovery, for field transplanting, as compared with *in vitro* plantlets.

The high perishability of both stakes and *in vitro* cultures is an obstacle for germplasm exchange. Under normal shipping conditions (without temperature extremes) either form of material can tolerate approximately two to four weeks in transit. Shipping methods and dates need to be carefully planned so that delays or adverse environmental conditions do not reduce viability. Many countries with interest in receiving *in vitro* cultures have already worked out logistics for effective exchange.

Stakes can normally be planted immediately, and evaluations taken in the first growing cycle. On the other hand, *in vitro* introductions first need to be propagated and grown in specialized conditions, resulting in some delay until agronomically useful evaluations can be made. Under ideal conditions, and using rapid propagation techniques, agronomic trials can be established within one and a half years after *in vitro* introductions. Normally, however, three or more years are required to obtain sufficient planting material (including one cycle of field propagation to obtain lignified stakes). Many scientists receiving

in vitro cultures have initially been too optimistic about the time required for regeneration and evaluation.

The amount of genetic variability that the breeder can manage is a major limitation for vegetative exchange. For large numbers of clones, the volume (for stakes), expense of preparation and difficulty of management by the recipient, may be prohibitive. This generally means that only a limited number of clones are sent in any given shipment, usually in the order of ten or less, but up to a few hundred in special cases.

6.2.2 Seeds

The two outstanding advantages of seed introductions are ease of handling broad genetic variability and the relatively high tolerance of seeds to storage and shipping. As one of the breeder's principal objectives is usually to evaluate as much genetic variability as possible, this first advantage of seed introductions may be decisive.

The fundamental property of seed introductions is that any plant derived from a botanical seed of cassava is a new, distinct genotype. There is no background of previous evaluations. The only indication of probable performance comes from evaluations of the parents. A second constraint for some programmes to utilize seed introductions is the need for specialized training for management of seed and seed-derived plants.

These disadvantages are minor in comparison to the potential benefits. Practically any country with a cassava variety evaluation and improvement programme should consider seed introductions as an option. Many programmes combine both seed and vegetative introductions, taking advantage of the positive features of each.

6.2.3 Future alternatives

Techniques now under investigation, either in cassava itself or in other crops, may offer new alternatives to cassava germplasm exchange for specialized purposes. Among these are exchange of meristem tips preserved in liquid nitrogen (cryopreserved), pollen preserved in liquid nitrogen or in other specialized environments (e.g. low relative humidity, low CO_2), protoplasts or other types of cell cultures, somatic embryos, and DNA constructs spliced into bacterial or other vectors.

6.3 QUARANTINE CONSIDERATIONS

6.3.1 Phytosanitary risks

The exchange of cassava stem cuttings through unofficial means (farmers, tourists, entrepreneurs) is probably the major means of disseminating pathogens and pests. The bacterial blight pathogen can survive in the xylem vessels of infested stems for months. Cassava viruses and mycoplasmas are efficiently harboured in stem cuttings from infected plants and readily transferred to new plants via infested cuttings. The cassava green mites, mealybugs and scale insects can survive for months, feeding on the lateral buds of stem cuttings. Introductions of green mites, mealybugs and the bacterial blight pathogen into Africa are important examples of the risks of inappropriate and unmonitored germplasm movement.

Some pathogens can be disseminated through botanical seeds. These fit into two broad groups: (1) those that infest the seed; and (2) those that infect it. Infestation may follow fruit dehiscence. If the seeds fall to the ground, the probability of infestation is higher than when the seeds are collected prior to dehiscence and stored under controlled conditions. Pathogens of cassava that can most effectively infest the seeds and survive on them are those producing abundant mucilaginous propagules, such as *Colletotrichum*, *Phoma* and *Diplodia spp*., and *Xanthomonas campestris* pv. *manihotis*. Infestation of storage containers is also a risk. Disinfested seed should be repacked in clean containers.

Pathogens that infect seeds include *X. campestris* pv. *manihotis*, *Diplodia manihotis, Fusarium spp*. and *Cladosporium spp*. However, the limited research in this area does not preclude the possibility of other fungal and bacterial pathogens.

Determination of the potential for seed transmission of all cassava viruses is essential for the safe interchange of botanical seeds, but information is far from adequate. The main virus concerns, namely, cassava mosaic virus, cassava common mosaic virus and frogskin virus, are apparently not transmitted via cassava seeds. Two more recently discovered nepoviruses, the cassava green mottle virus (apparently a minor virus limited to some South Pacific islands), and the cassava American latent virus, found in Brazil and Guyana, raise some concern about seed transmission in view of the type of virus.

A few mycoplasma-like organisms (MLOs) affect cassava, causing antholysis (leaf distortion) and witches' broom diseases. These MLOs are not seed-transmitted.

There are few insects that attack cassava seeds so the risk of disseminating arthropod pests is relatively low. Seeds may however be superficially infested, especially with mites. A seed insecticide/miticide treatment is recommended as a precaution, especially for any seed to be shipped internationally. However, some quarantine agencies, including that of Brazil, have expressed concern about exposing quarantine personnel to pesticides as they examine seeds.

6.3.2 Assuring phytosanitary status of germplasm

FAO and IPGRI jointly published technical guidelines that include general and technical recommendations for exchange (Frison and Feliu, 1991) (see summary in Appendix IV). These phytosanitary measures, independent of others legally established by quarantine regulations of importing countries, would reduce the risk of disseminating pathogens and pests through propagative material of cassava. Their effectiveness depends on the strict application of such measures by both the donor and the recipient. Technical recommendations are provided for: (1) seeds; (2) pathogen-tested *in vitro* cultures; (3) cuttings from pathogen-tested *in vitro* cultures; and (4) untested vegetative material.

The importing country should be especially cautious in the introduction of cassava propagating material from countries or areas where exotic diseases exist. For example, because of cassava mosaic disease, vegetative material should not be imported from Africa or India, except after very thorough virus indexing both at the source and in a third-party institution in a non-cassava growing country.

6.3.3 Virus detection methods

Detection methods can be based on the observation of symptoms in the mother plants, symptoms in grafts or indicator plants, or on the detection of virus particles and viral products. The reliability of detection methods based on plant symptoms can be increased by growing plants under optimal conditions for symptom expression. For example, the symptoms of cassava mosaic disease are poorly expressed at temperatures above 28°C. In this case plants may be grown in a cooler environment to enhance symptom development.

The bioassay of mechanically transmissible cassava viruses to indicator hosts is a sensitive indexing method if a very susceptible host is available, virus concentration in the test plant is high and environmental conditions are optimal for symptom expression. The Nigerian isolate of cassava mosaic disease produces a severe, systemic infection in inoculated *Nicotiana benthamiana* plants. The Kenyan isolate of cassava brown streak virus can be bioassayed on *N. debneyi*.

Grafting is a method for indexing viruses and virus-like agents that are not mechanically transmissible. Graft indexing is very sensitive if a highly susceptible indicator clone is used in the graft. The native Colombian clone *Secundina* is highly susceptible to frogskin disease (the same causal agent as for Caribbean mosaic). When a *Secundina* scion is grafted onto an infected rootstock, leaves express moderate to severe mosaic symptoms. Although a graft-indexing programme requires minimal facilities and training, the procedure is labour intensive and indexing results are not available for several weeks. Another major constraint can be the difficulty of maintaining virus-free stocks of the indicator clone.

Sensitive serological tests are available for viruses that have been isolated, purified and an antiserum produced. ELISA is a highly sensitive, efficient and rapid method for detecting CMD and cassava

common mosaic virus (CCMV). The immunoabsorbent electron microscopy (ISEM) test can also be used for detecting CMD and CCMV. ELISA is suited to a large-scale virus-indexing programme, where hundreds of plants can be tested in a day with results available within 36 hours. The preparation of test material and examination of grids is simple and rapid. Although ISEM is not as sensitive as ELISA, it has the advantage of providing results within several hours.

Nucleic acid or spot hybridization and isolation of viral-specific double-stranded RNAs (dsRNAs) can detect some cassava viruses. Spot hybridization has been adapted for detecting CMD. The procedure is based on the use of a radioactively labelled DNA molecule that is complementary to the viral genome, to probe spots of leaf sap for the presence of viruses. The test is highly sensitive and suited for processing large numbers of samples.

Isolation of dsRNAs is especially suited to detecting uncharacterized viruses for which an antiserum or nucleic acid probe is not available. The extraction and analysis of dsRNAs are somewhat laborious, making the test more appropriate for indexing a limited number of mother plants rather than as a general screening method.

6.4 PROCEDURES FOR EXCHANGE

6.4.1 Sources

There are few germplasm centres with the capacity to act as sources of cassava germplasm on a regular basis and provide the essential phytosanitary safeguards. These functions have been assumed mainly by the international centres – CIAT for Asia and Latin America, and IITA for Africa. Both centres use the latest indexing and preparation techniques to give the highest possible assurance that material being distributed is pathogen-free.

The Field Crops Research Institute of the Thailand Department of Agriculture, in collaboration with the CIAT Asia Cassava Programme, has distributed germplasm from various sources (mainly Thailand and CIAT breeding programmes) throughout Asia. Several quality *in vitro* laboratories are situated in the region, and the generally low level of problems of quarantine significance simplifies distribution. Within the context of a major international project for cassava breeding, the CNPMF and CENARGEN of Brazil have developed a protocol for distribution of cassava seed to Africa. A few other countries may respond to germplasm requests, but normally are not prepared to do so on a regular basis. The international centres can often act as intermediaries to facilitate germplasm exchange between two countries that may not have complete capacity for pathogen indexing.

The plant genetic resources conserved by CIAT are a component of the world designated collection of the Food and Agriculture Organization of the United Nations (FAO). Under a 1994 agreement with FAO, CIAT makes its germplasm available free of charge, upon request, to farmers, farmer associations, breeders, agronomists, extension agencies, universities and biodiversity institutes with a clearly articulated need. (www.ciat.cgiar.org/urg/).

6.4.2 How to request germplasm

Both CIAT and IITA periodically prepare detailed descriptions of advanced lines available *in vitro*, or the description of parents from which progeny are available as true seed. These lists are distributed widely to programmes working with cassava, or may be requested from either of the centres.

Clones available *in vitro* generally do not change with high frequency, because of the relatively slow rate of production of promising new clones and because of the time and resources involved in their *in vitro* preparation, virus indexing and multiplication. On the other hand, seed inventories change continually. New crosses are constantly being made and others discontinued. Variable seed production from different crosses makes it difficult to predict in advance which crosses will be available at any given time.

Germplasm requests should be accompanied by a short description of the target area for adaptation of the material (climate, soils, major pests and diseases) and manner in which roots (or foliage) will be

utilized. A breeder may request specific clones or crosses or request germplasm which will meet given criteria. Any special instructions for shipping should be given as well as the date by which the material is needed. Requests for specific crosses may require up to two years if there is no seed in stock. Requests for crosses with specific characteristics (as opposed to specific parent clones) can often be filled quickly.

6.4.3 Receipt

The supplier institution should send detailed information on how and when germplasm is shipped, to allow the recipient to follow up in the case of delay or loss. Prompt acknowledgment of receipt and a description of the condition of the materials is always appreciated. Any problems encountered should be reported in order to improve procedures for future shipments.

6.4.4 Storage

Ideally the request for germplasm and the shipment will be timed to coincide with a period when it is possible to process and plant the material immediately. However, there will be situations where some storage is required. Seeds may be stored for a few months at ambient temperatures without detrimental effects. If more than a few months of storage are required, seeds should be placed in a cold seed storage room at about 5-10°C and 60 percent or less relative humidity. If no such facilities are available, seeds may be stored in a standard household refrigerator, inside a sealed plastic bag or box containing a desiccant such as silica gel. The desiccant should be renewed periodically.

In vitro shipments should be stored for as short a period as possible to facilitate recovery. Storage, if necessary, should be at 15-20°C, with subdued light.

6.5 REGULATORY AND LEGAL ASPECTS

The international agricultural research system (including formal or informal collaboration among national programmes, universities, the private sector and international centres) has depended on the free exchange of materials and information for continued success. The results of plant breeding research, both from private and public sectors, are increasingly protected as various forms of intellectual property, including patents, material transfer agreements, plant breeders' rights and trade secrets. Since implementation of the International Treaty on Plant Genetic Resources for Food and Agriculture (www.planttreaty.org), the principal means of formalizing exchange of cassava germplasm has been material transfer agreements (MTAs), generally required for both basic germplasm and improved varieties (see example in Appendix V and at ftp://ftp.fao.org/ag/cgrfa/gb1/SMTAe.pdf). Patents and trade secrets associated with genetically modified plants or tissues are coming more into play, but to a far lesser degree than for crops important in temperate agriculture.

Africa, in particular, has less capacity to replicate research results patented elsewhere, for the benefit of poor farmers (Devries and Toenniessen, 2001). While there are many publicly funded partners who would be willing in theory to share their most important discoveries freely, they are often unable to do so because of agreements made with private donors who want to protect their market advantages.

7. CORE COLLECTIONS: ROLES IN GERMPLASM MANAGEMENT

Access to crop genetic diversity has always been a key to successful plant breeding. More effective ways to understand this diversity can contribute to all phases of germplasm management, collection, conservation, evaluation and utilization. This has been highlighted in recent years by the growing recognition of the need to incorporate new knowledge and introduce greater efficiencies and cost-savings into germplasm management. Breeders are interested in searching for specific new traits, such as variation in photosynthetic enzymes, amylose/amylopectin ratios, nutrient use efficiency, cyanogenic potential of roots and leaves, daylength sensitivity and resistance to pests and diseases. Some of these characters require highly specific and expensive evaluations, which may be difficult to apply to the entire gene bank.

The core collection concept grew out of the need to solve these problems. Originally conceived by Frankel (1984), a core collection would represent "with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives." These collections are normally 5-10 percent of the total. CIAT defined a core collection of 630 accessions based on geographic origin, morphological diversity, diversity of esterase isozyme banding patterns, common landrace varieties, and elite breeding lines (Hershey *et al*., 1994) (Table 5.3). CENARGEN, in coordination with CNPMF in Brazil, defined a core collection of Brazilian accessions.

Defining a core collection has several implications for management of the whole collection. Conservation strategies may be tailored to include a higher priority for the core. The core may be duplicated in several institutions, or it may be held in various forms (e.g. field and *in vitro*) while the remainder is kept only *in vitro* or in seed form. For example, based on recommendations of the Manihot Genetic Resources Network, CIAT sent the core collection to Brazil and Thailand for duplication. Longer-term plans call for Africa to also receive this subcollection, when introduction of vegetative material is accepted and managed more routinely.

A core collection permits a better understanding of genetic diversity in the whole collection, through more efficient use of resources for evaluation. Evaluation of the core for a given trait should indicate total diversity, and may direct the scientist to specific geographical areas or groups of germplasm with special promise for further study. It is a common experience in large collections that evaluation for a single trait can take years and considerable resources. Evaluation of the core as a first step can be far more efficient. CIAT began extensively evaluating the core collection soon after its formation, especially for traits that had previously been considered too costly to evaluate in the entire collection (Table 5.4).

As with any sampling procedure, a core collection definition is subject to sampling errors. The probability of a gene occurring in the core collection is significantly different from its frequency in the entire collection only if that gene is related in some way to the criteria for defining the core. One of the main risks is the difficulty of identifying rare genes that may escape inclusion in the core.

Defining a core collection is strategically useful only in large collections. While a number of factors may influence the decision, as a rule, defining a core collection may not be very worthwhile except in cassava collections of about 1 000 or more accessions, and where most of these accessions are landrace varieties.

8. INFORMATION MANAGEMENT FOR GENETIC RESOURCES

Generally, an institution assumes responsibility for germplasm management as a permanent, ongoing activity. An effective information management system becomes a critical part of the process. IPGRI recognizes six categories of genetic resources data (Perry, 1994): passport, characterization, preliminary evaluation, management and general information on collections. An information management system should be integrated across these categories, in recognition of their interrelatedness. A simple database package is adequate for most purposes.

Accuracy in information management is critical. For example, the cumulative effects of even a low error rate in the identification of accessions will have devastating effects on the validity of information in the long term. Historically, there is often considerable instability in the personnel responsible for conservation of collections, and this can contribute to some lack of consistency in information management. Information for many small collections is not well organized, and the evaluation data are of dubious quality.

A database that integrates information across all components of germplasm management (Perry, 1994) provides a means to:

- assess the current status of conservation and characterization of the genetic resources in all participating collections;

- provide an indication of gaps that may exist in geographical representation or phenotypic/genotypic variability inherent in the collection;
- provide an indication of duplication (including intentional security duplication) of material between collections;
- assess the regeneration requirements at an international level.

Table 5.3 Criteria for defining the CIAT core collection

					Proximity to centres of diversity		Country's total diversity in CIAT collection		Country's diversity of cassava ecosystems				Selected for specific parameters				
Origin	**No. of accessions**	**Local landrace varieties (%)**	**Est. level of duplic. (%)**	**Base no. of landrace accessions**	**Scale**	**Weight**	**%**	**Weight**	**Scale**	**Weight**	**Caorrction factor for collec. size[a]**	**Sum of weights[b]**	**Geogr. origin[c]**	**Morph. diversity[d]**	**Diversity of esterase**	**A priori selection[e]**	**Final no. in core[f]**
Argentina	16	40	10	6	1	1.00	25	0.75	2	0.40	1.00	2.15	2	4	0	3	8
Bolivia	3	100	0	3	1	1.00	5	0.95	2	0.40	1.00	2.35	1	2	0	3	3
Brazil[g]	1 637	95	20	1 244	1	1.00	40	0.60	5	1.00	0.20	0.52	110[h]	13	15	20	101
China	2	100	0	2	3	0.50	25	0.75	3	0.60	1.00	1.85	1	0	0	2	2
Colombia	1 907	95	20	1 449	1	1.00	75	0.25	5	1.00	0.20	0.15	111	15	13	14	14
Costa Rica	147	40	20	47	2	0.75	80	0.20	2	0.40	0.80	1.08	9	7	5	4	23
Cuba	74	90	20	53	2	0.75	80	0.20	2	0.40	0.80	1.08	10	5	1	2	18
Dominican Republic	5	100	10	5	2	0.75	10	0.90	3	0.60	1.00	2.25	2	2	0	4	5
Ecuador	117	100	25	88	1	1.00	50	0.50	3	0.60	0.80	1.68	25	6	0	4	32
Fiji	6	100	10	5	3	0.50	50	0.50	1	0.20	1.00	1.20	1	0	0	2	2
Guatemala	91	100	50	46	2	0.75	80	0.20	2	0.40	0.80	1.08	8	6	0	2	15
Indonesia	51	10	15	4	3	0.50	10	0.90	3	0.60	0.80	1.60	1	0	2	5	7
Malaysia	68	70	15	40	3	0.50	50	0.50	2	0.40	0.80	1.12	8	0	1	6	15
Mexico	100	95	30	67	2	0.75	75	0.25	3	0.60	0.80	1.28	14	6	0	2	20
Panama	42	100	20	34	2	0.75	75	0.25	2	0.40	0.80	1.12	6	2	0	2	9
Paraguay	192	100	20	154	1	1.00	80	0.20	2	0.40	0.60	0.96	25	8	3	7	40
Peru	405	95	20	308	1	1.00	60	0.40	2	0.60	0.60	1.20	63	10	3	2	76
Philippines	6	30	0	2	3	0.50	5	0.95	2	0.40	1.00	1.85	1	0	0	2	2

					Proximity to centres of diversity		Country's total diversity in CIAT collection		Country's diversity of cassava ecosystems				Selected for specific parameters				Final no. in core[f]
Origin	**No. of accessions**	**Local landrace varieties (%)**	**Est. level of duplic. (%)**	**Base no. of landrace accessions**	**Scale**	**Weight**	**%**	**Weight**	**Scale**	**Weight**	**Caorrction factor for collec. size[a]**	**Sum of weights[b]**	**Geogr. origin[c]**	**Morph. diversity[d]**	**Diversity of esterase**	**A priori selection[e]**	
Puerto Rico	15	40	15	5	2	0.75	60	0.40	2	0.40	1.00	1.55	1	2	0	4	7
Thailand	8	10	0	1	3	0.50	75	0.25	2	0.40	1.00	1.15	0	0	0	4	4
United States	9	0	0	0	3	0.50	100	0.00	1	0.20	1.00	0.70	0	0	0	4	4
Venezuela	240	95	20	182	1	1.00	60	0.40	4	0.80	0.60	1.32	41	9	3	3	55
CIAT hybr.	317	0	0	0									0	3	5	27	33
IITA hybr.	19	9	0	0									0	0	0	3	3
Totals	5 477			3 744									440	100	51	121	630[h]

[a] Correction factor for collection size: >1 000=2; >400-1 000=0.4; >100-400=0.6; >20-100=0.8; 1-20=1.0
[b] Sum of weights 1,2 and 3 x correction factor for collection size
[c] Number of accessions for core = (sum of weights x base no. of landrace accessions x constant), where constant = 0.17
[d] Clones included in CIAT/IPGRI in vitro Pilot Gene bank (IVAG)
[e] Selected by three criteria: included in CBN studies on the basis of diversity of geographic origin and agronomic value; common landrace varieties; and elite clones from CIAT and IITA. Final number may be less than the sum of columns, because the same clone may be repeated for different criteria
[f] Including 800 accessions in process of introduction at time of core collection definition
[g] 60 accessions to be included prior to introduction of 800 new accessions
[h] Actual total will likely be lower after testing for and eliminating duplicates within the core

Source: Hershey (1994)

Table 5.4 Evaluations in CIAT core collection, 1991-1995

Factor	Accessions evaluated	Mean	Min.	Max.	Ref. (CIAT Ann. Rep.)
Parenchyma total cyanogens (mg/kg DM basis)	566	314	164	4 126	1992
Peel total cyanogens (mg/kg DM basis)	566	1 871	1 638	8 415	1992
Parenchyma dry matter (%)	566	34	13	49	1992
Peel dry matter (%)	566	27	15	46	1992
Protein (%; dry basis)	515	36	1.4	10.4	1995
Starch (% of dry matter)	559	84	71	93	1994
Amylose in starch (%)	503	22	15	29	1992
Postharvest deter. (%)	491	4.2	0	73.4	1995
Harvest index	562	0.43	0	0.81	1995
Seasonal avg. LAI	30	1.2	0.7	1.9	1995
Top biomass (tonnes/ha DM)	30	4.4	1.3	10.2	1995
Root biomass (tonnes/ha DM)	30	9.7	4.7	15.5	1995
Cyrtomenus bergi damage[a]	175	not reported			1995
Aleurotrachelis socialis damage	563	not reported			1995
Mononychellus mite damage	230	not reported			1994
Avg. leaf photosynthesis (µmol $CO_2/m^2/s$)	53	23	14	32	1992
Intercellular CO_2 (µmol/mol)	53	137	73	195	1992
[a]*Group of low-cyanogen clones only*					

8.1.1 Field collections

Field collections require, at a minimum, a reliable method for identifying accessions (plot marker) and a fieldbook describing field management conditions, sources of planting material and observations during the growing cycle. Though this may seem basic and obvious, there are a surprising number of collections where plots are poorly marked and confused identity results by the end of the growing cycle. For a long season crop like cassava, the durability of the identification is especially important and the backup of a fieldbook with a detailed field map essential. The appropriate type of field identification will depend on what is locally available. To be avoided are stakes or tags subject to deterioration during the growth cycle. Small plastic or metal tags, marked with pencil or indelible ink serve the purpose well. One of the most common ways for errors to accumulate over time is through assigning underqualified persons to be responsible for marking plots or making fieldbooks. Given the critical importance of accuracy in this operation, it should always be executed by a qualified technician.

Other useful documentation of a field collection includes soil analysis, details of all field operations (soil preparation, planting system, weeding and pesticide applications), observations on pest and disease incidence, and distribution of planting material. All this information is a useful adjunct to effective conservation, but it does not have the same long-term critical importance as does the accurate identification of materials.

8.1.2 In vitro

In vitro conservation, by its more technologically advanced nature as compared with field conservation, may be assumed to be less vulnerable to errors in identification. Experience from several laboratories shows that extreme care is required in the information management of *in vitro* collections as well. There may in fact be a higher likelihood of errors in identification of *in vitro* materials because it is nearly impossible to catch an error by associating an accession name with its physical characteristics. On the

other hand, the use of computer-generated bar-code labels may be more practical for use in the laboratory as compared with the field. Their adoption should contribute to a reduced error rate.

8.2 CHARACTERIZATION AND EVALUATION

By definition, characterization involves traits with relatively stable expression. The conditions under which they are evaluated should be included in the database, but need not contain great detail. Date and location are usually adequate. On the other hand, most of the traits included in preliminary evaluation are significantly influenced by the environment. For meaningful comparisons among accessions, the evaluation environment must be well-described. This should, at a minimum, specify an exact location, including a field number within an experiment station, so that any relevant characteristics can be retrieved from archived records. The database itself should include some of the basic site characteristics, like soil analyses, rainfall, complete agronomic management practices and any other observations that may help to interpret behaviour of the accessions. Methodology for various types of evaluations and for organizing field sheets can be similar to that described in subsequent chapters on evaluations in breeding nurseries.

Chapter 6. Breeding and selection methods

A cassava breeder chooses from a wide array of options, or organizational schemes, for systematizing and carrying out genetic improvement. There is rarely a single correct pathway to the goal of improved varieties. More than one method can usually be applied effectively to meet a given set of objectives. Major classes of methods and the infinite variations on each, evolved out of a long history of experience with many crops. Breeders of any species can draw on this vast experience, but also need to tailor methods to the uniqueness of each crop and targeted client groups. Some of the factors influencing these choices are: form(s) of propagation of the crop, pollination and seed production, genetic consequences of the propagation system, genetic and cytogenetic characteristics, inheritance of target traits and expectations of growers, processors and consumers.

1. SOME DEFINITIONS

The terminology used for clonally propagated crops can be confusing. For the sake of simplicity, most breeders try to use terms that are also used with seed propagated crops, but these terms may need to be clarified. Throughout this text, the following terms will be used repeatedly:

Clone: The asexual progeny of an individual plant, or of any number of genetically identical plants.

Progeny: The offspring resulting from sexual recombination between two parent genotypes (unless specified otherwise).

F_1: The first-generation progeny of a cross between two genetically distinct parent plants. All known cassava plants are heterozygous and therefore the F_1 as defined here is a segregating generation.

Variety: A clone or set of clones of unique and identifiable characteristics, grown by farmers or officially released for that purpose. (Note: this term is used in preference to cultivar, a contraction of the term cultivated variety, to include those varieties which may be released but not cultivated, or abandoned landrace varieties. The distinction, however, is not of great importance and the two terms are generally considered interchangeable.)

Stake or **cutting**: A section of the lignified cassava stem used to vegetatively propagate the subsequent clonal generation.

Seed: Used only to refer to botanical or true seed, which is almost always the result of fertilization but in theory could result from apomixis.

Sprouting: Initiation of bud growth from vegetative propagation material, especially cuttings.

Germination: Initiation of growth of true seed. The term germination is often used incorrectly in the literature to describe sprouting of vegetative plant parts.

2. INHERENT INFLUENCES ON BREEDING METHODS

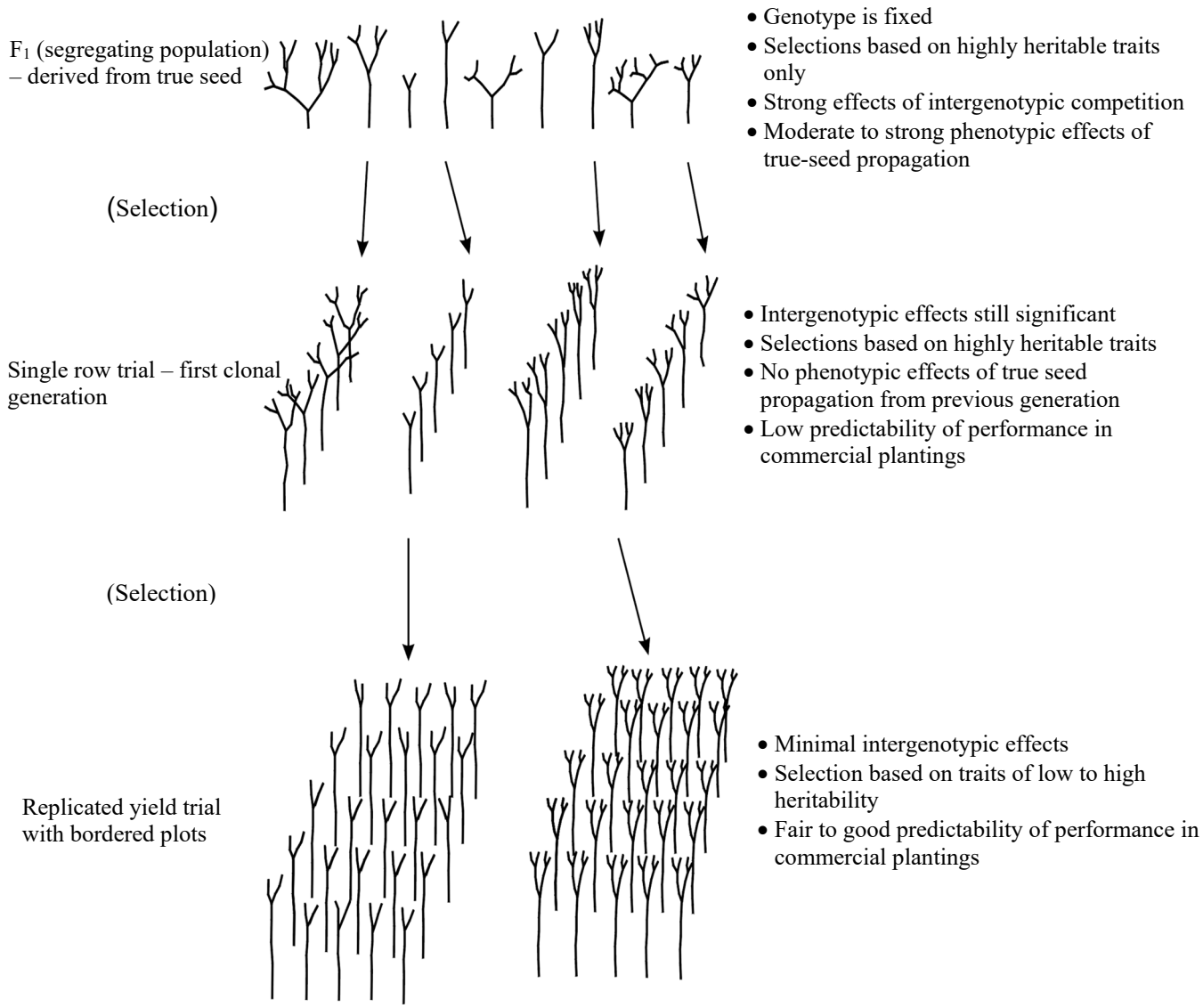
2.1 VEGETATIVE PROPAGATION

Cassava's vegetative propagation lies at the core of any breeding method. Vegetative propagation permanently conserves any genotype, whatever the level of heterozygosity (barring point mutation or spontaneous chromosome reorganization). This has the obvious advantage that a superior plant, identified at any stage of evaluation, can be cloned indefinitely. Genotypic integrity is maintained through successive generations of cloning. This makes vegetatively propagated crops among the easiest to breed in terms of methodology development. Figure 6.1 illustrates the basic premise of breeding a vegetatively propagated crop. The F_1 (progeny of a cross between any two genetically distinct clones) consists of a range of genotypes, each genetically distinct from the other. From this point, the breeder can vegetatively propagate any plant for as many generations as desired and no further crossing may be

required to develop a new variety. The genotype remains the same from one vegetative generation to the next, barring mutations or chimeras, which seem to be infrequent in cassava. While the expression of traits may vary from one generation to another, this variation will be totally due to environmental effects, since the genotype is constant.

However, some of the characteristics often associated with vegetative propagation may introduce complications, e.g. sparse flowering and difficulty in obtaining seeds, male sterility, chromosomal anomalies and inbreeding depression.

Figure 6.1 The genetic and environmental bases of cassava clonal selection



2.2 POLLINATION AND SEED PRODUCTION

Genetic improvement depends fundamentally on the presence of genetic variation. Most commonly this is obtained through sexual recombination. Cassava is monoecious (staminate and pistillate flowers separated on the same plant), which facilitates controlled pollination and makes open pollination schemes practical.

The larger the number of seeds a species can produce per pollination, the less will be the labour and material resources involved to create a desired range of variability. A prolific species like maize may produce 500-1 000 or more seeds from a single controlled pollination. Creating wide variability is relatively easy. Cassava is potentially able to produce only three seeds per pollination and in fact, usually produces an average of less than one seed in controlled crossing schemes. It is a highly labour-intensive procedure to obtain large numbers of F_1 seeds and this is frequently a serious limitation to success in breeding programmes.

2.3 HETEROZYGOCITY

Monoecious and vegetatively propagated crops nearly always consist of highly heterozygous individuals. Monoecy favours outcrossing and vegetative propagation obviates some of the advantages for the evolution of an inbreeding system. The wide segregation observed in progeny from any cross combination, or from selfing, is more immediate evidence for the highly heterozygous nature of cassava. There is little experimental evidence, however, to allow for a more precise quantification of level of heterozygosity. Such studies are only likely to be possible as biochemical and DNA markers are further analysed.

2.4 INBREEDING DEPRESSION AND HETEROSIS

A common feature of naturally outcrossing, heterozygous species is that they suffer yield depression as one effect of inbreeding (i.e. the effects of homozygosity at some loci). The decrease in yield is often proportional to the number of loci that are in a homozygous state. This may have practical relevance in terms of the potential to exploit heterotic effects expressed in the progeny from partially homozygous parents. Empirical evidence suggests that heterotic effects are not great for the large majority of crosses, indicating that most existing clones are already highly heterozygous. This is to be expected because farmers, through a long history of selecting for yield (among many other traits), will presumably have kept heterozygosity at high levels. It would, however, be interesting to research whether inbreeding depression has any influence on yield of important commercial varieties. It is quite reasonable to assume that in restricted gene pools, some inbreeding probably occurred. In Africa and Asia, a considerable proportion of current varieties has evolved from a limited introduced gene pool. Some degree of inbreeding in many of these varieties would not be surprising.

Limited studies have been reported on inbreeding in cassava. In one experiment Kawano *et al*. (1978) compared S_1 progeny on the basis of family means from 12 parents. On average, S_1 progeny yielded only 46 percent of the parent clones. There was, however, wide variation in the yield of the progeny and in one case, the S_1 yielded more than the parent. At IITA in Nigeria, selfed lines were taken to at least the S_5 generation (Devries and Toenniessen, 2001).

The Central Tuber Crops Research Institute (CTCRI) in India carried out selfing experiments to the S_4 generation, for about 30 percent of parents that were able to survive inbreeding. The response of the S_1 was similar to that reported by Kawano *et al*. (1978). Yield and total biomass were the factors most dramatically affected, with roughly a 50 percent reduction in each generation. Research continued on exploiting heterotic effects by appropriate parental selection in test-crosses and diallels (Joseph *et al*., 1994). The CTCRI continued an extensive inbreeding program and has developed commercial varieties using a topcross method. Heterosis for root yield was reported at 10 to 100 percent greater than the better parent (Abraham et al., 2002).

These results do not necessarily negate the possibility of finding relatively vigorous inbred lines of cassava. One should remember that in the early days of maize hybrid programmes, the normal response was similar. The early commercial hybrids were three- and four-way crosses, due to lack of vigour of the inbreds. Only after massive inbreeding and selection was it possible to derive inbreds with adequate vigour for commercial F_1 seed production. (See section on Breeding Methods).

For cassava, whatever breeding method is used, the final product for commercial use will need to be a highly heterozygous clone.

2.5 MALE STERILITY

Sterility is common in crops like cassava that has been vegetatively propagated for thousands of years. Cours (1951) studied the morphological variations of flowers in a large number of varieties and found that about 20 had deformed anthers and were male sterile. Magoon *et al*. (1968) identified varying degrees of male sterility and 35 types were completely male sterile. CIAT's germplasm collection has several male-sterile accessions. Pollen abortion, one mechanism of male sterility, apparently has various possible causes. These include non-disjunction of microspores, abnormal behaviour of the tapetum, cytological anomalies and a functional male sterility due to non-dehiscence of the anthers (i.e. pollen may be viable, but is not released from the anthers). Jos and Nair (1984) reported on the genetics of male sterility resulting from the non-disjunction of microspores from the tetrads. Fertility was dominant over sterility, showing monogenic inheritance.

A breeder can exploit male sterility in design of hybridization methods. The simplest applications are for open-pollination schemes. The use of male-sterile lines as female parents avoids all chance of self-pollination. CIAT has used male-sterile border rows to isolate distinct polycross blocks. More complex use of male sterility could be practical, especially if eventually there was an option for cassava to be commercially seed-propagated.

3. THE *NUMBERS* CHALLENGE

Even with the increasing precision of selection that new techniques allow, successful plant breeding usually relies on managing large numbers of progeny in order to increase probability of obtaining the desired gene combinations. Understanding the importance of managing appropriate population size is central to success. Status of germplasm flow is best documented for Asian programmes. Mr Kazuo Kawano, a CIAT breeder for 30 years, with over half that time spent in Asia, carefully recorded genotypes passing through each step of breeding in the principal programmes of the region. From 1975 to 1993, CIAT shipped about 350 000 hybrid seeds (each a distinct genotype) to nine countries of Asia (Kawano, 1995). Thailand received almost 40 percent of these and China, Indonesia and Viet Nam received most of the rest. Seed introductions from Latin America to Africa have been more sporadic, but were substantial in the 1990s under a project to increase the diversity of Africa's germplasm base. During this period, CIAT and CNPMF (EMBRAPA) in Brazil shipped in the order of 100 000 seeds for evaluation by IITA. Historically, CIAT has shipped 15 000-20 000 seeds per year to countries within Latin America (excluding 40 000–50 000 seeds per year used in CIAT's Colombia-based programme). The CIAT-Thailand office distributed an additional 10 000 seeds per year within Asia, many of these produced by the Thai national programme. IITA reports sending a few hundred thousand seeds per year to collaborating countries in Africa (IITA, 1993c).

Hoopes and Plaisted (1987) gave some revealing figures for potato breeding in North America and Europe. About 200 000 seedlings are grown for every variety released. Of these, only about one in four or five is successful. Hence, there is literally about a one-in-a-million chance that any individual seedling will become a new variety. These numbers are daunting indeed for cassava breeders, working with a crop where seed production is much more difficult.

How many seeds should a programme aim to manage? There is no clear-cut answer, but numbers from the past two decades indicate that in the order of 50 000 F_1 plants are evaluated for each commercially

successful variety produced (excluding formal releases of landrace varieties). For programmes managing just a few thousand seeds per year, these numbers may seem discouraging. However, many factors can influence success and the ability to manage only small numbers certainly does not doom a programme to failure. Factors that increase the probability of success where small numbers of seeds are managed may be: a sharply focused programme working on a few characters, finely tuned parental selection, optimum choice of selection environments and their appropriate management, and lack of competitive local materials. Nonetheless, as programmes gain expertise in the efficient management of seed populations, they are well-advised to maximize numbers.

4. SOURCES OF GENES

Breeding involves identifying superior genes among parents and combining them into a variety that is better than either of the two parents. There is a commonly accepted hierarchy of desirability of different sources of genes for breeding, based on ease of recombination and efficacy of producing desired recombinants. These are, in order of ease of management:
(1) agronomically acceptable, locally adapted genotypes of the same species;
(2) agronomically less acceptable, locally adapted genotypes;
(3) agronomically acceptable, exotic (introduced) genotypes;
(4) agronomically less acceptable, exotic genotypes;
(5) closely related species of the same genus;
(6) distantly related species of the same genus;
(7) other genera.

The basic logic of this hierarchy is not difficult to support: avoid complicated methods when effective simple solutions are available. A surprising number of plant breeders however, fails to follow this simple concept. Other disciplines often seem to be even more inclined than plant breeders to propose the use of genes from sources that may be very difficult to utilize. There is perhaps a certain glamour in working with the exotic and sometimes funding is available for wide crossing, even when more appropriate sources could better be used. There is also often a lack of information exchange among breeders that prevents them from being well-informed about germplasm evaluations and availability for breeding. As a result, a breeder may be unaware of the presence of useful genes in the most appropriate sources. Perhaps a classic example is the search for resistance to cassava mosaic disease, beginning in the 1930s in East Africa. The early searches indicated insignificant expression of resistance in local landraces and for over half a century, resistance breeding focused on wild species sources of resistance genes. It is now known, however, that resistance does exist in local landraces and this resistance is only recently being exploited in breeding (Devries and Toenniessen, 2001).

Table 6.1 Relative importance of different sources of cassava germplasm in African breeding programmes

	Relative importance (percent use)					
Country	**Direct selection of landraces**	**Crosses among landraces**	***In vitro* intro.**	**True seed intro.**	**Crosses among local & introduced**	**Crosses among introduced**
Benin	24	5	1	40	30	0
Ghana	15	5	0	40	30	0
Nigeria	0	0	0	10	0	85
Senegal	19	54	27	0	0	0
Sierra Leone	5	5	20	30	40	0

Source: Adapted from Bennett-Lartey (1994). Percentages may not add to 100 where other unspecified types of crosses are made

Bennet-Lartey (1994) summarized the use of different gene sources in West Africa (Table 6.1). Across the region there is a broad use of landraces for direct selection, landraces as parents, seed or *in vitro* introduction and local crossing with either local or introduced material. This diversity of approaches represents a very sensible attempt to explore and exploit many options and then concentrate on those that show the best opportunity for pay-off.

The caveat to the foregoing discussion is the emerging capability of genetic transformation via specific, isolated genes. With this capability, the phylogenetic relationship to cassava is less relevant and sources ranging from closely related species to bacteria may prove to be equally viable gene sources. Currently, however, transformation is only effective with a very small number of genes and the transfer of gene complexes is still only possible through sexual recombination.

5. GENETICS

Conventional genetic studies in cassava have been very limited, in part because of difficulties in producing populations to fit standard models and in part simply because of generally limited resources. Breeders have concentrated on obtaining some of the most basic information required for effective genetic improvement of the crop, but are only beginning to focus on a more complete genomic characterization of the species and its relatives. By the mid-1990s, a molecular map based on restriction markers was first developed through collaborative work within the Cassava Biotechnology Network. As this is refined towards a well-saturated map, it will be increasingly useful as a tool for elucidating cassava genetics and opening new avenues for their practical application. (See also Chapter 9 for an overview of quantitative genetics).

Table 6.2 Simply-inherited characters in cassava and their gene symbols

Genetic condition	Cassava traits	Reference
a/a	Albino seedlings (lethal)	Hershey & Ocampo, 1989
A/_	Normal chlorophyll	Hershey & Ocampo, 1989
z/z	Zigzag stems	Hershey & Ocampo, 1989
Z/_	Straight stems	Hershey & Ocampo, 1989
g/g	Dark green stem periderm	Hershey & Ocampo, 1989
G/_	Light green stem periderm	Hershey & Ocampo, 1989
y/y	White root parenchyma (flesh)	Hershey & Ocampo, 1989
Y/y	Light yellow root parenchyma	Hershey & Ocampo, 1989
Y/Y	Deep yellow root parenchyma	Hershey & Ocampo, 1989
ms/ms	Male sterile	Jos and Nair, 1984
Ms/_	Male fertile	Jos and Nair. 1984
v/v	Broad leaf lobes	Graner, 1942
V/_	Narrow leaf lobes	Graner, 1942
m/m	White root epidermis	Graner, 1942
M/_	Dark root epidermis	Graner, 1942
p/p[a]	Entire leaf margin	Hershey & Ocampo, 1989
P/_ [a]	Pandurate leaf margin	Hershey & Ocampo, 1989
[a]*Probable epistatic effects of other genes*		

Single genes control relatively few simply-inherited visually-expressed traits in cassava (Table 6.2). Several of these are commercially important. Based on experiences from many other crops, a further study should reveal many more characters showing Mendelian-type segregation patterns. Identification of single-gene controlled characters is important in the study of genome organization, linkages of traits and pollination habits. However, with the current possibilities for producing nearly unlimited numbers of DNA restriction markers, there is less need than in the past for a broad range of morphological markers.

In cassava there are two distinct leaf shapes – broad and narrow. Graner (1942) concluded from segregation patterns that narrow leaf is dominant to broad leaf and is monogenic. Graner (1942) also described light versus dark root periderm colour as monogenically controlled, with dark colour dominant to light. There are also clones with intermediate periderm colour, whose genetic constitution is unclear. Hershey and Ocampo (1989) described Mendelian inheritance for seedling albinism (a recessive, lethal character), stem periderm (collenchyma) colour (light green dominant to dark green), stem growth habit (straight dominant to zigzag) and root flesh pigmentation (yellow dominant to white, with an intermediate light-yellow heterozygote).

Prior to development of capabilities for DNA mapping, esterase isozymes proved extremely useful in germplasm characterization. CIAT produced six segregating populations from crosses involving eight clones. Four bands (19, 20, 21 and 22, according to CIAT's nomenclature [CIAT, 1990]) were consistently clear and constant. Segregation ratios supported the hypothesis that these four bands were determined by one locus with five different alleles, including one null allele (CIAT, 1991).

Inheritance of resistance to cassava mosaic disease, cassava bacterial blight, thrips, cassava green mite, and superelongation disease; root dry matter content; post-harvest root deterioration and root cyanogenic potential are all under multigenic control, with primarily additive effects. Experience from many crops suggests that most agronomically important traits will be inherited in a similar manner. Some of these traits may have one or a few particularly influential genes that can determine a major proportion of trait expression.

While it is certainly helpful to have good information on genetic control of traits being selected, a breeder has to strike some balance between making reasonable assumptions and committing resources to precise genetic studies. Probably for most characters of interest to the cassava breeder, the assumption of multigenic control, with mainly additive effects, will be valid. Using these assumptions to establish breeding methodologies can result in a large saving of time, while basic genetic studies are designed in parallel with a practical breeding programme. Quantitative genetic studies become most useful when a large number has been conducted with a range of genotypes and in a wide range of environments. Breeders should be cautious about placing too much weight on results of a few diallel crosses, tested in a few environments.

6. CYTOGENETICS

Cytogenetic information contributes to understanding a crop's genetic behaviour and may provide clues on methods and techniques for improvement. Bai (1987, 1992) compiled comprehensive reviews of *Manihot* cytogenetics. All of the species examined to that point (about 30) have a chromosome number of 2n=36. The completely paired pachytene bivalents vary in length from 19.3-40 µ. The haploid chromosomal complement has three functional nucleolar chromosomes and six chromosomal types in duplicate.

Meiosis was regular in a large number of the cultivated cassava types studied to date (Graner, 1935; Doughty, 1939). However, in a few types, occurrence of meiotic irregularities such as laggards, delayed separation of bivalents, non-orientation and non-congression of bivalents, restitution nuclei, monads, dyads, polyads, etc., has also been observed (Sohmer, 1968; Magoon *et al*., 1969b).

Considering the chromosome number of other genera in the Euphorbiacae, together with evidence from the meiotic studies in the species itself, Jennings (1963) and Magoon *et al*. (1969b) suggested an allopolyploid origin of cassava. Nevertheless, no tetraploid inheritance has yet been demonstrated for any trait. The lack of wild species having a chromosome number of 2n=18 may support either that the genus as a whole is of a very ancient tetraploid origin and has become essentially diploidized, or that the species are in fact of strictly diploid origin. This question should be resolved as the *Manihot* genome is better characterized through molecular techniques.

7. BREEDING METHODS

Practically any of the breeding methods developed for cross-pollinated crops can be applied to cassava. These will not be covered in detail, as they can be found in any basic plant breeding text. Breeding methods for cassava can be simplified, because heterozygotes are fixed through vegetative propagation. The breeder of a vegetatively propagated crop circumvents two of the major processes in breeding of seed-propagated crops: developing inbred parental lines (outcrossing species), or arriving at homozygosity after crossing (self-pollinators). Vegetative propagation also allows great flexibility for the design of more complex systems, especially for particularly recalcitrant breeding problems.

The following discussion may seem to complicate unnecessarily what is in fact normally a simple scheme. A number of alternatives is simply listed in order that breeders may be aware of possible solutions to special situations and to understand how improvement of cassava relates to that of other crops (Table 6.3).

Table 6.3 Comparison of breeding methods applicable to cassava[a]

Type of breeding practice	Resource demands	Complexity	Accumulated information on clones selected	Potential for progressive improvement	Quarantine complexity	Time for variety development/adoption
Selection among local landraces	*	*	***	*	*	*
Selection among introduced varieties	*	*	***	**	**	*
Selection from introduced F_1 populations	**	**	**	***	*	**
Hybridization – direct variety selection	**	**	***	***	*	**
Hybridization – cyclic improvement	***	**	***	***	*	***
Modified backcross	***	***	***	***	*	***
Inbred/hybrid system	***	***	*	***	*	***
Interspecific hybridization	***	***	**	***	*	***
Polyploidy	***	***	*	*	*	***
Induced mutations	***	***	*	*	*	**
Transgenics	***	***	**	***	*	***

[a] * = low; ** = medium; *** = high

7.1 SELECTION OF LOCAL OR INTRODUCED LANDRACE VARIETIES

The simplest of all methods is selection and release of superior existing landrace varieties. These may be locally selected clones with a long history in the target area or introductions from another region. Some may question the classification of this as a breeding method, but it is in fact the most common means of disseminating selected cassava varieties. In Asia, for example, at least 28 landrace varieties have been selected and released (Tan, 1994). Since the 1990s, however, nearly all new releases have been lines resulting from hybridization. The advantages of the method are that it is comparatively low cost and can make use of evaluation and selection already carried out by farmers and/or other institutions. Selection among local clones is a very conservative breeding approach. Significant impact is possible, but generally only if the selected variety is not already widely grown.
It is also possible in some situations to improve performance of existing varieties by cleaning them of systemic pathogens. This is not a breeding technique *per se*, but usually involves collaboration between breeders and pathologists. Chapter 22 discusses this option in further detail.

There is a logical sequence a breeder/agronomist should follow in designing a programme to introduce and evaluate existing varieties from outside the target region. An important part of this involves evaluation in well-designed and well-managed trials that permit effective statistical analysis to compare the traits of interest. Observations in farmers' fields alone, without controlled comparisons, can be misleading. Until one has good background knowledge of the attributes and deficiencies of locally available varieties, it is impossible to establish breeding objectives or make appropriate decisions on future introductions.

If, after evaluating local germplasm, the breeder sees a need to evaluate other clones, these should be introduced from agro-ecologically similar regions and where the roots are utilized for the same purpose. This improves the chances of adaptation and expression of full genetic potential. If such regions exist within the same country, introduction may be straightforward because international quarantine laws will not apply. However, there is also a need for precautions in moving clones among regions within countries if there are possibilities of disseminating pests and pathogens. CIAT and IITA can often facilitate movement of clones among countries, or from their own international germplasm collections.

Distribution of clones from CIAT-Colombia as *in vitro* cultures to most countries of Latin America or Asia is relatively straightforward. Currently, exchange of vegetative material between Latin America and Africa is only possible through the most stringent of procedures. Within Africa, clonal distribution is highly restricted. Direct exchange among national breeding programmes is infrequent, due to lack of facilities for adequately assuring plant health status. This is not a service most programmes are prepared to perform. Usually a request for a specific clone could be processed by an international centre, even if it were not in that centre’s collection. This is somewhat more difficult in the case of Africa because of generally more stringent quarantine laws.

7.2 DIRECT SELECTION FROM INTRODUCED F_1 POPULATIONS

With the introduction of seed populations from outside sources, the breeder need not be particularly concerned about the breeding methods used to obtain the material. Genotypes are fixed with vegetative propagation in all subsequent cycles and the breeder's main task is to select superior clones.

Both CIAT and IITA produce segregating F_1 populations for distribution to national research programmes. Currently these are the only sources for large-scale availability of such materials. As each seed represents a distinct genotype, the principal advantage of beginning a selection programme from true seed is wide variability. Whereas logistical constraints generally limit introduction of clones to a small number, large quantities of seeds are more easily introduced and managed. Larger numbers of seeds should translate into higher probability of finding all the required traits combined in an individual clone. A disadvantage is that no background information exists on specific clones arising from true seed, as they are totally new genotypes. Expression of many characteristics can, however, be roughly predicted on the basis of parental traits, where additive type inheritance predominates.

When segregating populations are introduced, the breeder must commit to a long-term programme to follow through with several years of evaluation and selection. Chapter 12 gives detailed alternatives for F_1 population management. Handling of segregating populations in a vegetatively propagated crop is simpler than for a seed-propagated crop (either autogamous or allogamous) and is in fact more like handling a large number of genotypes by clonal introductions.

As breeding programmes become more cognizant of the complexity of requirements for a new cassava variety to be successful, the importance of beginning a selection programme with as wide a range of variability as possible is becoming evident, and the simplest, most effective way to do this is often through true seed introductions.

7.3 INTRASPECIFIC HYBRIDIZATION

By far the most common means of creating new gene combinations in cassava is by crossing among distinct clones within the species. This is an appropriate strategy when: (1) no individual clone has been found that combines all the desired characteristics; (2) variability for the characteristics of interest have been observed within cassava; and (3) this variability is in part genetically controlled.

For vegetatively propagated crops, the most common breeding method involves selection of parents based on complementary characteristics, crossing and simple phenotypic selection of individual clones based on performance across years and locations. Some modifications of this procedure have been used or described. Vegetative propagation allows the use of certain methods applicable to either allogamous or autogamous species.

Mass selection is a simple population improvement methodology. Superior individuals are intercrossed and progeny from those plants are bulked to form a population for the next cycle. The process may be repeated for as many cycles as desired. The principal modification used in cassava is to propagate plants clonally over several seasons with continual selection, thereby giving higher confidence in the genetic value of individual genotypes than if they were selected only on an individual plant basis in the F_1.

Pedigree breeding is normally applied to self-pollinated species. Pedigrees on families and individual plants are maintained through the various segregating generations until homozygocity is reached. For cassava, a type of pedigree breeding could be described as maintaining full pedigree records on individual clones and partially basing selection on these records. Maintaining pedigrees is quite simple, as only one segregating generation is involved after each cross combination. Pedigree breeding may be used in combination with several of the other methods described.

Recurrent selection is a common population improvement method, used to accumulate favourable alleles in a population during a series of recombination and selection cycles. Most current cassava breeding programmes use some form of population improvement through modified recurrent selection. Evaluating progeny performance, as a measure of parental combining ability, is usually implicit in recurrent selection; i.e. parents are selected in part on the basis of performance of progeny resulting from a given mating design.

The salient steps in recurrent selection are: (1) production of progeny by any appropriate system of mating; (2) determination of parental values based on progeny performance; (3) parental selection; (4) recombination of the selected parents; (5) evaluation and selection of progeny; and (6) recombination of progeny to begin a new cycle. The method differs from mass selection primarily in that selection of parents is based on combining ability, as opposed to *per se* performance. When additive effects predominate, there is little practical difference between the two methods as applied to cassava.

Recurrent selection in cassava may be based either on half-sib or full-sib progenies. In half-sib selection, progeny are produced from an open-pollination mating design, where the female parent is known but the male parent is generally the result of a mixture of pollen from selected individuals.

Populations involved in recurrent selection may be either open or closed. Most cassava breeders prefer to work with open populations, allowing introduction of superior parents when they are identified. This is less restrictive than a closed population and generally allows more rapid genetic progress when the new introductions are well-planned.

Cassava programmes usually do not strictly adhere to the classical schemes of recurrent selection. For example, because overlapping generations are possible, recurrent selection can utilize progeny performance data for estimating parental values and at the same time, select superior progeny to include directly in crossing, as in mass selection.

The **backcross method** is normally appropriate only for self-pollinated crops, or for a breeding system that involves production of inbred lines. The objective is usually to introduce one or a few desirable alleles from an agronomically unacceptable genotype into one that is acceptable except for the locus or loci in question. The genotype to be improved is used as a recurrent parent and continually crossed onto the progeny carrying the desired alleles. The final product is a homozygous line having the new allele(s), but maintaining all or nearly all the other original characteristics of the recurrent parent.

As backcrossing to a single recurrent parent results in inbreeding, it is not well-suited for cassava. However, modified forms of backcrossing can be used that avoid inbreeding during backcrossing, or that restore heterozygosity in the final stage. Another disadvantage is that backcrossing is basically a conservative method, which relies on having an agronomically good recurrent parent or gene pool. The method allows for little advance in breeding for most characters while concentrating on only one or a few simply inherited traits.

Nearly all traits of importance in cassava are polygenically controlled, which is quite difficult to manage in backcross breeding unless heritability is high. However, with the continually more precise gene tagging methods being developed, backcrossing of polygenic traits could become more practical. Even with this higher precision in identifying genes, backcrossing of multiple genes will be a very time-consuming process in a long-cycle crop like cassava.

One form of modified backcross breeding involves transfer of the genes from a donor individual to a gene pool, rather than to a given genotype (illustrated in Figure 6.2). A set of clones (a gene pool) is identified having most of the desired traits, except one or a few. This missing trait is identified in an individual, either of the same or different species. The gene pool is used as the recurrent parent, with progeny from each generation successively crossed back to the group of individuals in the gene pool, until the trait(s) of interest are transferred and the undesirable genes are eliminated. Using a gene pool rather than an individual as the recurrent parent allows the retention of high heterozygosity throughout backcross generations. The best-known example of this method was the transfer of resistance to cassava mosaic disease from *M. glaziovii*.

7.4 INBRED/HYBRID BREEDING

The world's historically most productive breeding system involves developing commercial F_1 hybrids by crossing among inbred lines, in a crop that is naturally outcrossing and highly heterozygous. This crop, of course, is maize. Cassava breeders have long been intrigued by the possibility of capturing the advantages of an inbred/hybrid system. Nonetheless, cassava's vegetative propagation system already opens some of the advantages of inbreds/hybrids and few programmes have had the resources to move into the long-range and risky venture of developing inbred lines of cassava.

Homozygosity (complete or partial) can confer the following advantages in a breeding system:

- elimination of deleterious recessive genes (reducing genetic load);
- fixation of dominant alleles for future recurrent selection breeding;
- hybrids can be more precisely designed;
- consistent cumulative genetic progress is more feasible;

- useful recessive traits can be more easily discovered or created;
- basic genetic studies are greatly facilitated;
- backcrossing becomes possible as a breeding method.

In addition, specifically for cassava, as a vegetatively propagated crop:

- shipment and storage of germplasm is facilitated (via genetically stable seeds);
- sanitary concerns are reduced (especially important for viruses);
- breeding projects worldwide would be able to capitalize much more readily on each other's advances.

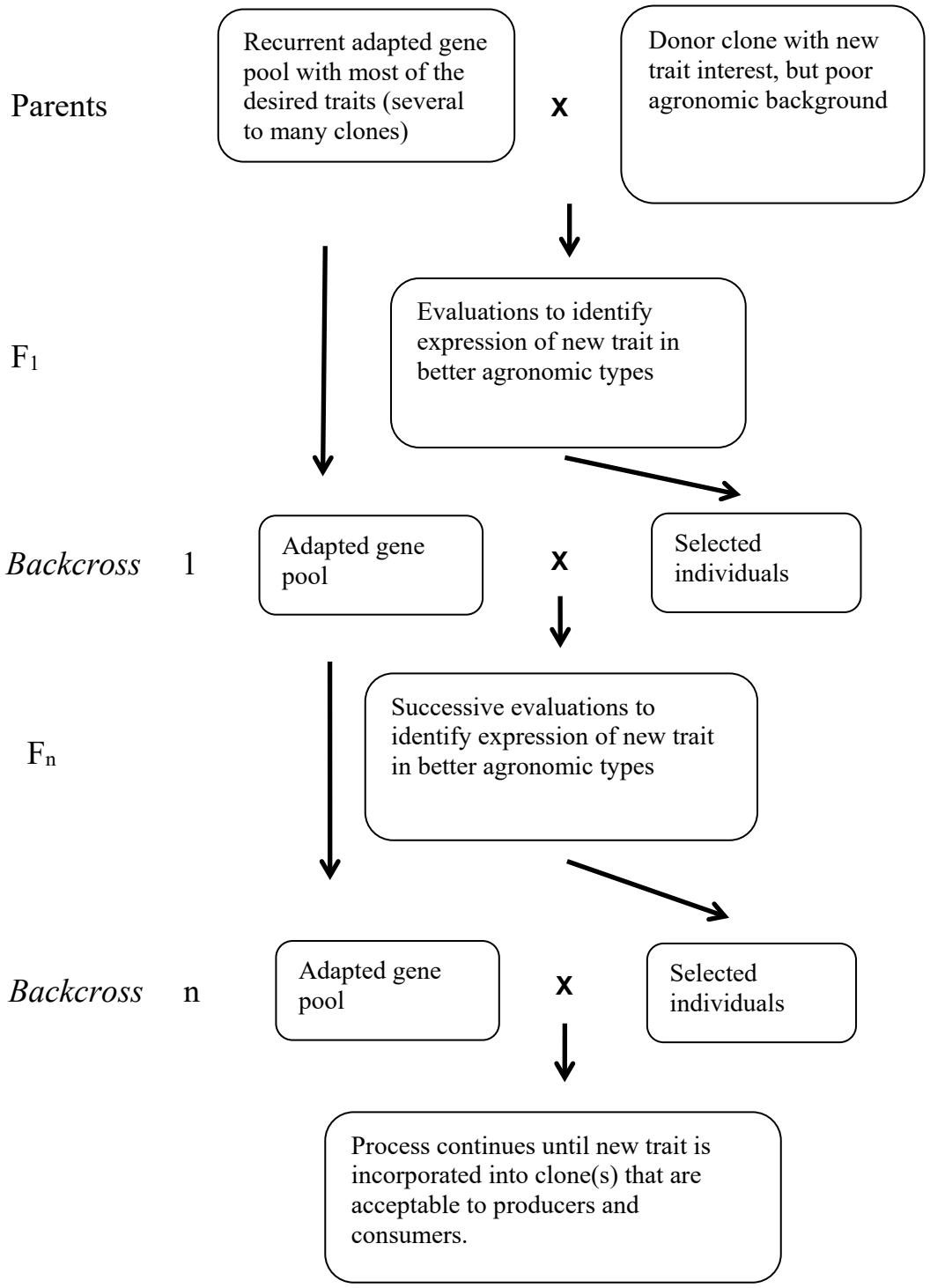
Maize is certainly a model system for the commercial use of inbreds developed from a heterozygous species. Historical developments in maize breeding can enlighten the possibilities of such a system in cassava. An initial constraint is the probable discouraging results of inbreeding, or of arriving at homozygosity in some other manner. Maize breeders initially developed many thousands of inbreds that were too weak to serve as sources of commercial seed in an F_1 hybrid system. Thus, most of the early maize hybrids were three-way or double crosses; the plant producing the seed was an F_1 hybrid. It was not until after about thirty years of inbred development that selection for inbred vigour was sufficiently successful to implement commercially successful F_1 hybrids for growers.

Koshy (1947) was one of the first to suggest inbreeding as a strategy for exposing hidden variability. The method's success relies on exploiting both additive and non-additive genetic effects, whereas population improvement methods in outcrossing species exploit primarily additive effects. Inbreeding and hybrid production are roughly the genetic equivalent of being able to identify an individual superior heterozygous plant in a population and multiply it indefinitely. This of course is already possible in a vegetatively propagated crop. The key difference is that inbreeding will allow elimination of deleterious recessive genes and this may have some effect on the potential of hybrids from inbreds as compared with intervarietal crosses.

There is already some experience by cassava breeders (primarily at CTCRI in India, at IITA in Nigeria and at CIAT in Colombia) showing that cassava is highly sensitive to inbreeding, but there is considerable variation, such that there is clearly the possibility of improving the vigour of selfed lines through selection. In this respect, cassava is probably similar to maize and cassava breeders should take encouragement from that fact and the possibilities of selecting reasonably vigorous inbreds.

Finally, if research moves cassava towards an option of commercial propagation through true seed, an inbred/hybrid system would be almost essential in the long term (Joseph *et al*., 1992; Iglesias and Hershey, 1994b) (see Chapter 24).

Figure 6.2 Schematic of modified backcross selection applicable to cassava



7.5 HAPLOID/DIHAPLOID TECHNOLOGY

A continually increasing number of species can be haploidized by generating plants from *in vitro* pollen or anther culture, or from unreduced gametes. These plants may spontaneously diploidize, or be induced to diploidize. These are referred to as dihaploids (diploids from doubled haploids). In theory, dihaploids should be 100 percent homozygous. In contrast, a residual heterozygocity remains even after many generations of conventional inbreeding. The practical advantage of reaching homozygosity via haploidy is principally a time factor. As compared with five or six generations of selfing required to generate inbreds conventionally (selfing), dihaploids can be produced in a single generation.

Doubled haploids found their first commercial application in rice. The technology was first reported in Japan in 1968 and a worldwide adaptation process for its mass application followed. More than 100 varieties have been released through this technology. It has also been used to introgress genes from exotic germplasm and related species and for molecular mapping. In maize the haploid/dihaploid system is aided by the *ig* gametophyte mutant system. This is a genetic option for haploid production and avoids what is usually a laboratory procedure. Whether or not this genetic system can eventually be transferred among species remains to be seen.

An international symposium in 2003 looked at all the ramifications and the potential for use of haploids and dihaploids in cassava. At that point the two main constraints were still the lack of a protocol for developing dihaploids and the expected severe effects of inbreeding depression once they were developed (Lentini and Ceballos, 2003). To date, whole-plant cassava haploids have not been generated, but a few laboratories have made progress. Both CTCRI in India and CIAT in Colombia succeeded in the initiation of callus, roots and embryoids from anther culture, but no plants (Joseph *et al*., 1992; CIAT, 1994). Experience in other crops shows a strong dependency on genotype for response to anther culture. There is no reason to expect cassava to be any different in this regard.

Cassava flowers are not ideal for haploid induction, in that there are only about 100 microspores per anther (1 000 per flower bud). This, like the low seed production per pollination, is mainly a constraint in terms of productivity of labour and supplies, rather than a genetic constraint.

The principal advantage of a haploid/dihaploid system compared with multigeneration selfing is time – one generation as opposed to six or seven to reach homozygosity. In reality, the multiple generations of selfing also give the breeder an opportunity to select for vigour and an array of other traits in each generation. For the dihaploids, theoretically a much larger population of homozygous plants would need to be evaluated to identify superior genotypes. The process of creating haploids and dihaploids may allow additional entry points for transformation of cassava; introducing genes into a haploid assures their expression will not be masked by dominance effects of other alleles at the same locus (non-expression for other reasons is, of course, still possible). Haploids may add options for the design of cassava polyploids. Mutation breeding is more effective if recessive genes can be observed in haploids.

7.6 INTERSPECIFIC HYBRIDIZATION

The potential worth of wild species and the need to look to wild *Manihot* species for cassava improvement, are subjects that elicit a wide range of viewpoints. Virtually all the early work on interspecific hybridization was based on limited species availability and there was no systematic evaluation of species for traits of interest. In addition, there was almost no information available on intraspecific variability for those species used as parents. Limited quantitative data support the assumption that intraspecific variability within the wild species will be very broad. Selection of genotypes within a species may be as important as the choice of species for breeding.

More recently, both the wild species collections and the information base about them are expanding rapidly. CENARGEN in Brazil has held about half the existing species in an *ex situ* field collection since the 1970s. CIAT has gradually increased its *Manihot* collection to include over 30 species by the mid-1990s. Since the late 1980s, IITA has been emphasizing evaluation and use of *Manihot* species. They successfully crossed many to cassava, with wild genotypes mainly as male parents: *M. epruinosa, M.*

chorosticta, M. glaziovii, M. leptophylla, M. brachyandra, M. tristis, M. tripartita, M. stricta, M. anomala, M. gracilis, M. catingae, M. pohlii and M. *neusana*. Success rates varied from about 1-30 percent. Both *cassava variety* as maternal parent and *species* as paternal parent had a strong influence on success; however, no fundamental incompatibility seems to be a barrier to crossing (IITA, 1993c). Olsen and Schaal (1999) discovered that cultivated cassava contained only 25 percent of the diversity of its wild progenitors, compared with 75 percent for maize.

It is clear that the tree species *M. glaziovii* was a source of genes for resistance to cassava mosaic disease (see Chapter 16). What is less clear, however, is the comparative rate of progress that could have been made using the variability for low levels of resistance known to exist within *M. esculenta* (CIAT, 1994).

For most of the pest and disease problems affecting cassava, where genetic resistance should be an important control component (as compared with cultural practices, chemical control or biological control) variability for resistance among landraces within the species *M. esculenta* appears to be adequate to make progress in breeding. Crossing with wild species does not appear to be necessary to improve resistance to most disease or insect problems.

Wild species are more likely to be useful in future cassava breeding for gross modification of certain characteristics. Currently the characterization of wild species is not well enough advanced to predict with confidence what traits might be targeted. Some have suggested that reduced cyanogenic potential or higher root protein levels may be possible through crossing with wild species. In the case of cyanogens, however, there is no evidence that traditional methods using intraspecific variability will be any less effective. Non-traditional techniques, such as mutation breeding (either *in vitro* at the cellular or tissue level), haploid screening or molecular techniques, should be more promising. Increased protein in cassava roots is still being debated as a breeding objective, on a physiological and nutritional basis. Modification of protein quality may also be justified.

A necessary precursor to effective utilization of wild species is to establish more comprehensive collections and thoroughly evaluate their characteristics. The breeder should be under no illusions as to the difficulty of exploiting genes from wild species. Obtaining products of commercial value with conventional crossing and selection schemes involves 15, 20, or more years. In spite of all the suggestions made on possible contributions of the wild species to cassava improvement, the only documented case so far is the incorporation of resistance to cassava mosaic disease in Africa. New techniques for transferring traits (e.g. transformation via gene insertion), or for more efficient mass screening, will probably accelerate the process somewhat.

8. POLYPLOIDY

Most crop species probably have evolved with an optimum ploidy level. Any increase is more likely to be detrimental rather than beneficial on balance. Use of induced autopolyploids has rarely been successful as a means of crop improvement, despite the fact that they have been studied in every major crop and in most minor crops (Fehr, 1987). Nevertheless, vegetatively propagated crops and those whose economic product is a vegetative part, have the greatest possibility of benefiting from an increase in ploidy levels. Some breeders become discouraged when the first generation polyploids are not obviously superior, without recognizing that a species with a new ploidy level should be viewed almost as a new crop, with weaknesses that need to be addressed in a lengthy breeding programme.

The following definitions apply to this discussion (adapted from Hahn *et al*., 1994):

Polyploid: Individual with three or more basic sets of chromosomes.

Autopolyploid: Individual with more than two complete sets of chromosomes of a single genome.

Allopolyploid: Individual with two or more complete genomes from well-differentiated species.

Somatic polyploid: Polyploid that originates through sexual means, either in a zygote to produce a polyploid individual, or in an apical meristem to produce a polyploid chimera. Often produced by colchicine treatment.

Sexual polyploid: Polyploid that results from cytologically non-reducing male and female gametes that combine to produce functional polyploid zygotes.

Boiteau (1941) reported on the natural occurrence of a polyploid series of cassava in Madagascar. Scientists, as early as the 1940s in Brazil and the 1960s in China and India, produced colchicine-induced tetraploids of cassava clones. As in other species, these polyploids generally exhibited the *gigas* characters, such as increases in leaf breadth and thickness, stomatal size, length and girth of petiole and flower size. Pollen sterility was high, but fertile pollen grains were much larger in size (180–196 µ) compared with diploids (125–140µ). The progeny of polyploids showed wide genetic variation. Some clones became weak and could not be maintained, while others were maintained easily for several generations. No programme has found stable, improved yield potential from autopolyploidy. In India protein content was increased initially by 42 percent, but this advantage disappeared with continued vegetative propagation.

At IITA, work focused on changes in ploidy by inducing unreduced gametes, mainly through interspecific crosses. From crosses between *M. pruinosa* or *M. glaziovii* and cassava, researchers isolated four spontaneous tetraploids and two triploids. A majority of the interspecific crosses produced 2n pollen, but their frequencies varied with cross-combinations and also with genotype within respective cross-combinations (Hahn *et al*., 1990).

IITA believes that the spontaneous polyploid cassava clones from interspecific crosses provide greater genetic variability and give an opportunity for radically new germplasm to evolve and diverge from the present ordinary cassava. The presence of multivalents in the polyploids suggests that pairing and crossing over are taking place between cassava and its related *Manihot* species.

IITA's programme involved the following steps:

Screening genotypes from a population with a relatively large genetic base, for their response to induced polyploidy through asexual or sexual pathways, particularly through 2n gamete formation. The wild species *M. glaziovii* and *M. pruinosa* and the variety TMS 30572 seem to form functional 2n male gametes at a relatively higher rate than others.

- Incorporation into improved tetraploids or a population with desirable genes conferring resistance to various biotic and abiotic stresses, adaptation to a wide range of agro-ecologies and high-quality yield.
- From the improved tetraploid population, identification of promising individual tetraploids and use either directly as varieties or indirectly as breeding material. Triploids had greater potential than tetraploids, probably because of an optimum number of chromosomes.

On the basis of many years of work with polyploid breeding, the CTCRI in India is also emphasizing triploids as the most promising approach. The programme concentrated on crossing diploids (2n=36) with induced tetraploids (2n=72). Selected clones display vigorous growth, erect plant type, broad leaves and thick stems. They have found these triploids to be especially promising for their potential for early root bulking, a factor of major interest to cassava farmers in many parts of the world. After evaluation of many experimental clones, CTCRI officially released *Sree Harsha* in 1996, the first triploid cassava variety developed for general cultivation (Sreekumari *et al*., 1999).

9. MUTATION BREEDING

Cassava should be well-suited to exploiting mutation breeding because vegetative propagation allows immediate fixing of desirable mutants. However, achieving expression of recessive mutants is a challenge, given the difficulties of selfing and attaining high levels of homozygosity. This will be further exacerbated with any traits inherited in tetrasomic fashion, though none has yet been identified. Probably the more common types of mutation effects will be from gross effects such as chromosome breakage.

Indian researchers began mutation studies in the early 1950s (Abraham, 1957). Gamma-irradiation of single-node cuttings was the most effective in producing viable mutants and high-cyanogenic potential types. Continued work with mutation eventually showed some promise, especially in higher photosynthetic rates and lower cyanogenic potential as compared with the parent M-4. Stability of expression of these traits is in some doubt (Joseph *et al*., 1992).

Mutation breeding may only be useful when it becomes possible to produce cassava haploids, so that all mutants are immediately expressed. However, as techniques for more directed genome manipulation evolve, mutation breeding with its very untargeted techniques and unpredictable outcomes is likely to fade even further in importance as a breeding tool. New methods of more targeted mutation may, however, become available.

10. MOLECULAR APPROACHES

Application of molecular techniques to crop improvement is not normally considered a breeding method, but rather a set of tools for creating, characterizing, or selecting specific genetic variability. Nonetheless, these methods are becoming such a basic part of a plant breeder's range of options that they deserve some discussion in the context of breeding methodologies. In this chapter some of the general principles of breeders' use of molecular techniques are discussed and in subsequent chapters on specific breeding objectives, recent advances and future perspectives of transgenics are included. In addition, Chapter 19 covers details of molecular assisted selection in cassava.

Molecular methods for breeding can be broadly divided into those that aid in identifying gene expression in order to make selection for those genes more effective and efficient, and those methods that involve inserting selected new genetic information into the crop genome through targeted systems, broadly known as transgenics.

Since the dawn of the biotechnology era for cassava, plant breeders have debated the appropriate role for molecular approaches. One school of thought suggests that since cassava (as well as other vegetatively propagated tropical crops) was bypassed by the Green Revolution, it may be among those crops with the most potential to benefit from transgenic technologies (Taylor *et al*., 2004b). An alternative line of thought asks whether a crop can appreciate significant genetic improvement if only one or a very few new traits are inserted into an existing genetic background that lacks responsiveness to modern management practices. Probably there are a range of realities across this spectrum, depending upon the circumstances of each specific country or region. While many publications in biotechnology seem to conclude that conventional breeding of cassava has had limited success, in fact many programmes have realized considerable success toward goals of improved yield, quality and pest resistance. For a number of reasons, some of these new varieties have had less success at the commercial production level, but many of these same factors, plus others, threaten to limit the success of transgenic technologies as well.

While the first major international thrust in cassava biotechnology began with the establishment of the Cassava Biotechnology Network in 1986 (see Chapter 1), it was not until a decade later that the first transgenic technologies were reported (Li *et al*., 1996; Raemakers *et al*., 1996; and Schopke *et al*., 1996). Both cassava and japonica rice were considered recalcitrant species in the mid-1980s, with regard to generating the totipotent tissues required to produce transgenic plants (Taylor *et al*., 2004b). Yet, a decade later, transformation of rice was so routine that it was considered a model system. The difference

is probably the level of investment; while rice had the benefit of dozens of laboratories, only seven laboratories worldwide worked on cassava transgenics (Taylor *et al*., 2004b).

Since the first successes at transformation, laboratories have worked to incorporate traits of agronomic and market value. These are reviewed in chapters that cover breeding for specific traits.

Many of the hurdles are now overcome for cassava. Transformation can be accomplished both by particle bombardment and by *Agrobacterium*-mediated gene integration, though the latter has become the preferred system (Taylor *et al*., 2004a). However, the range of genotypes that can routinely be transformed, was limited to just nine clones by 2004. These are MCol 22, MCol 1505, MCol 2215, MPer 183 and ICA-Negrita from South America; 60444, Bonaoua Rouge and L2 from Africa and Adira 4 from Asia (Taylor *et al*., 2004a). Although plant breeders made a strong point, since the founding of the Cassava Biotechnology Network, of the need to be able to transform many locally adapted varieties, this inability remains a major obstacle to field-level success.

The need for a capability to transform nearly any target clone highlights an important difference between vegetatively- and seed-propagated crops. For seed-propagated crops, a new gene needs only to be inserted into a model genotype and then backcrossed into the inbred or variety of interest. As described earlier with regard to backcrossing in cassava, there is no way to return to the original genotype in cassava. Thus, a gene inserted into a non-adapted genotype could only be deployed through an extensive conventional breeding programme that would probably include several cycles of recurrent selection. This would probably add a minimum of 15 years and probably more, to the time of deployment.

The need to transform locally-adapted varieties to successfully deploy transgenic clones, means that someone has to choose these target varieties. It is probably fair to say that laboratory-oriented biotechnologists are generally not the most qualified people to make this decision. Fortunately, this is generally recognized and they rely on plant breeders and extension personnel to recommend varieties. This is a decision that needs to be made with the greatest of care, since it may not be as simple as choosing the most common variety in the region. For example, there may be preferred clones that are not widely cultivated, for reasons that the new transgenic variety could correct (e.g. disease resistance). Generally, the decision on which clones should be transformed should be made by people within the target region. Scientists at international centres will have a broad international perspective on this issue and could also provide useful input.

Under more progressive commercial conditions, such as southern Brazil or Thailand, selection of best locally-adapted varieties for transformation may be more straightforward. In these situations, there tends to be widely-adapted varieties that are more oriented to industrial use.

Once this capability is achieved (of transforming almost any target clone), then transformation almost becomes a breeding methodology in itself. In this scenario, a major improvement can be made to an existing, locally adapted and accepted variety, by overcoming a basic weakness such as cassava mosaic disease susceptibility. While this will almost certainly be feasible from a technical standpoint, regulatory issues will slow the process considerably (see next section).

While transformation of specific local genotypes will be essential for the success of transgenic technologies in cassava, this is in some ways a conservative breeding approach. For many, it may seem incongruent to consider the application of sophisticated molecular techniques as being conservative. Transformation targets and modifies, only one (or at most, a few) trait(s). On the other hand, conventional crossing among diverse parents modifies many genes. Not all these changes will be in the desired direction, but the breeder works to keep the changes in a net positive direction, in order to achieve the long-term improvement of the crop that is needed to keep pace with advances in management technologies, with changes in processing techniques and with changes in consumer demand.

Transgenic cassava still faces many challenges, including further technical questions, intellectual property rights, the underdeveloped regulatory environment and biosafety infrastructures within the

target countries and accessing funding to solve these issues (Taylor *et al*., 2004b). As of early 2005, the first field trials were underway in the US Virgin Islands and confined tests in Colombia and Kenya.

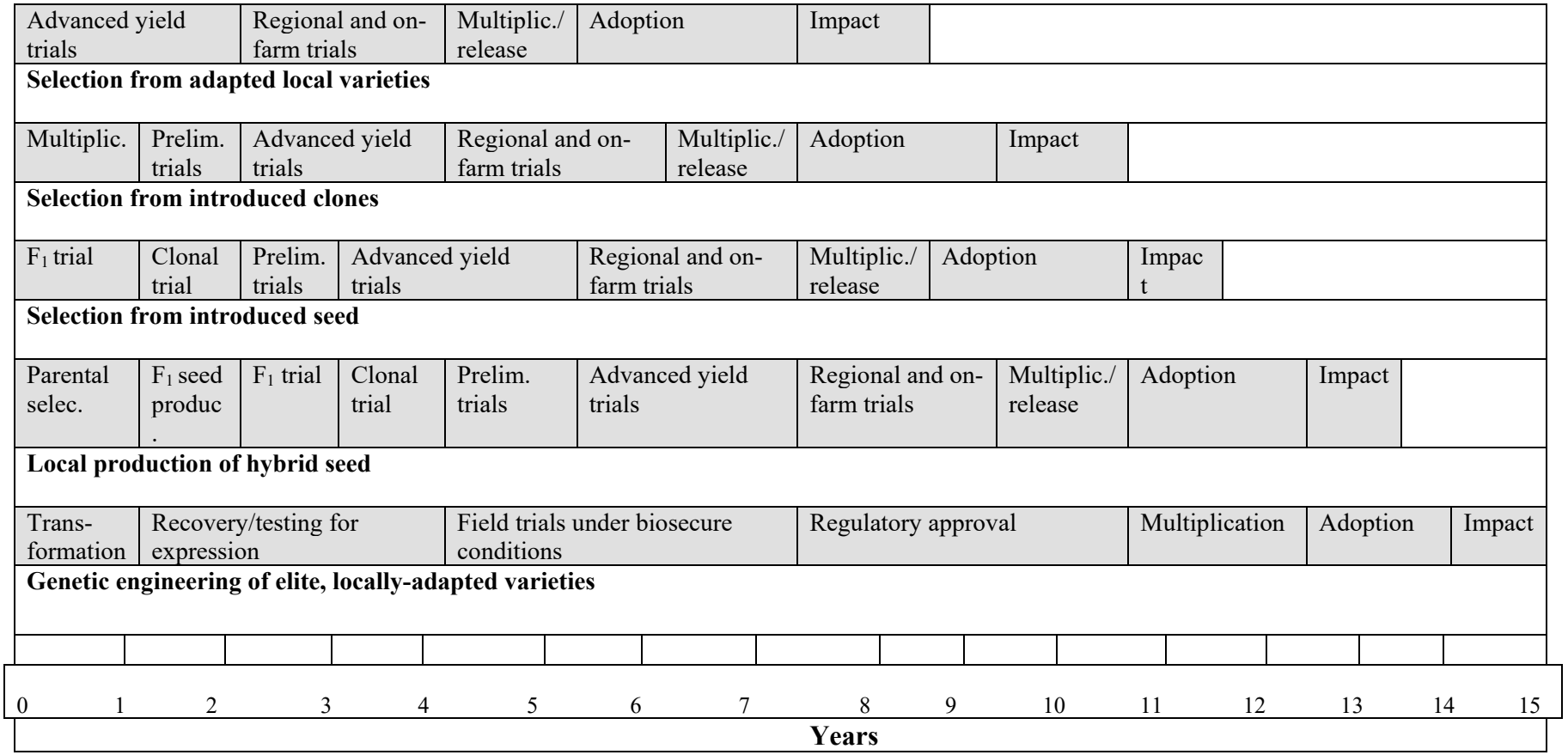
11. A TIME FRAME FOR GENETIC IMPROVEMENT

Every plant breeder is well aware that effective variety development is a long-term enterprise. Most people not closely associated with the field of breeding seem to have a limited appreciation of time as one of the fundamental components of success. Even the simplest of methods, such as selection within a set of adapted varieties, will typically require seven to eight years until impact can be registered. Figure 6.3 compares typical time frames for different breeding methods and strategies. What may seem surprising to many people is the projected time frame for genetically engineered clones. At a first glance, this may seem to be the most rapid method, involving inserting new genes into a known genetic background. First, the transformation of specific local or improved clones is still not possible, and how quickly this will progress is uncertain. In addition, the field testing phase is considerably more complex than with conventionally bred varieties This is due to the need to study efficacy of the transgenic trait in multiple trials, stability of expression of the new trait across environments, human and environmental safety and a range of other possible regulatory issues. In fact, early in the 2000s, very few countries had an approval process in place, and future interest by many countries in introducing genetically engineered crop plants is doubtful. Although more than ten thousand field trials of transgenic crop plants have been safely carried out in the United States since 1989, experience in cassava-growing countries is very limited. In sub-Saharan Africa, by 2004, outside of South Africa, only Burkina Faso, Kenya and Zimbabwe have carried out field trials of transgenic plants (of any crop), with fewer than ten across the three countries (Taylor *et al*., 2004b). In any case, if one assumes a regulatory system that functions reasonably well, the time for variety development and economic impact could be expected to be about 15 years.

This comparison of time frames is not an argument for or against any particular methodology. In a general way, the potential contribution of the breeding method increases as the likely time for commercial impact increases. Or maybe in colloquial terminology: “You get what you pay for.” Investing in comprehensive, longer-term programmes will ultimately have the greatest payback.

All the breeding methods are subject to improvements that will allow reducing the time requirements. MAS in particular promises to make breeding more efficient for any of the reviewed methods (see Chapter 19). Genetic engineering has the most potential for becoming more streamlined as it becomes more routine and when countries put into place efficient regulatory environments. The good news – for breeders, for the farmers who plant new varieties and for the organizations that fund breeding projects – is that experience in other crops suggests that cassava breeding can continue to make significant genetic advances for many decades.

Figure 6.3 Relative time requirements for alternative strategies for cassava varietal development



Chapter 7. The target area: role in breeding programme design

1. PRODUCTION AREA

For most breeders, the target production area is pre-determined by administrative or political considerations. It may be defined by state/provincial boundaries, include an entire country, or even cross-country borders. Having a geographically defined target area does not necessarily mean that it has been adequately characterized for purposes of setting breeding objectives and designing a breeding programme. Characterization is therefore the first step. If this process demonstrates high agro-ecological or market diversity, some subdivision may be required in order to make breeding more efficient.

1.1 CHARACTERIZATION

Experience shows that many programmes begin breeding research without adequately characterizing the target production and market areas. There is often then an iterative process whereby critical characteristics become known only through development of inappropriate varieties. Corrections are made for a particular trait, only to find another deficiency appearing in a later phase of development. Perhaps there is no way to avoid this process completely, because so many components of an environment interact in complex ways with plant genotype and consumer needs are often varied, complex and dynamic. Nevertheless, comprehensive planning at the outset can minimize the adjustments that need to be made later.

For discussion purposes, characterization of the target area can be divided into the categories of socio-economic, physical and biological. This summary is intended as a general outline of the factors that may influence a breeder's success in developing acceptable new varieties.

1.1.1 Socio-economic environment

Farmers' decisions on whether or not to adopt new varieties are influenced by an array of economic and sociological factors, apart from any improved agronomic value. The complexity of the socio-economic environment is exacerbated by the fact that cassava breeding programmes are commonly oriented towards adaptation to poor soils and climatic stresses and often for low income, small landholders. A principal influence on low-income farmers' decision-making is risk. Are there trade-offs to adopting the new varieties? Do they demand more fertilizer? Will they require costly modifications in cultural practices? Do they produce reasonable yields in bad years? Do they have the quality characteristics to consistently enter the intended markets?

Extensive studies are not usually necessary in order to begin a breeding programme. Nevertheless, at least basic background information should be available in all these areas. Initially it may be a bit obscure to the breeder how these factors can have any influence on the characteristics required of new varieties. However, even if some of the major breeding objectives can be quite clear-cut (e.g. need for resistance to an important local pest or improved yield potential) some of the fine tuning could very well hinge on less obvious socio-economic criteria.

1.1.2 Agro-climatic environment

There are established methodologies for classifying climate, soils and topography with reference to plant breeding programmes (Abou-El-Fittough *et al*., 1969; Boyd *et al*., 1976; Russell, 1982). In many countries extensive soil survey data are on file for public access. Similarly, virtually all countries have some system of gathering and compiling weather information. There is of course a wide range in level of detail and reliability of the information.

Basic soil characterization (structure, pH, major elements, possible minor element deficiencies or toxicities) should be carried out in all sites where breeding trials are conducted. Even where detailed soil survey data are available, the specific data from the experimental plots are important for analysing varietal performance. Even the most detailed regional soil surveys cannot capture the important micro-environmental variations observed from one small farm to another, or among fields within a farm. These types of data, accumulated over years, can give valuable insight into expected response to certain soil variations.

At a minimum, rainfall information should be gathered at the experimental sites, unless a weather station is located very close by (within a few kilometres). Data on wind speed, evaporation, hourly temperature readings, relative humidity and others can be useful complementary data, but generally require more sophisticated instrumentation than is available within most breeding programme budgets. Also, these factors do not vary as much as precipitation, and data interpolated from the nearest meteorological stations are usually adequate.

1.1.3 Biological environment

Commonly considered components of the biological environment include weeds, pathogens, mites and insects. Varietal resistance dramatically influences population dynamics and the damage that pathogens and arthropod pests can induce. Varietal traits influence weed competition more than many breeders or agronomists realize. Other vital but less obvious elements of the cassava plant's biological environment involve mycorrhizal associations with the roots and beneficial bacterial associations with both roots and leaves. The importance of varietal variations influencing these associations is still poorly understood.

Some biological factors can be locally significant, such as mammalian pests (e.g. wild pigs, which dig roots; elephants, which trample plants; and deer or monkeys, which eat the foliage). These types of problems are usually well known to local farmers and simple interviews can generally elicit their existence and severity. Although they can be among the most severe production constraints, they are rarely considered in establishing breeding objectives.

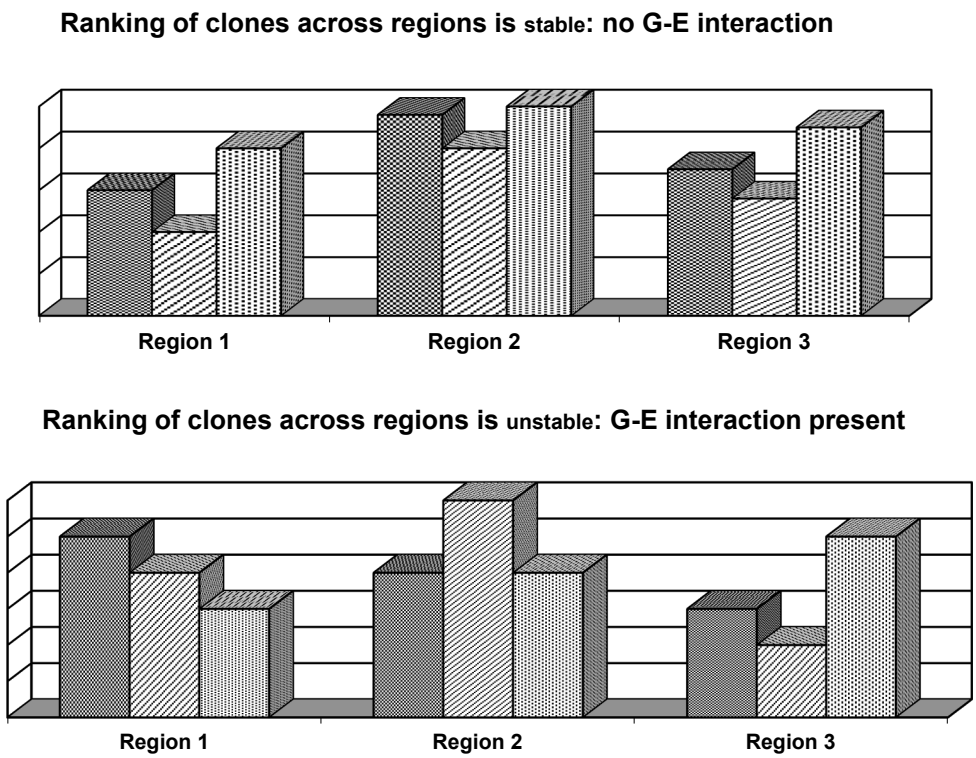
Within the biological environment, a cropping system strongly influences varietal performance. A cropping system specialist or agronomist should be called upon to characterize the most important cropping systems of the target region and goals for improving those systems in the future. A cassava breeder should establish objectives to coincide with those of the agronomist (and vice versa) so that alterations in either agronomic practices or variety will not have unexpected adverse effects on the total system. Biological and physical environment are closely interrelated. Shading and nutrient competition effects of an intercrop or weeds are common examples of such interrelationships.

1.2 GENOTYPE BY ENVIRONMENT INTERACTIONS AND BREEDING PROGRAMME DESIGN

Genotype by environment (G–E) interactions occur when genotypes respond differentially to factors in the environment. These factors may include a wide array of influences on plant behaviour, including ambient temperature, soil water availability, soil chemical and physical properties, photoperiod, light intensity/shading, pest and disease attacks and many others. G–E interaction is most easily conceptualized graphically (Figure 7.1). The fact of different performance in different testing sites does not *per se* constitute G–E interaction, but rather indicates an environmental effect. Interaction is said to occur when the ranking of genotypes changes from one testing site to another.

There are many statistical models for quantifying G–E interaction. The best seem to be the additive main and multiplicative interaction model (AMMI) and principal component axes model (PCA). The AMMI model quantifies a narrow-sense G–E interaction, while PCA quantifies the broad-sense G–E interaction (Yan and Hunt, 1998).

Figure 7.1 Presence and absence of genotype by environment interaction in cassava



1.3 THE BREEDER'S RESPONSE TO ENVIRONMENTAL VARIABILITY

Breeders often face a dilemma on how best to approach environmental variability. It remains one of the most debated topics of practical plant breeding. There are three broad options: (1) rely upon inputs (e.g. fertilizer, irrigation, chemicals) to decrease variability; (2) breed for wide adaptation across the range of variability of the target region; or (3) subdivide the environment and breed distinct varieties for each.

Stratification (or subdivision) may be necessary if the range of diversity within the target region is such that it will be difficult for the breeder to combine the necessary characteristics within a single variety in a reasonable time frame. If, for example, the target region includes both highland and lowland tropical areas (e.g. above 1 700 and below 500 masl) there is minimal possibility of developing a single variety suited to both extremes of temperature. The breeder might opt to develop lowland varieties and highland varieties simultaneously, allocating resources to the two projects on the basis of present and expected future importance in the target area.

More often, decisions on the need and the methodology for subdividing a target environment are not so straightforward. What degree of differences in soil type or climate justify subdivision? Are differences in pest or disease problems within the target region legitimate criteria for developing distinct varieties? It is not possible to generalize answers to such questions, as each individual situation is different. However, certain generalizations about means of approaching the question are possible.

The first principle to keep in mind is that the best measure of the environment is the response of the plant itself. This response should be based on cassava itself and not extrapolated from trials with other crops. The breeder's hypotheses of environmental differences are best tested by variety trials that compare a diverse set of clones over the range of variability encountered in the region. Emphasis must be given to the word diverse, because false conclusions could be drawn if only a narrow range of germplasm, showing a non-characteristic reaction, were used. From these trials, statistical analysis will detect the level of G–E interaction.

A significant G–E interaction is not in itself justification for subdividing breeding objectives. Virtually every study (and there have been many) of a common set of cassava genotypes planted across a set of locations has demonstrated significant G–E interaction. Some analysis of the factors causing the interaction is necessary. To do this successfully, one should observe trials carefully and frequently throughout the growing season. Without this intimate knowledge of the crop in the field, it is unlikely that statistical analysis alone will allow complete interpretation of results.

If the cause of the interaction is easily and inexpensively controlled, either by breeding or cultural practices, then subdivision of breeding objectives is probably unnecessary. One example could be where a strong statistical interaction is the result of variations in soil zinc levels. This might easily be corrected by treating planting stakes with a zinc sulphate solution. Breeding for distinct varieties for high and low zinc soils would be completely unjustified. Similarly, interaction caused by root rot problems on different soils might be eliminated by ridging to improve drainage on water-saturated soils.

One of the major limitations of subdivision based on varietal performance is that it describes a status quo situation. The breeder is more interested in making target area subdivisions on the basis of projected traits of new varieties and new agronomic practices. For example, thrips attack may be very serious in one region of a country and insignificant in another, with other environmental factors similar. A uniform variety trial including both resistant and susceptible clones would likely show a strong G–E interaction. In this example, if the breeder suspects that the major cause of interaction is thrips damage, he or she might plant trials in the same locations with insecticide-protected and unprotected plots. A lack of significant genotype–location interaction in protected plots may show that simply by incorporating thrips resistance in all new varieties, they would be adapted across the entire target region. If several factors are responsible for interactions, target area subdivision, with the objective of developing different varieties for subregions, is probably more efficient than making objectives too complex.

Soil analyses are commonly used for target area subdivision. Extensive databases already exist and there are widely accepted standardized soil classification systems. Often these systems have little to do with the reality of crop response, or are based on other crop species. Soil acidity may be over-emphasized because of the strong reaction of many crops to pH. Cassava is highly tolerant of low pH and target area subdivision on this basis probably is only justified for wide extremes of the environment in question.

Socio-economic criteria can also be a valid basis for subdivision, either independently or superimposed on physical/biological criteria. The form of utilization is a common example. If one market area requires roots of high cyanogenic potential and another, low cyanogens, there is little possibility of having a single variety meet both needs.

Breeders and others have often made the mistake of analysing target area characteristics based on differential performance of narrowly adapted landrace varieties. Their conclusion might be that cassava as a crop is widely adapted, but individual varieties show strong G–E interaction. As this conclusion has such profound implications for a breeding programme, it is essential that a breeder analyse his or her specific situation rather than assume any generalized situation.

Both CIAT and IITA have subdivided breeding objectives according to major agro-ecological criteria. It must be recognized, however, that these are continental or global classifications. Within any one of the defined zones there is very broad variation for a number of environmental factors, such that breeding for zone-specific adaptation does not imply narrow adaptation. It is also clear that some varieties are adapted across some of these broadly defined zones.

The reader may by now have the impression that appropriate target area subdivision for breeding purposes can only be accomplished after lengthy and expensive trials and sophisticated statistical analysis; or alternatively, by relying on uncomfortably unscientific measures. This probably is often true. Common sense judgments and overall familiarity with the crop's reaction to different factors in the environment may in the end provide nearly as good a basis for target area subdivision as a time-consuming series of trials specifically designed for the purpose. Uniform trials can add valuable

information to the database, but unless trials are planted in many sites and over several years, the basis for decision-making is probably too limited (Hershey *et al*., 1992). Usually breeders have neither the resources nor the luxury of time to embark on detailed baseline studies. In some crops with a longer, more extensive research history, it is possible to compile existing data and make appropriate analyses. For cassava, this is usually not possible. Firstly, research is generally rather limited and secondly, where data from varietal trials exist, the data are too sketchy to aid in determining with much precision the factors important in influencing yield.

In summary, the best alternative should be to:

- know the crop and its environments throughout the target area;
- compile data available on variety trials and corresponding environmental components;
- statistically analyse the data and test the environmental components most important in the G–E interaction, to the extent possible;
- hypothesize which of these might be relatively easily reduced by inexpensive cultural practices acceptable to farmers; which ones by genetic modification of the plant and finally; which ones will warrant subdivision of breeding objectives;
- establish a breeding programme on the basis of these hypotheses;
- simultaneously with the ongoing breeding programme, test the hypotheses either with standard evaluation trials or, if necessary, with trials designed to answer specific questions; and
- make adjustments in breeding strategy as new information becomes available.

By following this generalized strategy for subdivision of the environment, it is unlikely that grave errors in judgment will be made at the outset and the potential advantage to be gained by getting a head start in parental selection, crossing and preliminary selection can be enormous. This is not to say that breeding objectives can be set without foundation, but rather that the database for making these decisions is rarely totally reliable even if well-designed. Therefore, it is often counterproductive to wait for several more years of data before action is taken on breeding programme design.

1.4 EXAMPLE OF TARGET AREA STRATIFICATION

Table 7.1 illustrates a hypothetical analysis of various production constraints in different environments and their potential control through cultural practices and/or breeding. While more subjective than quantitative, this type of exercise serves as a preliminary basis for considering the need to subdivide breeding objectives by differentially weighting criteria for distinct agroclimatic regions.

CIAT, after some ten years' experience in cassava research, made a tentative broad classification of cassava-growing environments on a worldwide scale (Hershey, 1992; Table 7.2 and 7.3). This became one basis for subdivision of breeding objectives. The classification is a simple hierarchical subdivision based on the main environmental factors influencing cassava adaptation and productivity, factors that are more appropriately managed through breeding than through modification of the environment. This classification will certainly be subject to continual refinement well into the future as new information is added to understanding G–E interactions.

The first level of subdivision is based on temperature and photoperiod. Physiology studies and empirical observations have clearly demonstrated large G–E interactions for temperature (Irikura *et al*.,1979). As this is also one of the most predictable environmental components, it is a logical criterion for subdivision. Photoperiod appears to be an influence, but a lesser factor in cassava adaptation; individual varieties are commonly adapted to both tropical and subtropical day-length regimes.

Table 7.1 Hypothetical example of target area stratification for subdividing breeding objectives[a]

	Severity in region				Possibility of removing constraint through:	
Production constraint	A	B	C	D	Cultural practices	Breeding
Soil acidity	*	**	***	***	**	**
Low soil P	***	**	**	***	**	**
Drought	***	*	**	*	*	**
Bacterial blight	*	*	***	***	*	***
Cassava mosaic disease	*	**	***	***	*	***
Mites	***	*	**	**	*	***

[a] * = low; * = medium; * = high

Table 7.2 Description of agro-ecosystems defined by CIAT Cassava Programme as broad guidelines for gene pool development

No.	Description	Representative countries/regions	Principal constraints[a]
1	Subhumid lowland tropics	Northeast Brazil; Colombia (Atl. coast and Santanderes); North Venezuela; Mexico (Yucatan peninsula); northeast Thailand; East Java; subhumid belt of sub-Sahelian Africa; South India	Drought stress; mites; thrips; *Diplodia* and *Fusarium* root rots; mealybug
2	Acid soil, lowland tropical savannahs	Brazil (*Cerrado*); Colombia (*Llanos Orientales*); Philippines; West African savannas	Soil acidity; bacterial blight; superelongation disease; anthracnose; mites; mealybug; cassava mosaic disease
3	Humid lowland tropics	Amazon basin (Brazil, Colombia, Peru); West Java and Sumatra; Malaysia; southern Viet Nam; Equatorial West Africa	*Phytophthora* and *Fusarium* root rots; cassava mosaic disease; anthracnose; *Cercospora* and *Cercosporidium spp*.; mealybug
4	Mid-altitude tropics (800-1 400 masl)	Andean zone; central Brazilian highlands; Jos plateau of Nigeria; Cameroon; East Africa	Thrips; mites; root rots; mealybug
5	High altitude tropics (1 400-2 200 masl)	Andean zone; Rwanda; Burundi	Concentric ring leaf spot; low temperature; bacterial blight; anthracnose
6	Subtropics	Southern Brazil; northern Argentina; Paraguay; Cuba; China; northern Viet Nam; southern Africa	Low winter temperature; bacterial blight; superelongation; *Cercospora* and *Cercosporidium* leaf spots
7	Semiarid lowland tropics	Northeast Brazil; northeast Colombia (Guajira peninsula); semiarid belt of West Africa; the United Republic of Tanzania; Mozambique; Rwanda; Burundi	Drought stress; mealybug; mites

[a] Not all constraints are found in all regions of a given agro-ecological zone

Table 7.3 Cassava distribution among agro-ecosystems

	Latin America		Asia		Africa		World	
Climatic zone	%	000ha	%	000ha	%	000ha	%	000ha
Lowland humid tropics	15	417	18	690	34	3 033	27	4 112
Lowland subhumid tropics	33	918	41	1 604	38	3 390	38	5 850
Lowland semi-arid tropics	8	222	26	1 029	8	714	13	1 950
Highland tropics	15	417	0	0	10	892	8	1 281
Subtropics	29	807	15	598	10	892	14	2 242
Total	100	2 781	100	3 921	100	8 922	100	15 624

Source: Internal CIAT Cassava Program discussions and trip reports.

Two broad types of temperature variations influence cassava adaptation. In the tropical belt, little annual fluctuation occurs, but temperatures decrease with an increase in elevation above sea level. In the subtropics (e.g. southern Brazil, Cuba, southern China and Paraguay), temperature varies seasonally. Winter temperatures may fall below freezing and crop growth is halted or minimal in this season.

Temperature patterns delineate four distinct adaptation zones. Within the tropics, altitude defines three zones: lowland (0 to 800 masl; or >25°C mean daily temperature); middle altitude (800–1 500 masl; or 22–25°C mean daily temperature) and highland (1 500–2 200 masl; or 17–21°C mean daily temperature). Of course, there is no sharp gradient of adaptation between zones, but rather a continuum from low to high temperature adaptation. There does, however, seem to be a somewhat sharper delimitation at temperatures above, versus below, about 20°C. Within the subtropics, virtually all cassava is grown at lower elevations, so no further subdivision of temperature zones is made here.

The lowland tropics account for over three-quarters of the world's cassava production. The range of variation within this broad zone justifies a second-level subdivision. This is based primarily on rainfall patterns, but also includes some criteria for soil types: semiarid (6–8 months dry [<60 mm/month rainfall]); subhumid (low to moderate rainfall and a long dry season [3–5 months]); moderate to high rainfall with a long dry season on acid soil savannas; and high rainfall with a short or no dry season (<3 months).

Semiarid regions do not currently account for a large percentage of cassava-growing area, but have considerable potential for expansion, especially in sub-Saharan Africa. Low to moderate rainfall areas describe the most important cassava-growing regions of the world. This undoubtedly is a result of cassava's ability to utilize available water efficiently and to survive long drought periods. Moderate to high rainfall areas with a long dry season characterize some of the world's extensive acid soil grassland or shrub regions, like those of Brazil, Colombia, Mexico, Venezuela and West Africa. The high rainfall areas describe principally the tropical rainforest ecosystem.

Cassava is well adapted to the acid soil savannas. In the Americas (Llanos of Colombia and Venezuela and Campo Cerrado of Brazil) these are typically low population density areas, with poor infrastructure for supplying inputs or for marketing. Nonetheless, agriculture is expanding rapidly here, with accompanying infrastructure. West Africa's savannas are more densely populated and include key cassava-growing regions. Cassava is ubiquitous throughout the tropical rainforest ecosystems of all three continents, but population density here also tends to be low. In terms of practical breeding strategy, these two zones can possibly be combined into a lowland humid tropics target environment.
The need for subdivision of the lowland tropical environments is probably more critical for Latin America and Africa than for Asia. In Latin America and Africa the differences in climatic adaptation

are exacerbated by effects of pests and diseases, while in Asia these are generally less severe. The experiences in Asia in the past few decades, with extensive international movement of materials, are showing that quite broad adaptability is possible with selected varieties (CIAT, 1995), when biological stresses are low.

Other institutions have also developed criteria for subdivision and most coincide in a general way with the CIAT classification. However, as CIAT is the only cassava research programme with a global mandate, its classification is somewhat broader. As such, it will be used as a frame of reference throughout this text.

IITA has reoriented its breeding programme since 1989 towards an agro-ecological approach. Criteria for subdivision of breeding objectives were based on relative similarity of climate (rainfall distribution and amount, temperature), soil (acidity, nutrients and physical characteristics) and biological agents (disease and pest pressures). They also considered sociocultural practices (processing and utilization patterns, quality traits such as cyanogenic potential and mealiness, use of leaves as a green vegetable, population pressure and market access) and cropping systems (monocropping and intercropping). Ultimately, IITA defined four broad agro-ecologies for Africa: (1) humid forest; (2) humid forest-savannah transition combined with southern Guinea savannah; (3) northern Guinea savannah combined with Sudan savannah; and (4) mid-altitude (800–1 500 masl).

2. DEFINITION OF TESTING SITES

After completing characterization of the target production area and the target markets, specific testing/selection sites need to be chosen. The sites should be:

- closely representative of the target regions, in terms of the socio-economic, physical and biological criteria that will influence breeding objectives;
- accessible at all times of the year, including during heavy rainfall periods if these are common in the region;
- relatively uniform for soil conditions;
- available for research trials on a medium- to long-term basis;
- as free as possible from danger of theft, unintended farmer intervention in trial management or other human disruptions;
- free from entry of cattle or wild animals.

Many agricultural research institutions have a network of experiment stations for conducting research. It is often most convenient logistically and economically to use these same stations for selection purposes. However, experiment stations are rarely single crop stations and their suitability is often determined for crops other than cassava. They are often located in the most favourable soil and climatic conditions, as a way of demonstrating potential of new technology, while cassava is usually grown on the poorest soils. Varieties selected only in favourable environments of experiment stations may be unsuitable for more typical, stressful conditions. Naturally, this generalization does not always apply and each situation must be studied individually.

Advantages of an experiment station can include: an established infrastructure, more complete control of extraneous variables by the researcher and high probability of continuity of access to land and facilities. Often all of these criteria are not met even on well-run experiment stations and much less on farmers' fields. Ideally the breeder should allow flexibility to change testing sites if it becomes evident that a given site is unsuitable. This is especially important in the early years of a programme, when objectives or design are more likely to require adjustment to new information. It may be better to rent land from farmers rather than make large investments in purchased land and infrastructure, which could impose a long-term commitment to the use of that particular land.

Over time, the physical and biological environment of experimental sites may change as a result of research management practices that differ substantially from those used on farms. This may sometimes

be due to lack of appreciation by researchers of their target environment and sometimes by virtue of the inherent characteristics of research. Cropping systems on the experiment station might be dictated by space limitations, assignment of different crops to specific sections of the station or other criteria.
Experiment stations often practise continuous cropping, while farmers allow fallow periods or rotate crops. Continuous cropping could lead to abnormal build-up of pests and pathogens, giving the breeder an erroneous impression of the priorities to assign to resistance breeding. Breeding programmes nearly always work with a wide range of genetic variability. This generally includes variability for pest and disease resistance. Especially in the early selection stages, many highly susceptible host plants may be present, creating an environment conducive to abnormally high pest or pathogen build-up. This phenomenon, like that of atypical cultural practices, may result in a breeder assigning unrealistic priorities to certain targets for host plant resistance. One might consider that it is better to err on the side of breeding for unnecessarily high resistance levels rather than insufficient levels, but the breeder would not be using resources efficiently in either case. One advantage of periodically changing selection sites is to avoid this experiment station syndrome, where biological or physical environmental factors become progressively more unlike those of the on-farm environment and give misleading information to the breeder on selection priorities. Chapter 16 discusses in more detail the appropriate management of pest and pathogen populations for purposes of a breeding programme.

Characterization of the target area variability in relation to breeding objectives leads to alternative models for programme design. In the planning process, schematic representations can aid in better visualizing advantages and disadvantages of various scenarios. Diagrams showing the precise flow of materials through selection stages are often the best way to pinpoint potential management problems before they occur. The following section describes some hypothetical situations and appropriate evaluation schemes. Obviously, any number of variations on these models is possible and any given programme is not likely to conform exactly to a particular model. They are given as illustrations of starting points in planning.

MODEL 1: UNIFORM TARGET ENVIRONMENT

Where the environment (i.e. soil, climate, pest and disease problems, cultural practices, markets) is considered uniform, selection can proceed for various stages at a single site in the region. Figure 7.2 shows a possible scheme. In the real world there is no such thing as complete uniformity in natural environments, so for all practical purposes, a model of this simplicity will almost never be appropriate. Even in environments considered highly uniform, it is necessary to sample variation by having trials in at least a few sites at the later stages of selection.

MODEL 2: MODERATELY VARIABLE TARGET ENVIRONMENT

The majority of cassava breeding programmes probably fits this description. Preliminary selection to discard the most unacceptable genotypes can be carried out at a single site. At the intermediate and advanced stages, selected clones are evaluated across sites representing the range of environmental variability for the target region. Individual varieties can be selected for adaptation across the entire region (Figure 7.3).

MODEL 3: HIGHLY VARIABLE TARGET ENVIRONMENT

In this scenario, breeding for adaptation across the entire target region is not practical. Consequently, the region is subdivided into more uniform subregions and distinct varieties developed for each (Figure 7.4). Selection is decentralized from the earliest stages.

Figure 7.2. Hypothetical selection scheme for completely uniform target area

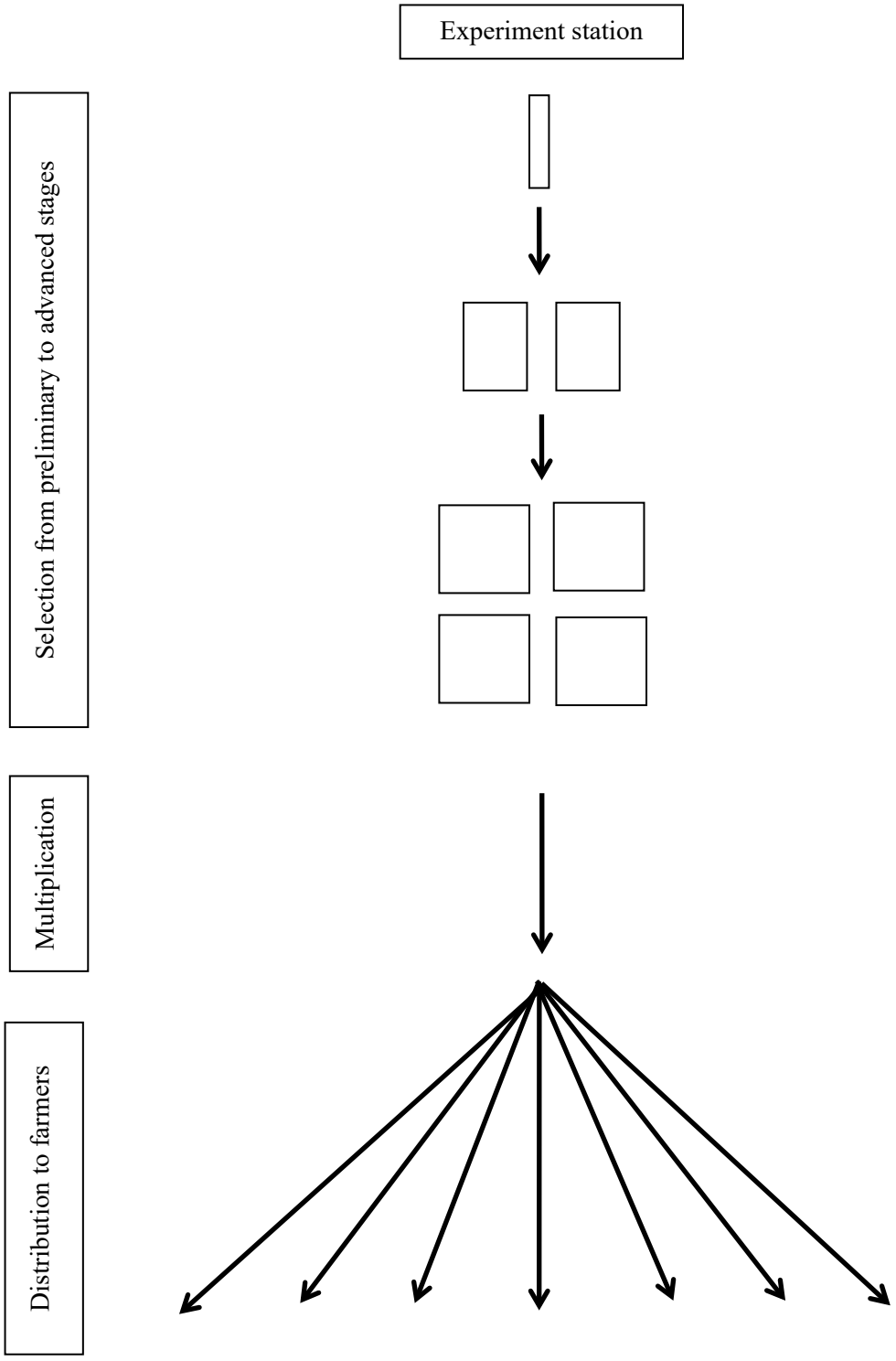


Figure 7.3 Hypothetical selection scheme to achieve adaptability across a moderately variable target region

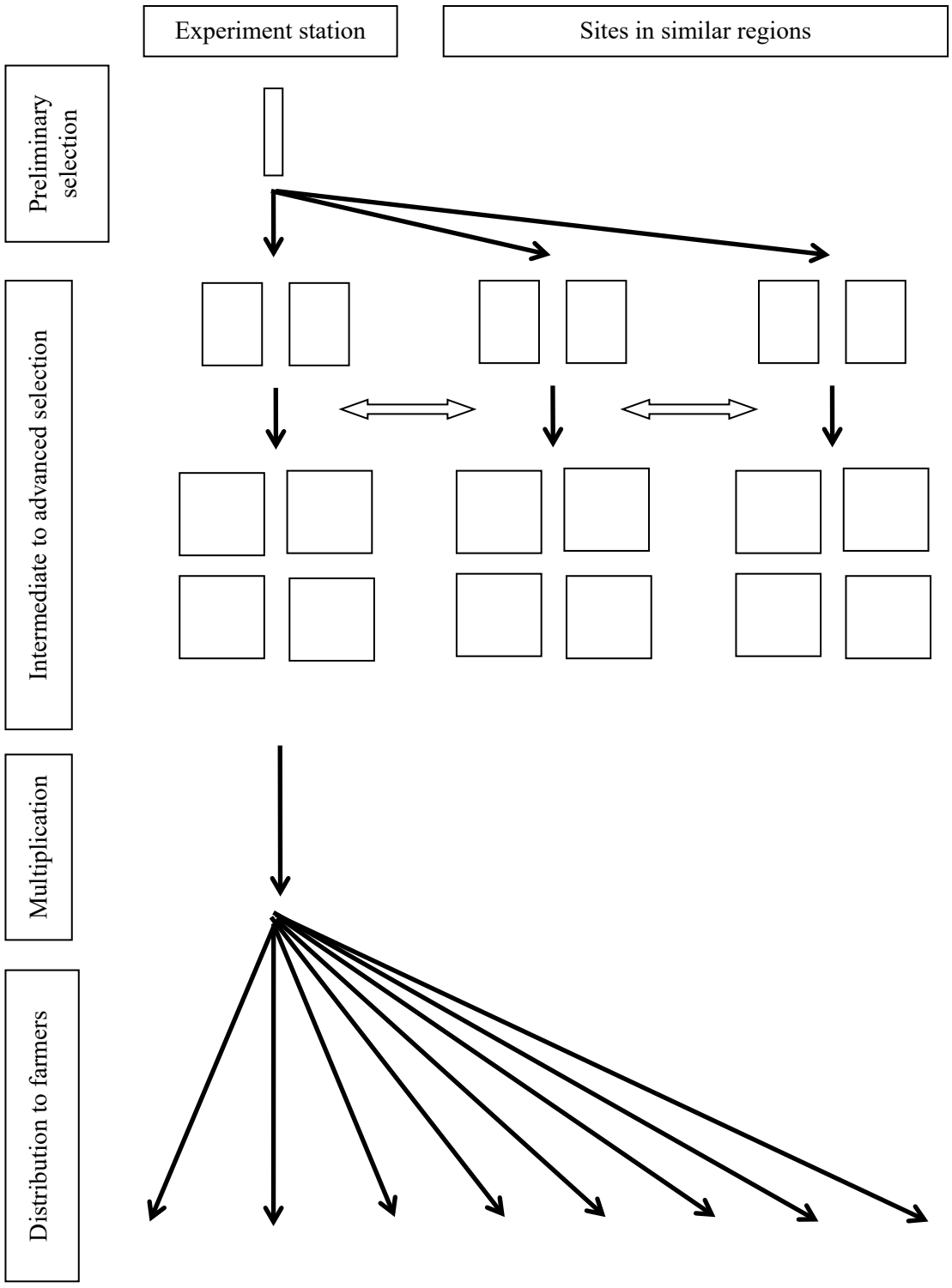
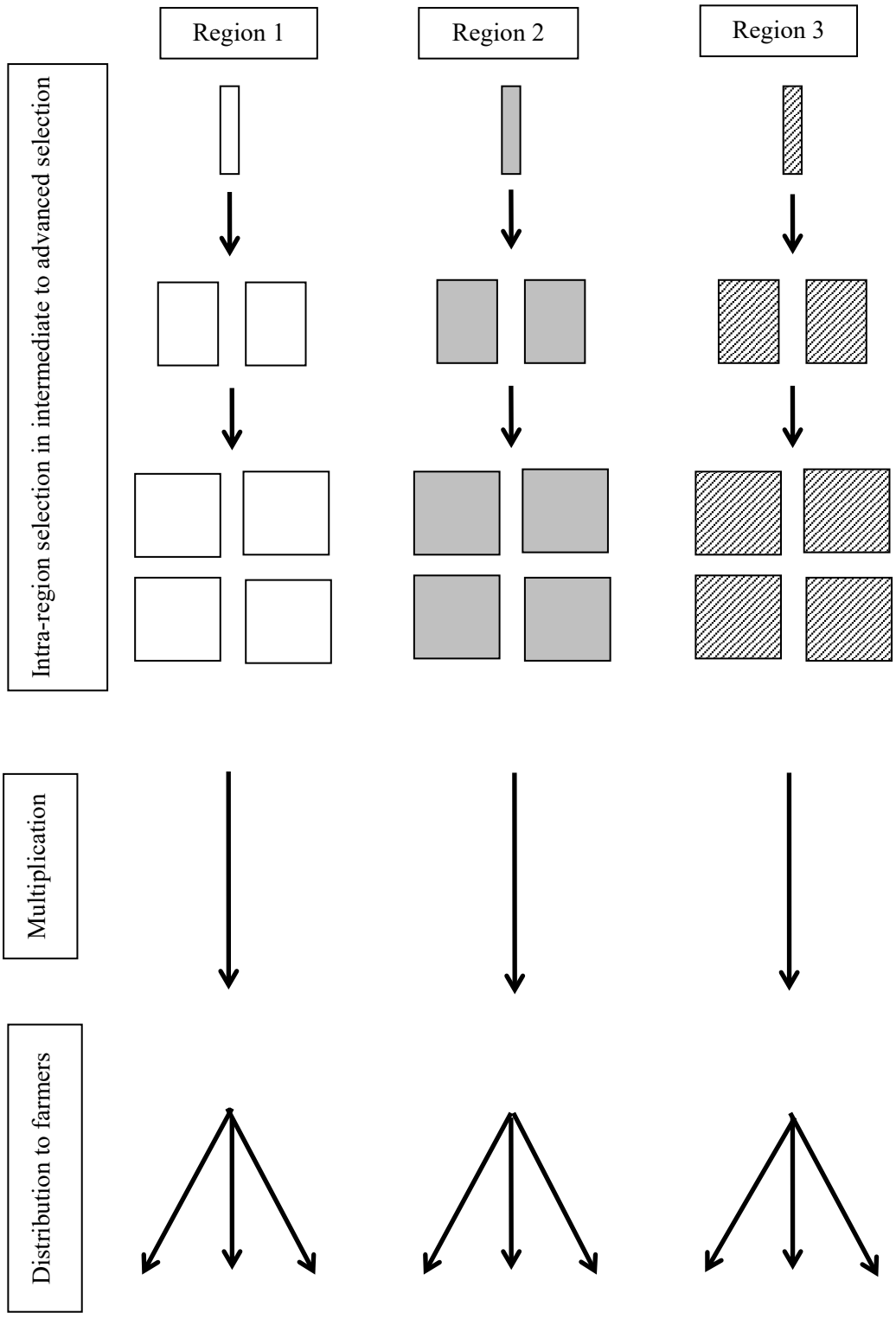


Figure 7.4 Hypothetical selection scheme for developing distinct varieties for agro-ecologically diverse regions



3. SITE COMPARISONS WITH VARIETY TRIALS

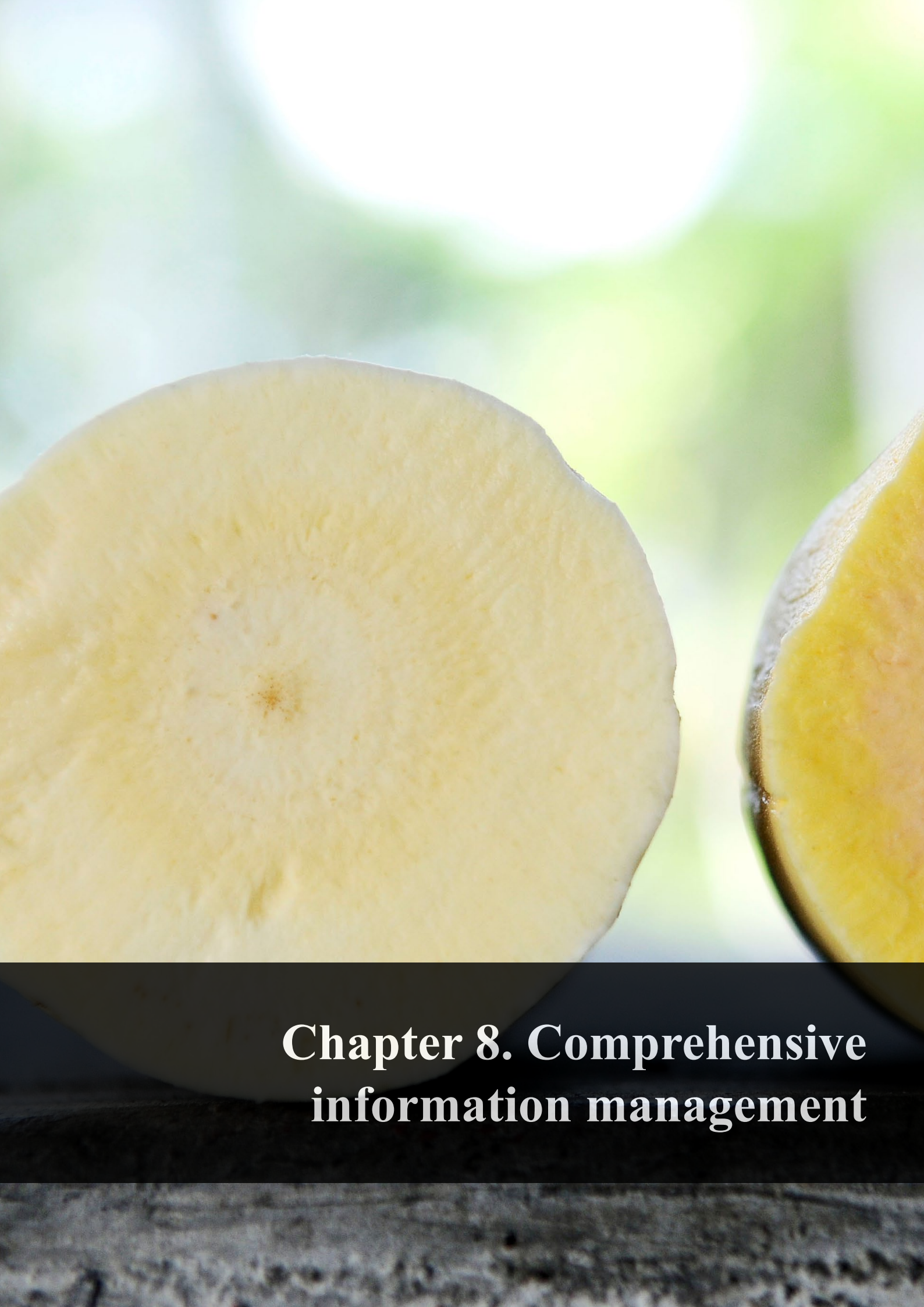
Whatever methodology has been applied for stratifying regions for cassava variety testing, breeders have frequently been disappointed to learn that variety performance often differs from expectations. The environmental effects within a region may still be very high and the correlations between trials within a region may be no greater than correlations across regions. Probably the main reason for this is the tendency for cassava to be grown in conditions where a number of stresses commonly affect growth and yield and these may differ substantially from one year to another, or from one location to another, even within a region defined as relatively homogeneous. This phenomenon appears to be more of an issue where more stresses exist and have a greater potential to interact with genotype.

In maize, wheat and rice, many thousands of international trials have aided in refining environmental classifications. In cassava, such trials have been very limited due both to the cost of managing bulky and perishable planting material and to quarantine restrictions on vegetative material. In the late 1980s and early 1990s, CIAT and CNPMF (Brazil) exchanged sets of germplasm with adaptation in distinct agro-ecosystems. These clones were tested in a range of sites typical of cassava-growing regions in both countries over a course of four years. Table 7.4 gives linear correlations for root yield among sites in Brazil and sites in Colombia, according to edaphoclimatic zone (ECZ) description. Significant correlations of performance between sites were the exception rather than the rule. One of the encouraging results from the study was the relative consistency throughout years within a site (whether correlations were significant or insignificant). On the other hand, there is not a clear pattern of relationship between trials within the same ECZ, across countries. This suggests either that more extensive trials need to be carried out in order to better elucidate relationships among sites; that selection needs to be carried out locally, since no site is consistently a good predictor of performance in another; or that the definition of edaphoclimatic zones is incomplete. These types of results are typical of trials in cassava when a broad germplasm base is tested across diverse environments.

Table 7.4 Linear correlations for root yield among sites representing different edaphoclimatic zones (ECZs)[a] for a set of clones tested in Brazil and Colombia

			Brazil testing sites[b]						
			ECZ 1/7						**ECZ 2/3**
			CN88	**CN89**	**PC87**	**PC89**	**PC90**	**IT89**	**UN89**
Colombia testing sites[c]	ECZ 1	ML87	0.57	0.62*	-0.17	0.10	0.08	0.30	0.20
		ML88	0.56*	0.57*	-0.12	0.26	0.26	0.28	0.00
		CB88	0.70**	0.52	0.28	0.53	0.48	0.54	0.43
	ECZ 2/3	LL88	0.61*	0.44	0.30	0.20	0.31	0.71**	0.60*
		LL90	0.66*	0.62*	0.21	0.34	0.35	0.23	0.62*
		CR87	0.11	0.22	-0.15	0.27	-0.12	-0.05	-0.25
		CR88	0.45	0.50	0.35	0.26	0.37	0.33	0.43

[a]Zone 1: lowland subhumid; Zone 2: acid soil savannas; Zone 3: humid rainforest; Zone 7: semiarid
[b]CN=Cruz das Almas; PC=Pacajus, IT=Itaberaba, UN= Una
[c]ML=Media Luna; CB=Carmen de Bolivar; LL=La Libertad; CR=Carimagua
Source: Data from combined results of CIAT (Colombia) and CNPMF (Brazil) trials, reported in CIAT Annual Report, 1992



Chapter 8. Comprehensive information management

Data are the basic product of most scientific research. Raw data are organized, analysed and interpreted, and thereby make the transformation from data to information. The same data can often be processed and presented to provide different information for different purposes. For example, information used to select genotypes in a breeding nursery may be based on the same data as information included in a research progress report.

The term information management is used in the context of this discussion to include the entire system of planning, gathering, storing, manipulating, interpreting and communicating data and information. This chapter presents a broad overview of various features of information management as it relates to cassava breeding, including discussion of components of information, and objectives, principles and strategy for information management. Chapters 12, 13, 20 and 21 include sections that examine information managed in specific types of breeding programme trials.

The first objective of information management is to enable effective and appropriate selection leading to genetic improvement and variety adoption. These activities are viewed here in the broad sense, to include the range, for example, from assembly of a germplasm base, to release of new varieties. The second broad objective is to communicate accurate information to scientists, donors, users and others with an interest in the process.

The information revolution of the late twentieth century grew out of technological advances in information storage, processing, access and transfer. Personal computers played an immense role and are now accessible in nearly all research environments. Telecommunications technology is allowing immediate and universal access to information. The internet became a vital part of information sharing in the early 1990s. This revolution will continue unabated and the tools will be constantly changing. This chapter looks at principles of information management in a cassava breeding programme, principles that should by-and-large remain valid through technological advances.

1. PRINCIPLES AND STRATEGY

Information management should be comprehensive, covering and integrating all relevant aspects of the breeding programme. It should be a central part of a programme's strategy for meeting its objectives. Several basic precepts govern effective information management: accuracy, timeliness, relevance, clarity and cost. Unfortunately, most formal training of plant breeders focuses on techniques of statistical analysis and places less emphasis on the very important area of designing information management systems. A breeding programme should not be managed solely as a series of unrelated experiments. The breeder needs a system that is comprehensive and relates information across stages of selection, locations and years. Yet it must be managed relatively easily, so its use does not occupy an inordinate amount of time.

Standardization of data (e.g. rating scales, criteria for measurements) across years and trials is highly desirable, so that comparisons can be made on the basis of uniform criteria. On the other hand, a rigid system should not limit the breeder's flexibility to modify methodology as new information is obtained or new goals are elaborated. For example, it is convenient to use the same system for evaluating pest resistance throughout the years, but if research indicates need for modification, some sacrifice in ease of data processing or interpretation may be justified.

Breeders usually do not directly take all the data they use for selection. Scientists of other disciplines – pathologists, physiologists, entomologists and others – frequently provide specialized evaluations. Due to the fact that it is often the breeder who is responsible for integrating this information, it is his or her responsibility to convince colleagues to contribute to an integrated information management system. History shows the sharing of information often to be a point of contention among co-workers. Individual researchers may jealously guard what they consider to be their personal data, and therefore these data never get fully utilized. Some institutional environments are certainly more conducive than others to free sharing of data. Beyond the individual, it is an institutional responsibility to create appropriate

incentives for data-sharing. But if these incentives do not exist, breeders may need to take the initiative. Most scientists will only be persuaded to share if they are convinced that they will get proper recognition for their work. In no case should the breeder attempt to present the work of others as though it were his/her own. The data integrator, the breeder, needs to be especially sensitive to giving credit to others if he or she expects continued good collaboration.

2. TOOLS FOR EFFECTIVE INFORMATION MANAGEMENT

The tools available for information management have the potential to make research far more efficient and productive than was imagined possible a generation ago. Perhaps the best starting point for application of these tools is to take a problem-solving approach, determine management objectives and then search for the best way to meet them. Many breeders fall into the trap of using programmes or techniques because they have access to them, and they provide a sense of sophistication, while maybe only minimally useful in helping to meet research objectives.

By the early 1990s desktop computers had become a basic part of the functioning of most cassava breeding programmes. They are a tool to facilitate some of the tasks that have always been carried out in breeding programmes – data storage and retrieval, transformation and analysis. Via communication linkages, they are also indispensable tools for communication of research results. Use of electronic processing facilities is now the norm in plant breeding programmes, though many poorly funded cassava programmes have only the barest minimum of computing facilities. In any case, this continuing advance will mean that recent, but not necessarily cutting edge, information management technology will be comparatively inexpensive and broadly available.

Breeders make considerable use of codes and rating scales that need careful interpretation to be understood by others. These shortcuts are necessary to enable efficient data-processing, but for effective communication, all the data taken and all the information generated from its analysis must be transparent. All codes should be clearly interpreted and a written record kept of all procedures. There are numerous examples of breeders retiring or transferring to another position, and the incoming scientist is unable to use much of the data because they had not been properly documented.

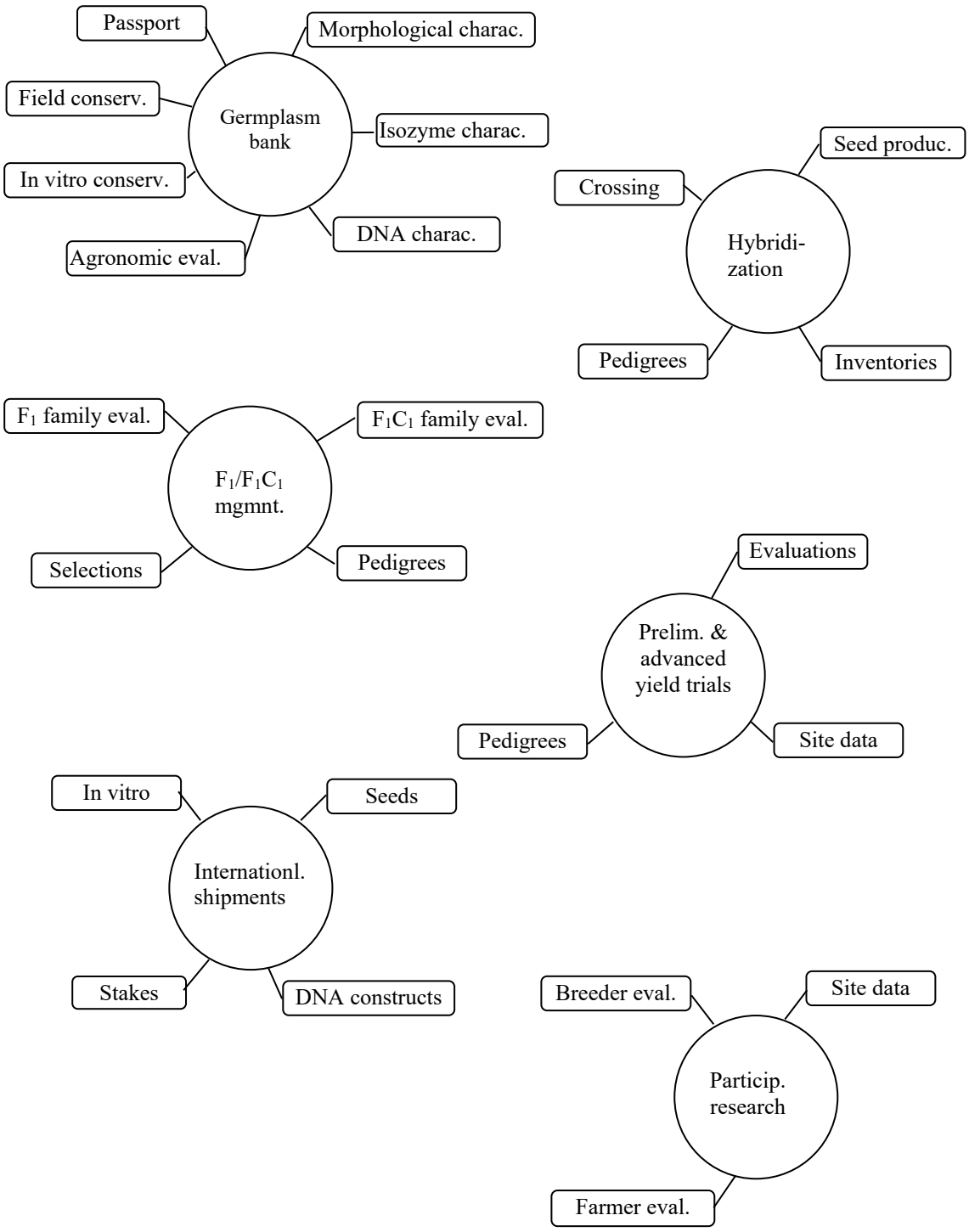
3. COMPONENTS OF INFORMATION MANAGEMENT

3.1 PLANNING

Few programmes have the luxury of planning a comprehensive information management system from the outset. It often evolves by bits and pieces over time, into a less-than-optimum system. As breeders generally collect massive amounts of data, changes in the way those data are handled can signify considerable time and energy inputs. One has to justify the time required for planning and developing a new system by the gains in efficiency and time saved later.

The breeder should develop a comprehensive plan for all the management areas within the varietal development system. The characteristics of these areas will vary widely from one programme to another. Figure 8.1 illustrates the components of a scheme that might be managed by an international programme, or a large national programme. Linking all the management areas in a comprehensive information management system requires considerable coordination and collaboration, especially when several scientists are in charge of the various areas, and more so when different institutions are involved. One of the main requirements of integrating the various components is the appropriate coding of experiments and genetic materials. This is the basic information that allows linkages throughout the information system.

Figure 8.1 Schematic representation of breeding management areas and subareas for design of an information management system[a]



[a]*All areas and subareas are integrated and linked together in the information management system*.

Most breeders will dedicate the majority of their resources to managing selection in a series of trials, with the goal of identifying new varieties to recommend to farmers. Figure 8.2 illustrates how information management for such a plan might look. This example shows three broad types of evaluations, based on the stage of selection and the breeder's choice of emphasis to give to different varietal traits. There is a nearly infinite number of variations on this example. Subsequent chapters give more detail on selection criteria and breeding trial management.

Commercial software packages are available for managing breeding trial information. Some breeders find they can adequately manage information with simple spreadsheet and database programmes developed in-house. An advantage of the commercial packages is that they usually integrate capabilities for trial design (e.g. plot randomization), fieldbook generation, data analysis and data archiving. This integration across the different facets of information management can contribute immensely to overall programme efficiency. Flexibility of programmes for modification over time and broad compatibility with software used by other breeders, should be considered.

3.2 MANAGING ORIGINAL DATA

Breeders normally manage original (unprocessed) data within two broad categories: in fieldbooks or data loggers, and in office files. Both may be either in digitized (computer-managed) or hard-copy form, or some combination of these. As so many of a breeder's activities revolve around data collection and processing, it is worth having a very well thought-out strategy.

3.2.1 Trial descriptions

A complete, standardized description of all trials should be recorded and filed. This information should be kept both in office files and in fieldbooks. These register the type of trial, materials included, location, date of planting and basic agronomic practices.

A coding system for naming trials can be a very useful organizational tool. This might include codes for the institution responsible, type of trial, year of planting or, simply be a consecutive sequence spanning years and locations. These codes are especially helpful as a means for computer programmes to link information.

3.2.2 Data logging

A great deal of a breeder's time is spent with fieldbook in hand, observing trials and recording data. The fieldbook is a central tool for management of a breeding programme. Personal taste plays a large role in what sort of fieldbook is most appropriate for a given breeder, but there are also many utilitarian considerations. Figures 8.3a to 8.3e are examples of fieldbook sheets that can be adapted to the needs of specific programmes.

Ease of use. The essence of a fieldbook is to facilitate recording and maintaining accurate records. The format should allow easy registering of data and observations, with rows and columns clearly marked for respective data. Size and design are important for ease of use. Usually a book has to be held in one hand while taking notes with the other, which means it cannot be very large. A format no larger than standard 21.7 x 28 cm should be used, and a smaller size is probably more convenient. The binding should allow insertion and removal of pages, and for the fieldbook to open nearly flat for ease of data entry and transcription.

Figure 8.2 Example of data profile for a complete range of breeding trials[a]

F_1 and F_1 C_1	F	F		F				F				F								F				F	F
Single row trial	A	A		A	A	A	A	A	A	A								S	S	S	S	S		A	A
Preliminary yield trial	A	A	A	A	A	A	A	A	A	A	S	S	S	S	S	S	S	A	A	A	A	S	S	A	A
Advanced yield trial	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	Germination	Initial vigour	Flowering	Pest and disease incidence	Lodging	Plant height	Height of first branch	Levels of branching	Leaf retention	Number of planting stakes produced	Ease of harvest	Root length	Root neck length	External root colour	Root flesh colour	Root form	Root constrictions	Number of commercial roots	Percent of rotted roots	Root yield	Leaf and stem yield	Root specific gravity	Root cyanogenic potential	General evaluation: shoots	General evaluation: roots

[a] *Based loosely on CIAT trials; evaluation criteria and breeding trial design are dynamic, and this table is given as an example only*

F = Evaluation on a family basis

S = Evaluation of pre-selected clones only

A = Evaluation of all clones in the trial (selected and discarded)

Figure 8.3a Fieldbook – trial description

Figure 8.3a. Fieldbook - trial description.

TRIAL TYPE

01 -	GERMPLASM BANK	07 -	PRELIMINARY YIELD TRIAL
02 -	POLYCROSS	08 -	ADVANCED YIELD TRIAL
03 -	CONTROLLED CROSS	09 -	REGIONAL TRIAL
04 -	F_1	10 -	MULTIPLICATION
05 -	F_1C_1	11 -	PARTICIPATORY RESEARCH
06 -	SINGLE ROW TRIAL	12 -	OTHER

SITE

01 -	PALMIRA	07 -	CARIMAGUA
02 -	QUILICHAO	08 -	POPAYAN
03 -	MEDIA LUNA	09 -	FLORENCIA
04 -	CARIBIA	10 -	EL CARMEN
05 -	FONSECA	11 -	PIVIJAY
06 -	VALLEDUPAR	12 -	OTHER

Figure 8.3b Fieldbook – evaluations during the growing season

Figure 8.3b. Fieldbook - evaluations during the growing season.

PROJECT: ____________________

PROJECT CODE [ ][ ][ ][ ] (1 2 3 4) YEAR [ ][ ] (5 6) FORM NO. [2] (7)

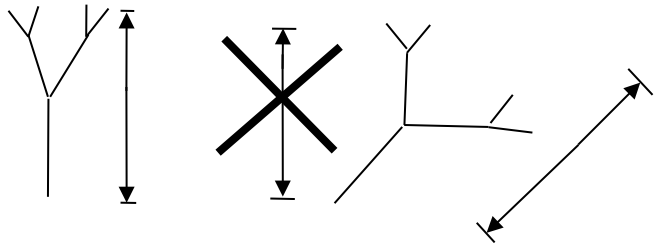
PLOT				CLONE CODE													ENTRY				REPL		NO. SPROUTED		VIGOUR	FLOWERING	PLANT HEIGHT			FIRST BRANCH HEIGHT			BRNCH LVL	STEM LENGTH W/ LEAVES			STAKES PER PLANT		LODGING	ADDITIONAL EVALUATIONS														
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62

Figure 8.3c Fieldbook – descriptors for Form 2 (Fig. 8.3b)

Early vigour
1. Very low
2. Low
3. Medium
4. High
5. Very high

Flowering
0. None
1. Sparse
2. Moderate
3. Abundant

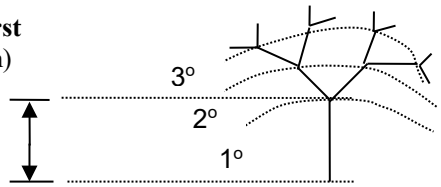
Plant height (cm)



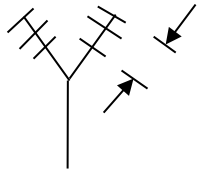
(Upright plant) (Lodged plant)

Branching levels

Height of first Branch (cm)



Canopy length (cm)



No. of stakes per plant (good quality and 20 cm long)

Lodging
1. None or very little
2. Intermediate
3. High

Pest and disease damage
1. No, or very few, visible symptoms
5. Severe damage

Figure 8.3d Fieldbook – evaluations at harvest

Figure 8.3d. Fieldbook - evaluations at harvest.

PROJECT: _____

PROJECT CODE (1 2 3 4) YEAR (5 6) FORM NO. 3 (7)

8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
	PLOT			PLANTS HARVESTD			HARV EASE	ROOT LNGTH	PED LNGTH	ROOT CLR	FLSH	ROOT FORM	CONSTRIC	FOL. EVAL	ROOT EVAL	NO. COMMER. ROOTS			ROTTED ROOTS (%)		SHOOT WEIGHT (KG)				ROOT WEIGHT (KG)				HCN	SELECT	DRY MATTER (%)			SEL ZONE 1	SEL ZONE 2	SEL ZONE 3	ADDITIONAL EVALUATIONS																			
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62

Field 8.3e Fieldbook – descriptors for Form 3 (Fig. 8.3d)

Ease of harvest
1. Easy
2. Intermediate
3. Difficult

Root length
1. Short
2. Medium
3. Long

Peduncle (neck) length
1. Short
2. Intermediate
3. Long

Root surface color
1. Light
2. Light brown
3. Dark brown

Flesh color
1. White
2. Cream or light yellow
3. Yellow
4. Deep yellow to orange

Root form

1. Conical 

2. Conical-cylindrical 

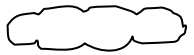
3. Cylindrical 

4. Irregular 

Root constrictions

1. None or few 

2. Intermediate 

3. Many 

General root or shoot (foliage) evaluation
1. Excellent
2. Good
3. Fair
4. Poor
5. Very poor

Cortex pigmentation
1. None
2. Slight
3. Intermediate
4. Intense

Formula for determining percent root dry matter content:

158.3 x [weight in air /(weight in air – weight in water)] – 142

Comprehensiveness. The fieldbook should contain all the information the breeder needs to know when evaluating a trial. At a minimum, this includes a field map, basic information about the site (soil analysis, agronomic practices) and a list of materials and their plot numbers. It is often useful to have some background information on the materials in the trial, especially pedigrees and a summary of performance in previous trials. However, unless this information can be computer-generated, it may not be worth the extensive effort that would be required to include it in the fieldbook manually. An alternative could be to carry separately the summarized previous years' evaluations for consultation as needed.

Clarity. Organization of a fieldbook should be sufficiently clear for use by a number of different people, either for taking or interpreting data. Formats for entering data, basic explanation of rating scales and other measures should be included in the fieldbook itself for ease of reference. Rows and columns should be easy to follow horizontally and vertically so that errors in placing data are minimized. Clarity can be improved by using coloured ink, such as green or blue, for printing fieldbooks, thus making data entered in pencil clearly visible.

Standardization. Effective communication of results requires the use of certain standards that everyone can understand. Individual breeders may have strong feelings about the best way to take certain types of observations, and that needs to be respected. For the most part, however, following internationally accepted standards is beneficial to everyone. Uniformity throughout the years and sites is necessary for comparing data among trials at any level, local, regional or international. At the second network meeting of Latin American cassava breeders in 1990, participants agreed on a series of standards for information management. These may be a useful model also for programmes on other continents (Table 8.1).

Flexibility. The flip side of standardization is flexibility. The information needs of most research programmes change with time. Usually, if enough thought goes into the original fieldbook design, such changes will be infrequent, and easily incorporated.

Durability. Cassava is a long-season crop, and consequently fieldbooks may need to be more durable than those used for short-season crops. The breeders need to take into account likely exposure to the elements. In high rainfall regions, for example, some degree of water-proofing may be helpful. Durable covers and heavy-weight pages that will tolerate some abuse are needed. All data should be taken in pencil. Ink pens should never be used because they are difficult to correct, and more importantly, can smudge if wet.

Fieldbooks are the main vehicle for recording and conserving raw data. They should become a permanent part of a breeder's archives. Probably, after data are transcribed and analysed, they will rarely if ever be consulted, but they should always be available if needed.

Electronic data recorders may be an alternative to printed books for some programmes, for digitizing data directly in the field and for preliminary analysis. The major advantages are elimination of the need for transcription of data and ability to store and recall considerable background data on breeding lines in the field. Disadvantages may be initial cost, potential for higher error rate (easier to punch a wrong key than to write the incorrect number long-hand) and need for trained technicians for up-loading trial design information and down-loading evaluations. There are possibilities for combining both manual and electronic systems and many programmes that use data loggers probably will, initially at least, make this choice. As more people become familiar with the use of these devices and they become more user-friendly, more powerful and less expensive, there is little doubt that they will gain in popularity.

Table 8.1 Minimum data suggested by the Pan-American Cassava Breeders' Network, for describing performance of clones in advanced evaluation trials

1. **Site data**
   - site name
   - latitude
   - longitude
   - altitude
   - chemical and physical soil analysis
   - monthly precipitation
   - monthly mean, mean minimum and mean maximum temperatures
   - other special conditions

2. **Cultural practices**
   - soil preparation
   - fertilization
   - former crop
   - incidence of pests and diseases
   - pest and disease control
   - planting and harvest dates
   - weed control
   - irrigation
   - planting system
   - plot size and design
   - spacing
   - experimental design
   - other locally relevant data

3. **Clone performance**
   - plant height (cm)
   - levels of branching
   - root yield (tonnes/ha)
   - harvest index
   - root dry matter (percent)
   - root starch content (percent)
   - cyanogenic potential (quantitative or semi-quantitative; one to nine scale for latter)
   - eating quality (where relevant for market; one to five scale)

4. **Comparative statistics**
   - trial means
   - check means
   - least significant difference at 5 percent (LSD 5 percent)
   - coefficient of variation (CV)

Note: It is recommended, in the case of Latin America, to use the standard check *Mantiqueira* (syn.: CMC 40, MCol 1468, *Manihoica P-11*), along with other local checks.

Source: Adapted from Iglesias and Fukuda (1992)

3.3 DATA STORAGE

Data storage involves concepts of security, economy and accessibility. The need for secure data storage is obvious. The necessary degree of security will depend on the specific situation of each programme. Secure storage is often best achieved by having a duplicate of all data kept in different locations (sites or buildings). In particularly high-risk situations, such as impending social turmoil, duplicates should be kept outside the at-risk region. Duplicates of original data should not be stored in the same room, so that any localized catastrophe would not destroy both copies. Raw data will usually be maintained in original fieldbooks, archived to become a permanent record. These data need to be transcribed, either electronically or manually, and this can be the second copy of original data. In addition, various types of analyses may be stored. Cost of information storage, at the level generated by a breeding programme, is not likely to be a major constraint. Storage in various forms of digital media (disk, tape, flash memory) is very inexpensive, and storage in hardcopy form is a matter of having some shelf space.

Accessibility depends primarily upon having files, and all the information they contain, clearly labelled and described. The breeder should abide by the principle that any file and all the information in it should be accessible to, and understood by, someone who does not have inside knowledge of the programme. This is not to say that everyone should be granted access to data files. In the case of departure of a breeder from a programme, all the information he/she leaves behind should be accessible to and understood by a successor. Loss of information during transition between breeders is all too common. Breeders should give adequate thought to organizing archival data in a manner that makes it easily accessible and clearly understood for years to come.

3.4 DATA PROCESSING

3.4.1 Verification

Each step of data processing involves the possibility for introduction of error. Errors can occur in a number of ways, some of the most common being: misreading or misunderstanding a measurement, placing data in the wrong position in a fieldbook and transcription errors while passing data from fieldbook to another form. The best way to control errors is to limit their introduction in the first place. Most errors are avoidable. A breeder has a personal responsibility to be extremely careful while working with data and to instil the same passion for accuracy among all collaborating personnel.

Some level of data verification is helpful after each step of processing. The type of verification will depend upon the format in which data are held and a knowledge of the types of errors commonly found in that particular format. Fieldbooks can be scanned visually for errors in column placement and large inconsistencies in magnitude. Ideally, this should be done after each evaluation, so that errors can be corrected at the appropriate time. For example, if one notices a yield recorded as 120 kg when all neighbouring plots are between 5 and 15 kg, there is high probability of error. If the data can be verified in some way, it should be corrected; if there are strong doubts, it is best to leave a data point as missing.

If fieldbooks are transcribed to electronic media, the transcription should be verified. The most accurate procedure is to transcribe the same data twice (by two different people) and electronically compare duplicate files. The assumption is that the same input error on the same data point in both files by two different people is highly unlikely. If inconsistencies are found, they can be checked against original data. Electronic verification was more common when specialized data keypunchers did most of the transcription from fieldbooks. This system of verification seems to be used rarely by cassava breeders, as more scientists enter their own data on computers. Of course, if data are entered electronically in the field, there is no need for transcription, and one possible step for introducing errors is eliminated. Many scientists do not systemize the search for errors in data, but it can be a very effective practice if it is designed efficiently.

Data that fall outside a normal or expected range of values can often be searched by introducing procedures into the computer data management software. Such a programme can scan data and note any points that fall outside predetermined limits. For example, one may determine that dry matter content rarely goes above 40 percent or below 15 percent, and thus programme a search for values not fitting

these criteria. Any suspect value need not automatically be discarded, but would alert one to recheck original data. A less complex approach to reach the same objective could be to order data independently by various criteria. A quick scan of high and low values will show if any are unrealistic and need to be reconfirmed.

3.4.2 Transformation

Data transformation may follow a decision based on statistical parameters, done usually for the purpose of validating the application of a given statistical procedure to data that, in their original form, do not meet the necessary assumptions. Most statistical analysis software has transformation routines incorporated. As many types of transformations are mathematically quite simple, they can also be carried out manually.

3.4.3 Analysis

This discussion will only point out a few principles especially relevant to cassava breeders. Most breeding programmes use fairly simple statistical designs in the routine selection trials. They are normally adequate for the types of comparisons among genotype performance in which the breeder is interested. Randomized complete blocks and lattice designs are probably the most common. In the early stages of selection, a given genotype may be represented by a single plant or a single plot without replication.

Given that the same design is usually used over sites and years, there may be possibilities for automating analyses, i.e. programming many of the parameters that are repeated year after year in order to make analysis more rapid and efficient. Standardizing analysis and report generation not only saves time but makes comparisons across trials much easier.

3.4.4 Communication

Breeders commonly generate vast quantities of data for internal use, and only a small proportion is used for eventual publication or distribution to other scientists. For refereed journals, standards for data reporting are generally quite specific. The following comments are oriented more toward data organization and reporting for internal institutional use, or for informal reporting among a network of breeders.

Breeders commonly report each parameter in a separate table, one each for yield, dry matter, pest and disease reactions, and so on. Perhaps this produces an impressive number of tables, but it does make data interpretation very difficult. Usually one wants to make comparisons among genotypes based on the full range of traits, and the easiest way to do this is to create two-way tables, listing entries as rows and evaluations of a range of traits as columns. Of course, for specialized reporting needs, other forms of presentation are appropriate.

As with analysis, reporting across sites and years is most efficient and much more easily interpreted when standardized. Use of standard units, preferably based on internationally agreed-upon conventions, will greatly facilitate interpretation in the literature (see for example Tables 8.2a and 8.2b). Another practice that makes data easier to view is to standardize the number of significant digits. As a rule of thumb, no more significant digits should be presented than were recorded in the original data, or what is a common-sense value. For example, when yields are extrapolated from small plots to tonnes/ha, there is rarely justification for including more than one decimal point. The common habit of directly transferring results from computer-generated lists that have four or more decimal points should be avoided, not only because it is misleading, but because it makes tables of data unnecessarily complex.

Table 8.2a Example of an easy-to-read summary of trial results

Clone	Yield (tonnes/ha)	Root DM (%)	Mite damage rating (1-5)	CBB damage rating (1-5)	Plant height (cm)	Harvest index
MCol 22	24.6	34.3	2.0	4.6	143	0.58
CM 3616-4	33.6	33.6	3.1	2.8	204	0.48
CM 4312-8	38.1	32.3	3.1	2.7	173	0.61
CM 8214-3	37.8	35.0	2.4	3.3	186	0.53
SM 849-2	40.5	32.1	2.6	2.4	203	0.43
SM 1637-12	36.1	29.7	2.9	3.0	190	0.53
Secundina (local check)	21.3	36.3	2.3	3.8	185	0.43
Trial mean (35 clones)	29.4	33.2	3.3	3.4	192	0.51
LSD (0.05)	5.8	3.2	0.6	0.8	20	0.05

Table 8.2b Example of a difficult-to-read summary of trial results

Clone	Yield[a]	Root DM[b]	Mites[c]	CBB[c]	Plant height[d]	Harvest index
MCol 22	24.635	34.311	2.088	4.686	143.78	0.582
CM 3616-4	33.621	33.623	3.178	2.821	204.54	0.483
CM 4312-8	38.153	32.354	3.165	2.728	173.93	0.618
CM 8214-3	37.883	35.065	2.476	3.360	186.86	0.534
SM 849-2	40.529	32.194	2.626	2.474	203.58	0.439
SM 1637-12	36.187	29.738	2.964	3.076	190.34	0.535
Secundina[e]	21.349	36.322	2.304	3.870	185.14	0.435
Trial mean (35 clones)	29.433	33.283	3.483	3.451	192.80	0.517
LSD (0.05)	5.833	3.264	0.676	0.867	20.78	0.052

[a] *Tonnes/ha*
[b] *Percent dry matter*
[c] *1-5 scale (1 = zero or very low damage; 5 = severe damage)*
[d] *Centimetres*
[e] *Local check variety*

Chapter 9. Introduction to quantitative genetics[2]

2 Contributed by Hernán Ceballos

1. INTRODUCTION

Basic knowledge about the inheritance of traits is fundamental for efficient and effective crop genetic improvement. Most economically important characteristics are controlled by many genes and strongly affected by the environment. However, only a few articles relative to the inheritance of quantitative traits in cassava have been published (Cach *et al*., 2005; Easwari *et al*., 1995; Easwari and Sheela, 1998; Losada, 1990; Perez *et al*., 2005; Perez *et al*., in press). Cassava's situation is unique in that while a molecular map has already been developed (Fregene *et al*., 1997; Mba *et al*., 2001), knowledge of traditional genetics lags considerably behind.

2. BASIC CONCEPTS OF QUANTITATIVE GENETICS

In the analysis of genetic variation, the pioneering research by Mendel focused on traits that segregated in contrasting classes (i.e. tall versus dwarf, purple versus white flowers, etc.). In fact, it was the sharp and distinctive phenotypic classes observed in these traits that helped Mendel to reach his breakthrough discoveries. The inheritance of these traits, identified as qualitative, is easy to study and predict because of the large effect of different alleles on the phenotype, which results in distinctive phenotypic classes, and because of the negligible effect of the environment on their expression.

There is, however, additional variation that was not originally addressed by Mendel, which is certainly less obvious and refers, for example, to the differences in plant height within the normal-height plants, or within the dwarf ones. This kind of variation does not result in clearly distinguishable classes but in a continuous variation between the extreme phenotypes and is, therefore, called quantitative. Quantitative traits are controlled by several genes (in this context several may mean as few as five genes, but generally refers to many more). The effect of the information contained at each locus, on the phenotype, is relatively small and therefore, it is difficult to track individual alleles in segregating progenies. In addition, the environment frequently affects the expression of quantitative traits. It is important to emphasize that quantitative trait alleles are inherited and segregate according to Mendel's laws. The difference is that their individual segregation cannot be tracked based on the phenotypes.

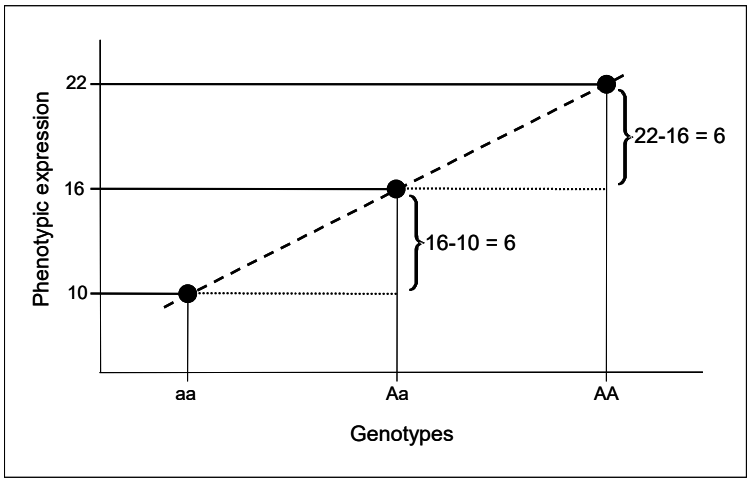
The analysis of the inheritance of qualitative traits is relatively simple, with obvious, clearly distinguishable contrasting phenotypes and negligible interaction with the environment. These traits are typically analysed by determining the segregation ratios of the two or three classes that, for example, a single gene inheritance typically determines. On the other hand, understanding the mechanisms behind quantitative inheritance is much more complex, because: the segregation of individual alleles cannot be properly tracked, there are a large number of genes involved, there are interactions within and between loci and the environment confounds the expression of the trait under study.

G.V. Yule (1906), E.M. East (1908) and G.H. Shull (1909) first developed the principles of quantitative genetics in the early 1900s, at the dawn of the age of modern plant breeding. R.A. Fisher (1918) and S. Wright (1921) were key scientists to incorporate some of the new information on gene behaviour early in the twentieth century. In the ensuing years many scientists added to the understanding of quantitative genetics: Comstock (1952); Comstock and Robinson (1948); Falconer (1981); Hallauer and Miranda (1988); Hayman and Mather (1955); Lynch and Walsh (1998); Mather and Jinks (1977); and Vencovsky and Barriga (1992). According to Lynch and Walsh (1988), the impact of the early quantitative genetics theory profoundly influenced the evolution of modern theoretical and applied statistics, facilitating development of the theory behind regression and correlation analyses and the principles upon which the analysis of variance is based. A brief description follows of the most important concepts of quantitative genetics in relation to plant breeding.

3. ADDITIVE, DOMINANCE AND OVER-DOMINANCE EFFECTS IN SINGLE-GENE INHERITANCE

Figure 9.1 illustrates a hypothetical model of different types of gene action, where there are three possible genotypes at a given locus, and their respective phenotypes. The homozygous genotypes are identified as **aa** and **AA,** and the heterozygote as **Aa**. The phenotype of the heterozygous genotype (**Aa** = 16) is exactly halfway between the two phenotypic values defined by the homozygotes (**aa** = 10 and **AA** = 22). The mode of inheritance depicted in Figure 1 is called additive. In our hypothetical situation, each dose of an "**A**" allele will add six units to the phenotypic expression of the trait. Hence the shift from genotype **aa** to **Aa** resulted in their respective phenotypes increasing from 10 to 16, and shifting from genotype **Aa** to **AA** also resulted in a phenotypic increase of six units.

Figure 9.1 A hypothetical case of the relationship between genotypes and phenotypes for a trait whose inheritance is fully due to additive effects



The situation illustrated in Figure 9.2 is similar to that shown in Figure 9.1 for the two homozygotes. The phenotypic value for **aa** is 10 and that of **AA** is 22. The phenotypic expression of the heterozygote (**Aa**), however, is identical to that of the homozygote **AA**. In the heterozygote, the allele **A** exerts a dominance over allele **a**, and therefore, genotypes **Aa** and **AA** express the same phenotypes. This is the typical situation analysed by Mendel in his pioneering work and is known as complete dominance. The dominance can be exerted either by the allele that increases the expression of the character or by the one that reduces it. The hypothetical model in Figure 9.2 showed **A** dominating over **a**, but the opposite situation could have been chosen without affecting the conclusions.

The difference between actual and expressed value of the heterozygote, and the expected value in the additive model, is called the dominance deviation (Figure 9.2). Finally, Figure 9.3 illustrates another situation frequently observed in nature. In this case, the trait shows overdominance. The overdominance (or transgressive) inheritance is characterized by a heterozygote with a phenotype outside the range of variation defined by the two homozygotes. In this example, the range of variation defined by the two homozygotes was between 10 and 22, and the phenotype of the heterozygote was 25. Depending upon the trait, the overdominance can result in the phenotype of the heterozygote to be above or below the range of variation observed in the homozygotes. Overdominance plays an important role in the heterosis or hybrid vigour shown by many crops, including cassava.

Figure 9.2 A hypothetical case of the relationship between genotypes and phenotypes for a trait whose inheritance shows complete dominance of the allele (A) that increases the expression of the trait

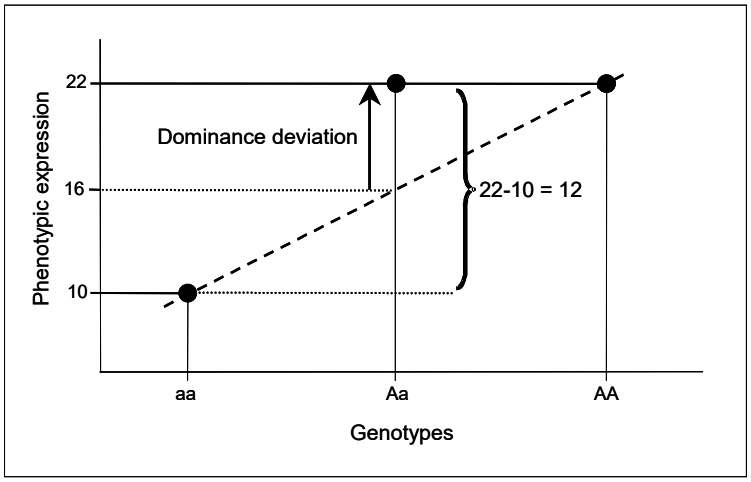
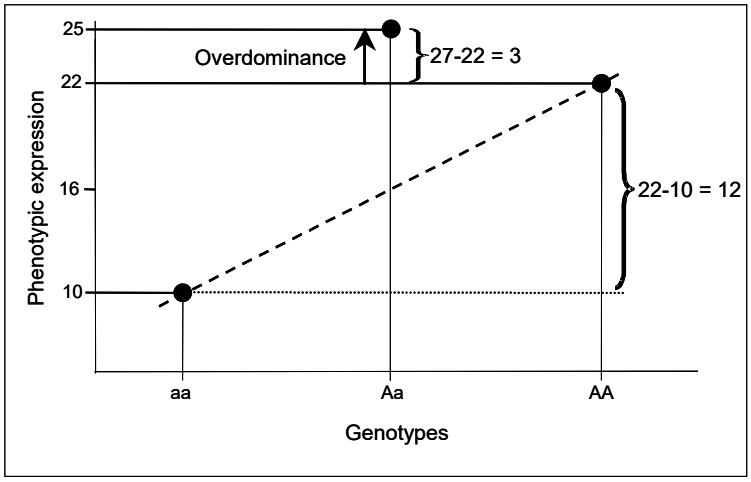


Figure 9.3 A hypothetical case of the relationship between genotypes and phenotypes for a trait whose inheritance shows overdominance of the allele that increases the expression of the trait (allele A)



4. ADDITIVE, DOMINANCE AND OVERDOMINANCE EFFECTS IN A TRAIT CONTROLLED BY SEVERAL GENES

In the phenotypic expression of quantitative traits several genes segregate simultaneously and each of them may show any of the gene actions illustrated in Figures 9.1 to 9.3. In addition, a confounding effect can occur when the allele that reduces trait expression is dominant in one locus, while in other loci the opposite is true. Quantitative genetic analysis evolved with the purpose of explaining this type of situation, where:

- several to many genes are involved in the expression of a trait;
- there are no clearly distinguishable phenotypic classes;
- there is strong genotype-by-environment interaction.

It soon becomes evident that the involvement of more than one gene in the expression of a given trait greatly complicates the analysis. Allard (1960) provided the example presented in Table 9.1. In the first model presented (additive), the phenotypic expression is defined by the number of capital letters present in the genotype (capital letters representing the allele that increases the phenotypic expression of the character). The contribution of **A** is slightly higher than that of **B**. It should be apparent that this model is very simple and, to a large extent, predictable. A key feature of the additive model presented in Table 9.1 is that the substitution of one allele by another results in predictable increases or decreases in the phenotype and this is true regardless of the other genes present. In every case, when one allele **A** replaces another allele **a,** the phenotype increases by two units. Similarly, when one allele **B** replaces **b**, the phenotype increases one unit, regardless of the status in the locus **A/a**. Two important properties of this model are:

- the effect of replacing **a** by **A** (or **b** by **B)** is the same regardless if that happens in the homozygote or in the heterozygote. Dominance effects, therefore, are absent;
- the effect of replacing **a** by **A** (or **b** by **B**) is the same regardless of the status of the other locus. There is no interaction among loci, i.e. epistatic effects are absent.

Model II from Table 9.1 illustrates the typical case of two dominant genes. Genotypes **AA** and **Aa** have the same phenotype, in contrast with that of **aa**. The same can be seen with genotypes **BB** and **Bb**, whose phenotypes are identical but differ from that of **bb.** Although the model introduces some changes in relation to the simple additive model, the relationship between genotype and phenotype is still relatively simple and predictable:

- the effect of replacing **a** by **A** (or **b** by **B**) is different depending on the circumstances. If the replacement occurs from **aa** to **Aa** (or from **bb** to **Bb**) there is a drastic effect on the phenotype. If the replacement occurs from **Aa** to **AA** (or from **Bb** to **BB**), on the other hand, there is no effect;
- the effect of replacing alleles in locus **A/a** or in locus **B/b** is the same regardless of the status of the other loci. Epistatic effects, therefore, are still absent.

Model III in Table 9.1 introduces an additional complexity. There are only two phenotypes possible: those that have at least one capital letter allele at each of the two loci and those that have capital letter alleles at one or no locus. In this model the individual effect of alleles present in locus **A/a** cannot be determined unless there is information about the status of locus **B/b**. This is the typical case of complementary gene action, which is one of the simplest epistatic effects observed in nature. In spite of the dependency of the genotype at one locus on other loci, the relationship between phenotype and genotype is still relatively simple and predictable.

The complications derived from epistatic effects are more clearly illustrated in Model IV from Table 9.1. The first column for Model IV (**BB** in every case) illustrates full dominance of **A** for the different allelic combinations for locus **A/a**. The second column (the other locus always **Bb**), however, shows overdominance with the heterozygote **Aa** having a higher phenotypic expression than either homozygote. Finally, the third column (**bb** is common in the three genotypes) illustrates full dominance of **a,** for the different allelic combinations for locus **A/a**.

Table 9.1 Alternative hypothetical models for the segregation at two loci (adapted from Allard, 1960)[a]

I. Additive model				**II. Dominance model**			
AABB	AABb	AAbb	**AA--**	AABB	AABb	AAbb	**AA--**
7	6	5	**6**	4	4	2	**3½**
AaBB	AaBb	Aabb	**Aa--**	AaBB	AaBb	Aabb	**Aa--**
5	4	3	**4**	4	4	2	**3½**
AaBB	aaBb	aabb	**aa--**	aaBB	aaBb	aabb	**aa--**
3	2	1	**2**	3	3	1	**2½**
--BB	**--Bb**	**--bb**		**--BB**	**--Bb**	**--bb**	
5	**4**	**3**		**3¾**	**3¾**	**1¾**	
III. Complementary epistasis				**IV. Complex epistatis**			
AABB	AABb	AAbb	**AA--**	AABB	AABb	AAbb	**AA--**
3	3	1	**2½**	4	2	3	**2¾**
AaBB	AaBb	Aabb	**Aa--**	AaBB	AaBb	Aabb	**Aa--**
3	3	1	**2½**	4	3	1	**2¾**
AaBB	aaBb	aabb	**aa--**	aaBB	aaBb	aabb	**aa--**
1	1	1	**1**	3	2	1	**2**
--BB	**--Bb**	**--bb**		**--BB**	**--Bb**	**--bb**	
2½	**2½**	**1**		**3¾**	**2½**	**1½**	

[a]*Numbers indicate the genotypic value for each genotype. The border rows and columns represent the mean genotypic values for the three conditions possible at each locus (assuming a gene frequency of ½ at each locus)*

Segregation at locus **B/b** (when state of locus **A/a** is constant**)** results in a different set of reactions. The first row always has genotypes **AA--**, and in this case segregation at locus **B/b** shows underdominance. In the second row (all genotypes **Aa--**), segregation at the **B/b** locus reveals partial dominance and in the third row (all genotypes **aa--**) gene action for locus **B/b** is completely additive. Many examples of epistatic relationships between genes in different loci have been reported. An interesting review, which is relevant to cassava, illustrates the complexities derived from the interaction among different genes involved in the synthesis of the two polymers present in starch: amylose and amylopectin (Jobling, 2004).

The situations illustrated above led Fisher (1918) to propose the three main gene action effects that are the subject of quantitative genetics studies:

- additive variance or effects were initially defined as the differences between the homozygotes, but in genetic designs are generally related to the breeding value of an individual, which is described below;
- dominance variance or effects, are basically derived by the interactions among alleles in the same locus (intra-allelic interaction);
- epistasis variance or effects, are associated with interactions among alleles at different loci (inter-allelic interaction).

These gene actions can then be summarized in a classical formula for partitioning genetic variance in its components as follows:

$\sigma^2_G = \sigma^2_A + \sigma^2_D + \sigma^2_{AA} + \sigma^2_{AD} + \sigma^2_{DD} + \sigma^2_{AAA} + \sigma^2_{AAD} + \sigma^2_{ADD} + \sigma^2_{DDD} + \ldots$

where:

σ^2_G = Total genetic variance

σ^2_A = Additive genetic variance (associated with breeding value)

σ^2_D = Dominance genetic variance

σ^2_{AA} = Digenic epistatic variance between additive effects

σ^2_{AD} = Digenic epistatic variance between additive and dominance effects

σ^2_{DD} = Digenic epistatic variance between dominance effects

σ^2_{AAA}, σ^2_{AAD}, σ^2_{ADD} and σ^2_{DDD} = Trigenic epistatic variances among different effects.

5. ADDITIVE EFFECTS, GENERAL COMBINING ABILITY AND BREEDING VALUE

A major constraint in the study of quantitative genetics is the impossibility of tracking individual alleles. As complete as the model developed by Fisher was, it remained a theoretical development with little practical relevance until the concepts of breeding value and average effect of gene substitution, were defined (Falconer, 1981).

The average effect of gene substitution is closely associated with the additive model. In the first example of Table 9.1 (additive model) the average effect for the gene substitution of **a** by **A** is two units. In effect, replacing one **a** allele by the **A** allele in the homozygote **aa--**, results in the heterozygote whose average phenotypic value shifts from 2 to 4 (see right column for that model). Similarly, replacing the **a** allele in the heterozygote **Aa--** by an **A** allele, results in the **AA--** homozygote and a phenotypic increase, again, of two units (from 4 to 6). For locus **B/b** the same trend can be observed, with the only difference that allele substitutions result in smaller phenotypic changes (one unit for each allelic substitution).

If all the genes affecting a quantitative trait were considered, the average effects of all the alleles present in a given progenitor would determine the mean genotypic value of its progeny (Falconer, 1981), which is directly related to the concept of breeding value. The breeding value of a given progenitor is defined by the average effects of the alleles it possesses and relates to a clearly defined reference population.

It should be clear that the average effects of the many alleles involved in the inheritance of a given trait cannot be measured. On the other hand, the breeding value of that progenitor can in fact be measured through the relative performance of the progeny it produces. In practical terms the breeding value of an individual is related to a better-known parameter, general combining ability.

When a given individual is randomly crossed with a large number of mates from the same reference population, the breeding value of that individual will be twice the average deviation of its progeny from the population mean. The logic behind this relationship is that this individual contributes with only half of the gametes of its progeny, the other half of the gametes being produced by the mates it crosses with. The variation in breeding values has been associated with the additive effects of genes, as described above, although strictly speaking they are not the same. A major advantage of the breeding value is that it can actually be measured.

By the middle of the twentieth century, breeders and geneticists had established most of the principles of quantitative genetics. Many different publications demonstrated that, if certain conditions were met, the genetic variation in a given population could be partitioned into its additive and dominance components using different family structures. One major limitation in these studies is the frequent assumption that epistasis (interaction between different loci) is negligible.

Several genetic designs have been developed to measure the relative importance of additivity, dominance and epistasis in the expression of different traits, in view of the information provided in Table 9.2. Generation mean analysis (Mather and Jinks, 1977) is a design favoured by breeders and geneticists working with self-pollinated species. Diallel crosses and North Carolina Designs I and II are the most common approaches used in allogamous crops (Hallauer and Miranda, 1988). Regardless of the kind of study, a common assumption in most of these studies is the absence of epistasis in the expression of the traits analysed. Depending on whether genetic effects are considered fixed (Model I) or random (Model II), the studies will focus on effects or variances. In fixed models the parents in the genetic design are themselves the reference population; conclusions are therefore only relevant to the genotypes evaluated and cannot be extrapolated to some hypothetical larger reference population. In random models, on the other hand, the parents are a sample of genotypes from a reference population clearly defined. Results in these cases are applicable to the reference population from which the genotypes evaluated are just a random and unselected sample (Hallauer and Miranda, 1988; Steel and Torrie, 1960).

Table 9.2 Distribution of the genetic variation into its additive and dominance components in a population with different family structures (Hallauer and Miranda, 1981; Venkovsky and Barriga, 1992)

Type of family	Among families		Within families	
	σ^2_A	σ^2_D	σ^2_A	σ^2_D
Half-sib families	1/4	0	¾	0
S_1 families from half-sibs	3/8	0	5/8	0
S_2 families from half-sibs	7/16	0	9/16	0
Full-sib families	1/2	1/4	1/2	3/4
S_1/F_3	1	1/4	1/2	1/2
S_2/F_4	3/2	3/16	1/4	1/4
S_3/F_5	7/4	7/64	1/8	1/8
S_4/F_6	15/8	15/256	1/16	1/16
S_5/F_7	31/16	31/1024	1/32	1/32
S_6/F_8	63/32	63/4096	1/64	1/64
…	…	…	…	…
S_∞/F_∞	2	0	0	0

Most of the designs listed above focus on the between-family variation. The within-family variation is seldom analysed because it usually does not provide any relevant additional information. Cassava and other crops with vegetative propagation, however, have the advantage that individual genotypes can be cloned. By cloning, the within-family variation can be partitioned into its genetic and environmental components. Moreover, the interaction between genetic and environmental components of variation can also be measured. This is a decided advantage given the large proportion of the total genetic variance that generally remains in the within-family component (Table 9.2) and by doing so, the relative importance of epistasis can be measured indirectly (as will be shown later in this chapter).

Genetic studies analysing the importance of epistatic effects are not very common, particularly in annual crops. Holland (2001) published a comprehensive review on epistasis and plant breeding. Several cases of significant epistasis have been reported in self- and cross-pollinated crops. Finding significant epistasis seems to be easier in self- than in cross-pollinated species and in designs based on the contrasts of means rather than the analysis of variances (Holland, 2001). In general, however, reports on the relevance of this kind of gene action are not as frequent as those on additivity and dominance, and they have generally taken advantage of the vegetative multiplication that some species offer (Comstock *et al*., 1958; Stonecypher and McCullough, 1986; Foster and Shaw, 1988; Rönnberg-Wästljung *et al*., 1994; Rönnberg-Wästljung and Gullberg, 1999; Isik *et al*., 2003). Many of these reports are on forest trees. Due to the complexities of these analyses and the costs involved, the scarce reports in the literature on epistasis are frequently based on a limited sample of genotypes, which consequently may result in contradictory or unreliable results.

6. RELEVANCE OF THE COMPONENTS OF GENETIC VARIANCE TO CROP BREEDING

At the beginning of this chapter it was mentioned that knowledge on the inheritance of traits is fundamental for efficient and effective genetic improvement of crops. After this description of the different components that make up the total genetic variance, the implications that this information has on breeding in general and cassava in particular, is discussed.

The concept of additive effects (or variance) has been redefined so that it can be measured by the most common quantitative genetics designs and it is directly associated with general combining ability (GCA) and the breeding value of an individual when used as progenitor in a breeding nursery. Breeding value, in turn, is closely related to the mean performance of the progeny of a given parent, compared with the overall average performance across all the progenies evaluated. Additive effects are relatively simple to estimate and to improve, since most breeding schemes will properly exploit them. The main concern that a breeder should have is that enough additive variation is available for success. While it may appear to be a contradiction, complete dominance gene action can strongly influence GCA effects. In this case, however, a single dominant gene does not produce a truly quantitative segregation.

The dominance effects associated with heterosis (or hybrid vigour) are typically those in which many genes are involved in control of the trait. Epistasis and dominance are frequently grouped together and renamed as the non-additive fraction of the genetic variance. As mentioned above, dominance and epistasis represent the within- and the between-loci interactions, respectively. The successful exploitation of these non-additive effects requires a special breeding scheme. Several such schemes, known as reciprocal recurrent selection, have been developed and successfully used in maize breeding (Hallauer and Miranda, 1988; Pandey and Gardner, 1992). A common feature of the different schemes is the presence of two (or more) heterotic or reciprocal populations. The goal of reciprocal recurrent selection is to increase the performance *per se* of the populations and, more importantly, of the crosses among them. In theory these schemes tend to improve the complementarity of the two reciprocal populations, in such a way that when they are crossed the number of heterozygous loci is maximized. Heterozygosity, as explained above, is responsible for the heterosis or hybrid vigour observed in many plant species. Not all loci necessarily contribute to hybrid vigour, however, since certain regions of the genome are likely to have more influence than others.

The breeding schemes used for improving additive and non-additive traits should be different. The breeding value (or general combining ability) of a given parent depends on the genes it contains (as well as the frequency of these genes in the reference population). Additive effects depend on good genes that can be properly identified and, more importantly, transmitted to the progeny. On the other hand, non-additive effects (or variances) depend on specific gene combinations, which cannot be transmitted, as such, to the progeny through sexual reproduction. The gametes (pollen and ovules) can transmit genes but not gene combinations. Therefore, good gene combinations need to be reconstituted every time there is sexual reproduction. Reciprocal recurrent selection can exploit genetic effects that depend on gene

combinations (dominance effects within the loci and epistasis effects between loci) because it facilitates the reconstitution of desirable gene combinations after sexual recombination has taken place.

Furthermore, several reasons justify the introduction of inbreeding in the genetic improvement of cassava (Ceballos *et al*., 2004). Current breeding systems rely on the crosses among predominantly heterozygous parents. Use of inbred parents would facilitate the gradual fixation (cycle after cycle of selection) of the appropriate genes in the complementing inbreds in such a way that the consistent improvement of gene combinations becomes feasible. This is particularly true when reciprocal recurrent selection is implemented. The advantage of this system is that, once a pair of lines that combine well is identified, they can be further improved to better complement each other when crossed to produce hybrids (Cach *et al*., 2005; Ceballos *et al*., 2004; Perez *et al*., 2005). Each of the two inbred clones, for instance, can be crossed with related germplasm (only limited genetic variability is required so the good combining ability between the two lines is not jeopardized) and the segregating progenies crossed with the "reciprocal" clone to identify those that produce better hybrids.

It is critical that the inbred parents are crossed with a related germplasm to maintain many loci at the homozygous stage and generate variation in just a few loci. Otherwise the gene combinations already fixed and responsible for the good heterosis between the two "reciprocal" clones would be quickly lost. The entire process described above aims at improving the parental lines in order that when they are crossed with the reciprocal gene pool they produce an outstanding hybrid. This is the hybrid that the farmer will plant and multiply vegetatively as it is ordinarily done in cassava.

Figure 9.4 illustrates the advantages of reciprocal recurrent selection, particularly when inbred lines are involved. The process may start with the formation of two heterotic populations (A and B) that complement each other well, i.e. they produce outstanding hybrids. Parent A is crossed with a related line to produce progenies that segregate only for a restricted number of loci. Parent B is also crossed with a related line. Some of the progenies from these crosses of related lines have a better combining ability, and complement each other better than the original parental lines. As a result, the hybrid produced by the improved versions of parents A and B shows more heterozygocity and a better performance than the original hybrid. Genetic progress is more directed, consistent and predictable.

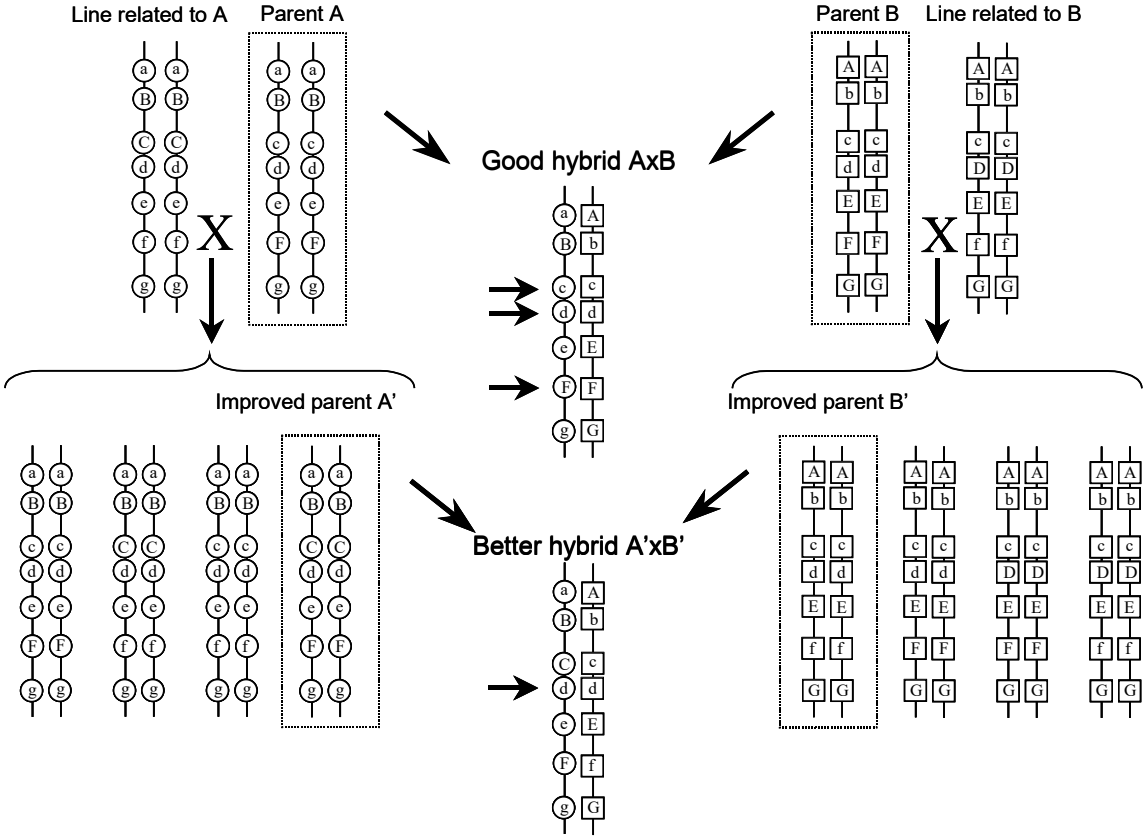
If no such case is found (where the two heterotic populations are found to complement each other), the populations can be defined based on other criteria. One approach could be to use genetic distances determined by molecular markers. Inbred lines are derived from each population. In the process, selection for good agronomic performance – for example, plant type and resistance to pests and diseases – may be exerted. The segregation may also allow for the identification of useful recessive traits, particularly for starch properties (i.e. waxy starch), nutritional characteristics (i.e. acyanogenesis), modified plant type or disease/insect resistance.

The inbred lines from population A are crossed with inbred lines from population B. Eventually a pair of lines will be identified because of the outstanding hybrid they produce (AxB). The hybrid may be released to farmers who will multiply it vegetatively. The fact that this hybrid is the result of the cross between two inbred lines offers additional advantages. The inbred lines can be stored and/or shipped to other cassava-breeding projects as botanical seed. The hybrid can be reconstructed each time the same inbred parents are crossed. Therefore, tissue culture approaches to clean the planting materials after several cycles of vegetative reproduction are unnecessary (Ceballos *et al*., 2004).

Improved gene combinations can be obtained to produce a better hybrid than the original AxB cross. Inbred line A is crossed with related lines from the same population and, in the process, a segregation restricted to a limited number of loci will occur (Figure 9.4). The same is done with inbred line B. It is important that a limited number of loci segregate because the original AxB hybrid is already an excellent cultivar and it is desirable not to lose the good gene combinations that it possesses. In this particular case (which is greatly simplified), there were three loci in the AxB hybrid that were not in the heterozygote conditions (loci **cc**, **dd** and **FF**). Hybrid vigour depends largely on a maximized number of loci in a heterozygous state (Crow, 1999); therefore, the improvement of the inbred parental lines

should focus (in this example) on establishing contrasting states for these three loci in the two parental lines. In doing this the heterozygosity of the F_1 can be maximized, particularly at those loci responsible for heterosis.

Figure 9.4 Illustration of a reciprocal recurrent selection based on the development of inbred parental lines



Several inbred lines are obtained from each population in order to produce genotypes that will be better parents than the two lines originally used to produce the hybrid AxB. The lower half of Figure 9-4 depicts the segregation of inbred lines from populations A and B. Among these lines, two show a better performance, when crossed, compared with the original hybrid. As a result, only locus **dd** remains in a non-heterozygous condition: a subject of interest for a new cycle of selection that could eventually solve this remaining undesirable situation.

This scheme is ideal for gradually and consistently fixing desirable gene combinations. In the process the individual alleles cannot be tracked and the whole process is done "blindly" through phenotypic evaluations of the resulting hybrids. This scheme has been used successfully by different companies developing hybrid maize and has resulted in constant genetic gains in maize genetic productivity during the last 70 years (Duvick, 1984).

7. ESTIMATION OF GENERAL COMBINING ABILITY EFFECTS

One of the most relevant decisions taken by any breeder is the selection of parents used to produce a new generation of segregating progenies. This section introduces the application of quantitative genetics to parental selection, and Chapter 10 treats the subject of parental selection more broadly.

In cassava, this decision has been mainly based on the *per se* performance of each clone, complemented by (usually) empirical knowledge obtained over the years on the quality of progenies produced by different parents. This lack of organized information on the breeding values of parental lines used in breeding projects is partially due to the fact that, in most instances, no data are taken during the first stages of selection, particularly in the first clonal evaluation; or at best, data are incomplete. Chapter 10 describes a system for evaluating parental values in segregating populations, but this does not allow the formal estimation of quantitative genetic effects. In the early 2000s CIAT put into place an evaluation system that allows better estimation of GCA, or breeding value of parents used to generate segregating populations. The following sections describe early data from this system, as an example of the types of information and conclusions that are possible when improved quantitative genetic parameters are obtained.

Table 9.3 provides information from the first clonal generation of a segregating population (clonal evaluation trial, or CET) conducted in the acid soil savannas in the Meta Department of Colombia. There was a total of 49 families of which ten were full-sib and the rest half-sib families. A variable number of genotypes represented each family, ranging from 4 to 60, with an average of about 25 clones per family. To get a better estimation of family performances, they were divided into three groups (or *blocks*) which acted as replications for the family performance. Each replication contained a different sample of clones for each family (Ceballos *et al*., 2004). Results are averages across the three blocks in which the CET was divided. In some families, not a single clone was selected, whereas in a few, more than 30 percent of sibling clones performed well enough to pass to the next stage of selection. Table 9.3 presents the results of the best and worst four performing families. For each family, the percentage of selected clones is closely associated with the mean selection index (SI) value, which ranged from –22.47 to +15.97. The overall mean SI value should be 0.00 (as it is for each of the three blocks) but deviates slightly when averaged across the entire experiment. Further details regarding the use of the selection index were provided by Ceballos *et al*. in 2004. The sharp differences among families suggest large differences in the genetic value of the parents that produced them. Moreover, the origin of these differences can be understood by analysing individual traits such as disease reactions, harvest index and dry matter content.

The bottom of Table 9.3 provides the averages for each of the three blocks. The differences in these means measure the environmental variation that is removed in the experimental error in the estimation of family means. Since selection is conducted within each block separately, this environmental variation does not affect the selection of the best clones either. For instance, the average fresh root yields for Blocks 1, 2 and 3 were 20.9, 21.7 and 22.3 tonnes/ha, respectively. This implies about a 10 percent variation for this important trait. It is to be expected that many testing sites will have much larger variation, and this can be removed by stratification into blocks.

Similar results were obtained for the subhumid environment on the northern coast of Colombia, and are presented in Table 9.4. The main problems in this region are the short rainy season, low soil fertility and different species of mites and/or thrips. A total of 50 families was evaluated with an average of about 44 genotypes per family. Pressure from mites and thrips was relatively low during this evaluation. Data presented in Table 9.4 have been further consolidated by grouping the performances of all progenies derived from a given parent. This is possible because progenitors are used in more than one type of cross, and therefore participate in more than one family. The average performance of all the clones derived from a given progenitor (across different families) is presented in Table 9.5.

Table 9.3 Results of the four best and four worst families of a clonal evaluation trial for the acid soil savannas agro-ecological zone (Meta Department, Colombia), harvested in May 2003. Averages across three blocks in which a total of 49 families were evaluated[a]

	Family size (no. of clones)	Selected (%)	Plant type (1-5)	Fresh root yield (tonnes /ha)	Harvest index (0-1)	Root dry matter (%)	Selection index	Super-elongation disease
Information across the entire trial								
Mean	25.2	13.3	3.4	21.5	0.49	31.4	-1.3	2.9
Minimum	4	0.0	2.4	16.9	0.39	27.5	-22.5	1.8
Maximum	60	37.5	4.4	25.3	0.58	34.8	16.0	4.3
Family rank	**Averages by family**							
1	24	37.5	2.5	22.6	0.54	33.3	9.4	1.8
2	15	33.3	3.5	24.1	0.56	30.5	3.5	3.1
3	46	32.6	2.8	24.2	0.52	33.4	12.9	2.2
4	14	28.6	2.7	23.0	0.54	31.3	8.9	1.9
46	15	0	4.2	19.0	0.49	29.0	-16.1	3.9
47	9	0	3.8	16.8	0.39	31.3	-16.7	3.3
48	4	0	3.3	17.8	0.43	27.5	-18.1	3.5
49	22	0	4.3	17.1	0.48	27.8	-22.5	3.5
Averages by block								
Block 1	412	14.6	3.3	20.9	0.50	31.6	0.00	2.75
Block 2	412	14.6	3.3	21.7	0.49	31.2	0.00	2.83
Block 3	411	14.6	3.5	22.3	0.50	32.4	0.00	3.00

[a] *Selected = percent of selected clones within a given family or block; Plant Type score 1=excellent to 5=unacceptable; Harvest Index = fresh root biomass/total fresh biomass; score for superelongation disease where 1=very low damage and 5=high damage*

The best three families in Table 9.4 were all derived from germplasm developed in Thailand for Asian subhumid conditions (which are relatively homologous to those found on the northern coast of Colombia). Out of 50 families, this analysis could place these three families on top, one after the other. Moreover, the information in Table 9.5 places the progenitors of these three families (Rayong-5 [R-5], R-60 and R-90, and Kasetsart University 50 [KU-50]) as the best regarding the proportion of their progenies selected. Therefore, it can be concluded that the progenitors that gave rise to these families should be preferentially selected as parents in the crossing nurseries for this type of environment. These examples also provide evidence of the power that this analysis offers for properly distinguishing the genetic potential of the materials evaluated.

This approach also allows for the identification of useful germplasm for particular traits. The best progenitors for resistance to diseases, insects and different types of abiotic stress can now be identified much more precisely, not only based on the *per se* performance, but more importantly, based on the performance of the progenies they produce.

8. INHERITANCE OF IMPORTANT TRAITS IN CASSAVA

Knowledge on the inheritance of traits of agronomic relevance in cassava is limited. Very few studies have been conducted and published and therefore the cassava breeder has to work without the advantages of a clear understanding of the way the traits to be improved are inherited. Chapter 10 further discusses rudimentary genetic studies with regard to parental selection. This section focuses on the methodology for obtaining heritability information through standard quantitative genetics studies.

CIAT conducted diallel studies for each of three contrasting agro-ecological zones in Colombia (acid soil savannas, subhumid environment and mid-altitude valleys). The study involved nine or ten parents, and 30 clones each representing F_1 crosses. Field evaluation involved two locations in each zone with three replications at each location. Each plot consisted of a single plant, for a total of six plants representing each genotype. Therefore, for each F_1 cross the analysis of the thirty clones can also involve the within-family segregation, since replications for each individual genotype are available.

Table 9.4 Results of the four best and four worst families of a clonal evaluation trial for the subhumid agro-ecological zone (Atlántico Department, Colombia) harvested in May 2003. Averages across three blocks in which a total of 50 families were evaluated[a]

	Family size (no. of clones)	Selected (%)	Plant type (1-5)	Fresh root yield (tonnes/ha)	Harvest index (0-1)	Root dry matter (%)	Selection index
Information across the entire trial							
Mean	44.4	13.0	2.9	13.7	0.46	26.4	-0.7
Minimum	10	0.0	2.1	9.6	0.36	21.5	-16.0
Maximum	83	61.6	3.3	21.4	0.60	30.5	23.5
Family rank	**Averages by family**						
1	73	61.6	3.0	21.4	0.58	30.5	23.5
2	32	53.1	2.8	20.7	0.60	28.4	20.6
3	32	40.6	2.9	17.5	0.56	30.1	16.4
4	22	36.4	2.6	17.2	0.49	27.0	7.8
47	56	0.0	3.0	12.4	0.47	23.9	-7.7
48	53	0.0	2.4	11.1	0.36	21.9	-16.0
49	33	0.0	3.1	11.9	0.43	26.2	-5.5
50	35	0.0	3.0	11.9	0.41	25.4	-10.4
Averages by block							
Block 1	749	13.4	2.9	14.2	0.50	26.1	0.00
Block 2	746	13.4	2.9	14.4	0.46	27.2	0.00
Block 3	705	14.2	2.9	12.9	0.44	26.3	0.00

[a] *Selected = percentage of selected clones within a given family or block; Harvest index = fresh root biomass/ total fresh biomass*

The study allowed the standard estimation of two genetic parameters for the set of genotypes involved: (1) the average performance of parents in crosses, estimates GCA and is related to the additive variance (σ^2_A); and (2) the deviation of individual crosses from the average performance of parents, due to specific allelic combination, dominance effects, or specific combining ability (SCA) which is related to the dominance variance (σ^2_D). The statistical model is described in different articles (Cach *et al*., 2005; Perez *et al*., 2005; Perez *et al*., in press). In these evaluations, in addition to the usual between-family variation, the vegetative propagation of cassava allowed the analyses of the within-family variation. By cloning individual genotypes, they could be planted in two locations with three replications in each location, making it possible to partition the within-family variation into its genetic, genotype by environment, and the environmental components. The within-family analysis allows the obtaining of information on the relative importance of epistatic effects as suggested by Hallauer and Miranda (1988).

Table 9.5 Number of progenies evaluated and selected from each progenitor. Data from the clonal evaluation trial evaluated in Santo Tomás, Atlántico, described in Table 9.4

Progenitor	Family size	Progenies selected		Progenitor	Family size	Progenies selected	
		Number	Percent			Number	Percent
R 90	73	45	61.6	CM 4365-3	41	4	9.8
KU 50	64	30	46.9	SM 1657-14	21	2	9.5
R 60)	73	34	46.2	SM 1210-10	83	7	8.4
R 5	32	13	40.6	SM 1201-5	37	3	8.1
SM 1068-10	68	20	29.4	SM 1422-4	51	4	7.8
SM 2192-6	50	12	24.0	CM 7389-9	103	8	7.8
SM 1411-5	97	23	23.7	SM 1521-10	42	3	7.1
CM 7514-8	118	24	20.3	SM 1754-21	28	2	7.1
SM 1657-12	52	10	19.2	SM 1210-10	101	7	6.9
SM 643-17	32	6	18.8	SM 1619-3	29	2	6.9
MVEN 25	53	9	17.0	CM 8027-3	46	3	6.5
SM 1665-2	57	9	15.8	MNGA 19	215	12	5.6
CG 1141-1	33	5	15.2	CM 2772-3	28	1	3.6
SM 1511-6	87	13	14.9	SM 1600-4	61	2	3.3
SM 890-9	69	10	14.5	CM 7395-5	42	1	2.4
SM 1433-4	213	26	12.2	SM 805-15	73	1	1.4
SM 1565-17	108	13	12.0	CM 6438-14	53	0	0.0
CM 3372-4	52	6	11.5	CM 7514-7	56	0	0.0
CM 6754-8	49	5	10.2	SM 1431-2	33	0	0.0
SM 1438-2	109	11	10.1	Average			13.6

Reaction to arthropod pests such as mites (*Mononychellus tanajoa*), white flies (*Aleurotrachelus socialis* Bondar) or thrips (*Frankliniella williamsi*); diseases such as superelongation disease (*Sphaceloma manihoticola)* or cassava bacterial blight (*Xanthomonas axonopodis* pv. *Manihotis)*; plant architecture and general root appearance were scored using a 1 to 5 scale, where 1= resistant or excellent and 5= susceptible or very poor. Plants were hand harvested individually.

Tables 9.6, 9.7 and 9.8 present the summary of the most relevant information from the diallel analyses conducted in the mid-altitude valleys, subhumid environments, and acid-soil savannas, respectively. The large genetic variation contained in the within family component is remarkable and reflects the observations in the field, particularly when breeders evaluate the different clones from the same family in CETs.

Genetic parameters for fresh root yield were similar across the three different agro-ecological zones. In general, dominance effects were much more important than the additive ones. Moreover, the test for epistasis was significant in the three environments further highlighting the relevance of non-additive genetic effects in the expression of this complex trait. Ultimately, these results support the theoretical justifications for the implementation of reciprocal recurrent selection based on inbred parental clones, as described above.

In contrast with the lack of importance of additive effects observed for fresh root yield, at least compared with those for the non-additive effects, the reaction to pests showed a heavy dependency on additive effects. Table 9.6 shows the results for the reaction to mites and white flies. In both cases additive variances were significantly different from zero and considerably larger than the dominance variance, which failed to reach statistical significance in the case of white flies. Epistatic effects were negligible for both pests (negative variance estimates). This situation illustrates the case mentioned above – that traits with strong dominance effects (as is the case of resistance to white flies) will be expressed as additive effects in this type of analysis. In the acid-soil savannas (Table 9.8), the reaction to

superelongation disease showed a similar trend, with large additive effects, smaller dominance effects and negligible epistasis. In the subhumid environment (Table 9.7), the reaction to thrips also showed significant additive effects whose variance was about twice as large as that for dominance effects. For thrips, however, the epistatic effects reached statistical significance.

Table 9.6 Variances and test for epistasis from the evaluation of a diallel set combining data from two locations (Jamundí and Palmira) in Valle del Cauca Department, Colombia. The standard error for each estimate is in parentheses (Perez *et al*., 2005)

Genetic parameter	Fresh root yield (tonnes/ha)	Harvest index (0-1)	Dry matter content (%)	Reaction to mites[a] (1-5)	Reaction to whiteflies[a] (1-5)
σ^2_G (Between F_1)	42.8 (13.3)	0.0016 (0.0004)	1.19 (0.43)	0.271 (0.067)	0.345 (0.115)
σ^2_G (Within F_1)	288.9 (19.2)	0.0029 (0.0002)	2.25 (0.21)	0.188 (0.107)	0.119 (0.120)
σ^2_A	11.9 (24.7)	0.0029 (0.0015)	1.43 (1.33)	0.571 (0.271)	0.994 (0.467)
σ^2_D	152.1 (49.1)	0.0018 (0.0008)	2.47 (0.89)	0.170 (0.065)	-0.210 (0.132)
Epistasis test[b]	168.9 (40.2)	0.0001 (0.0010)	-0.32 (0.92)	-0.225 (0.179)	-0.221 (0.279)

[a] *Score for reaction to pests, where 1=very low damage and 5=high damage*
[b] *Test for epistasis based on H_o: test of epistasis = 0*

Table 9.7 Variances and test for epistasis from the evaluation of a diallel set combining data from two locations (Pitalito and Santo Tomás) in Atlántico Department, Colombia. The standard error for each estimate is in parentheses (Cach *et al*., 2005)

.Genetic parameter	Fresh root yield (tonnes/ha)	Fresh foliage yield (tonnes/ha)	Harvest Index (0-1)	Dry matter content (%)	Dry matter yield (t/a)	Thrips (1-5)[a]
σ^2_G (Between F_1)	13.09	11.53	0.0010	0.772	0.694	0.225
σ^2_G (Within F_1)	127.21	131.86	0.0037	5.556	9.977	0.641
σ^2_A	17.82 (13.75)	11.93 (12.59)	0.0009 (0.0010)	1.452 (0.985)	0.741 (0.933)	0.419 (0.211)
σ^2_D	23.87 (11.15)	27.02 (10.00)	0.0027 (0.0011)	0.765 (0.497)	1.589 (0.919)	0.231 (0.068)
Epistasis test[b]	100.40 (12.74)	105.64 (11.84)	0.0013 (0.0009)	4.257 (0.673)	8.414 (0.990)	0.259 (0.119)

[a] *Score for reaction to pests, where 1=very low damage and 5=high damage*
[b] *Test for epistasis based on H_o: test of epistasis = 0*

Table 9.8 Variances and test for epistasis from the evaluation of a diallel set from ten parents combining data from two different edaphic environments at CORPOICA – La Libertad (Villavicencio) in Meta Department, Colombia. The standard error for each estimate is in parentheses (Cach *et al*., 2005)

Genetic parameter	Fresh root yield (tonnes/ha)	Fresh foliage yield (tonnes/ha)	Harvest Index (0-1)	Dry matter content (%)	Plant type score	Super-elongation disease (1-5)[a]
σ^2_G (Between F_1)	1.649 (2.954)	1.325 (3.094	0.0010 (0.0006)	1.600 (0.664)	0.089 (0.039)	0.237 (0.055)
σ^2_G (Within F_1)	21.082 (2.297)	38.557 (3.242)	0.0030 (0.0003)	3.216 (0.169)	0.121 (0.012)	0.088 (0.066)
σ^2_A	-1.485 (6.321)	1.172 (8.035)	0.0015 (0.0016)	3.379 (2.399)	0.160 (0.144)	0.523 (0.234)
σ^2_D	9.028 (7.930)	3.384 (6.594)	0.0011 (0.0013)	0.873 (0.666)	0.096 (0.033)	0.092 (0.050)
Epistasis test[b]	15.054 (6.740)	35.433 (6.858)	0.0014 (0.0012)	0.872 (1.294)	-0.031 (0.077)	-0.242 (0.139)

[a] Score for reaction to pests, where 1=very low damage and 5=high damage
[b] Test for epistasis based on H_o: test of epistasis = 0

For harvest index and dry matter content, results from the three different agro-ecological zones generally agree (Tables 9.6 to 9.8). Dominance variance tended to be significantly different from zero whereas neither additive variances (although larger than dominance) nor epistasis reached statistical significance. Results from fresh foliage yield for the subhumid environment (Table 9.7) and acid-soil savannas (Table 9.8) show a similar trend, with negligible additive effects, increasingly important dominance effects (which reached statistical significance in the subhumid environment) and strong epistatic effects in both environments.

Quantitative genetic information is useful for planning a more efficient evaluation and selection scheme. When additive effects are important (as in the case of different traits related to plant health), the information generated from the GCA effects described above is very relevant and can be effectively used to predict the performance of the progenies to be derived from each progenitor. When dominance effects are important, the particular cross in which a given parent is used, is relevant information. In this case, a specific combination of progenitors yields better (or worse) performing progenies that one would expect based solely on the breeding value of the progenitors. Finally, the epistatic effects will be responsible for the variation among clones from the same family, further reducing the predictive value of GCA or breeding value.

It is also important to note that quantitative genetic studies become more useful as a large volume of information is accumulated from diverse germplasm sources and across a wide array of environments. This has been very difficult to achieve for cassava, given the low level and relative low number of plant breeders, and the limited resources with which they work. Every trial adds an important building block to the breeders' repertoire of breeding tools. However, until the structure is more complete, one needs to recognize the limitations of interpreting limited individual trials too broadly in establishing a breeding scheme.

Chapter 10. Parental selection

Plant breeding involves two basic activities: the assembly or creation of genetic variation and selection within this variation to achieve defined goals. Chapters 4 and 5 describe the assembly and management of existing genetic variation. After evaluating these materials, the breeder decides whether creation of additional variation is necessary to attain goals.

Theoretically there are many methods by which new genetic variation can be created, including sexual recombination, somaclonal variation, mutation, ploidy manipulation and targeted gene insertion (transformation). By far the most commonly used method is sexual recombination through interpollination of selected parents. Where other methods are used, crossing is typically supplemented rather than it being replaced. This chapter and Chapter 11 focus on the steps that follow a decision to create new variation through crossing: selection of parents, design of a hybridization scheme, pollination techniques and information management for crossing and seed production.

There is a considerable advantage to influencing the course of genetic variation a breeder creates, as opposed to random mating among unselected parents. Even the earliest farmers, during the process of crop domestication, probably had a sense that choosing the best plants as seed producers was the way towards progress. Choosing appropriate parents continues to be a crucial activity of the breeder who embarks upon a crossing programme. Some of the concepts and factors entering into the process of parental selection are: characteristics of materials in which genes of interest are found (sources), heritability, combining ability, complementarity and heterosis. Given that incomplete information exists on all of these parameters, the task of successfully selecting parents may indeed appear daunting. There are, nonetheless, some generalizations that seem to apply to cassava, which simplify procedures for many traits that breeders may want to improve.

1. GENE SOURCES

Conventional wisdom in plant breeding suggests that in order to transfer characteristics into a breeding population, the starting point is the identification of gene sources. These are genotypes that express the trait of interest at a high level. If such traits are heritable, then the genes controlling their expression can be transferred to the progeny, with the aim of achieving a similar high level of expression.

If a trait is controlled by a single gene, then clearly that allele conferring the desired trait must be present in order to transfer it to the progeny. There are, however, comparatively few important agronomic traits in crops under single gene control. Normally the breeder needs to plan a strategy for transferring multigenically controlled (quantitatively inherited) traits. For these, the choice of gene sources is less straightforward. When genotypes with very high expression of the trait of interest also include an array of unfavourable traits, they may be poor parents. The genes controlling undesired traits will be transferred to the progeny along with the desired genes. The breeder may never succeed in completely eliminating the undesirable genes even after several cycles of crossing and selection. Sources do not necessarily need to have high trait expression to be of value as parents. For most traits and for most situations, parents can best be selected with a view towards a total genetic value, rather than considering only a high level of expression of one or a few traits.

The strategy for defining best gene sources in the case of pest and disease resistance may be particularly complex owing to the interaction of two living organisms: host plant and pest. Generally, pathogen mutation is more likely to overcome single-gene resistance, while resistance resulting from the accumulated minor effects of many genes can be more stable. Low to intermediate resistance levels from several sources can probably increase chances of developing stable resistance. It should be noted, however, that in cassava, single-gene resistances appear to be rather rare. There is no hard evidence that selecting sources with highest resistance levels will result in instability of resistance in cassava. Nonetheless, given many examples of negative results in other crops, a breeder is well advised to be cautious with single gene resistance. Chapter 16 further discusses strategies for pest and disease resistance as related to gene sources.

The cumulative effects of genes with minor effects can combine into major effects. Traditionally, one difficulty has been that it was virtually impossible to determine whether genes from one source are the same or different from those of a different source, if the phenotypic expression is the same. In this situation the value of accumulating several or many genes for controlling a given resistance trait is mainly theoretical. With the expanding possibilities of gene tagging, this limitation will be slowly overcome, at least for some of the priority traits. Molecular markers will allow clear identification of different genes that control the same trait.

Logically, one would expect to increase the chances of diversifying genes by choosing parents of varied geographic origins and characteristics. Clones with the same level of expression of a trait are more likely to have similar genes for that trait if they are from the same region than if they are geographically distant. The possibilities of achieving transgressive segregation should be higher if the generally more diverse genotypes are recombined.

A common error in plant breeding programmes is to make premature conclusions on available genetic variation. A breeder is often interested in rare traits, which by definition may only be found by careful evaluation of a very broad germplasm base. Erroneous conclusions about presence or absence of a trait in a crop species can lead to great inefficiencies in design of a breeding programme. A decision to resort to wide crosses (i.e. with wild species), when a trait exists within the cultivated species, could add many years to a breeder's timeline. If a trait is not found within local germplasm, inquiries may be made to other curators of some of the world's larger collections, such as EMBRAPA in Brazil or CIAT, about the possibility of germplasm exchange or even a collaborative screening project.

2. HERITABILITY

Heritability is defined either as broadsense (h^2_b) or narrowsense (h^2_n). Broadsense heritability is that part of the observed total variation that results from genetic effects (total variation = genetically controlled + environmentally controlled). Estimates are dependent both upon genotypes and the conditions of evaluation. Broadsense heritability of a trait is of minor interest as a criterion in the selection of parents and will not be further discussed in this regard. It is, however, a critical statistic in determining selection criteria in breeding nurseries.

Narrowsense heritability describes the proportion of variation due to additive genetic effects. This parameter is of paramount importance in estimating the expected level of expression of quantitatively inherited traits in a population resulting from the recombination of two parents. There are various means of estimating h^2_n and details can be found in most basic plant breeding texts. In cassava, most of the available estimates are from parent-progeny regressions (see Table 10.1).

For traits with high h^2_n, there is a corresponding high probability of observing the trait in the progeny, providing that an adequate population sample size is taken. Progeny means will be near the mean of the two parent clones, for each trait controlled by mainly additive effects. There will also be a range of values, often following a normal distribution around the mean. High and low values may approach (or even exceed) the parental values. The latter is known as transgressive segregation. Whether distinct traits segregate independently or tend to be correlated will depend on the degree of genetic linkage and possibly other factors such as epistasis. With no linkage and high h^2_n, the breeder can expect that among the progeny from two parents, each with high expression of a different trait, there will be some proportion of individuals combining high expression of both traits, at least in a large population. As the number of traits of interest increases, the difficulty of combining all traits in a single individual increases exponentially.

As heritability estimates depend on the genetic material in question as well as environmental conditions, values reported in the literature may be of limited value unless they are accompanied by a careful description of the experimental conditions. For highest confidence, values should be obtained across a range of environments. Most cassava breeding programmes find it difficult to channel already-limited

resources to basic studies that do not directly lead to the production of new varieties. However, because these data are crucial to success, they will need to be obtained in some manner. Usually it is possible to include genetic studies as part of an overall breeding effort, without the need for substantial additional investment. Using the same populations created for the selection programme to carry out genetic studies is an efficient use of resources. Through networks, it should be possible for breeders to allocate research problems, such as basic genetic studies, to different institutions having specific interests and expertise, where all can benefit from shared resources and information.

Table 10.1 Narrowsense and broadsense heritability estimates of some important agronomic traits in cassava

Trait	h^2_n	h^2_b	Source
Root yield	0.40		Kawano (1977)
		0.34	CIAT (1994)
		0.19-0.51	Iglesias & Hershey (1994)
		0.49	Mahungu *et al*. (1984)
No. of thickened roots		0.11	CIAT (1994)
		0.51	Mahungu *et al*. (1984)
Harvest index	0.68		Kawano (1978)
		0.72	CIAT (1994)
		0.35-0.70	Iglesias & Hershey (1994)
		0.49	Mahungu *et al*. (1984)
Plant height		0.53	CIAT (1994)
Dry matter or starch	0.62		Kawano (1978)
		0.66	CIAT (1994)
		0.08-0.69	Iglesias & Hershey (1994)
		0.52	Mahungu *et al*. (1984)
	0.06		CIAT (1995)
Post-harvest root deterioration		0.44	CIAT (1993)
Resistance to cassava bacterial blight	0.63		Umemura & Kawano (1983)
		0.01-0.33	Iglesias & Hershey (1994)
		0.21	Mahungu *et al*. (1984)
Resistance to superelongation disease	0.60-0.79		Kawano *et al*. (1983)
		0.30-0.57	Iglesias & Hershey (1994)
Resistance to cassava mosaic disease		0.54	Mahungu *et al*. (1984)
Resistance to *Mononychellus* mites	0.78		CIAT (1981)
Root cyanogenic potential	0.87-1.07		Kawano, de la Cuesta & Gomez, unpublished data
	0.62	0.51	CIAT (1994)
		0.35	Mahungu *et al*. (1994)
		0.18-0.67	Iglesias & Hershey (1994)
		0.94	CIAT (1995)
Leaf cyanogenic potential		0.32	Mahungu *et al*. (1994)

3. COMBINING ABILITY

Combining ability describes the performance of a genotype as a cross parent. The term is closely associated with narrowsense heritability, but does not have a precise genetic explanation. Combining ability is referred to either as general or specific. General combining ability (GCA) is the average performance of a genotype as a parent in a series of crosses. Specific combining ability (SCA) is the deviation from predicted performance, of a specific cross combination, relative to general combining ability.

Obtaining general combinability estimates of a clone necessarily means observing performance of progeny from crosses with many other clones, over environments (usually years and locations). Usually cassava breeders arrive only tangentially at combinability estimates because they rarely obtain reliable family (cross combination) mean values for traits. More commonly, they obtain data only on the plants selected in the F_1, or sometimes a later stage of selection, which of course should not represent the population mean if selection was successful. Consequently, the information on combining ability usually comes from knowledge of the proportion of superior selections from crosses involving a given clone. For example, a parent clone that appears in a high frequency of selected progeny is a good general combiner. While this does not completely exemplify the classical definition, it certainly has practical value in breeding.

Hahn *et al*. (1977) at IITA suggested determining breeding values by general combining ability in test crosses to a low-yielding local variety. In this scheme, the test cross is made by growing the germplasm collection and the local test variety in isolation. The tester is the universal pollen parent and all male flowers are removed from all plants of the genotype being evaluated. Seed from the testcross is planted over seasons and locations. There is no record that this method has been applied elsewhere, perhaps because the benefits may not outweigh the complexity and time requirements as compared with simpler methods.

SCA may be of equal or greater importance than GCA to the cassava breeder. Clones in specific combinations that result in progeny substantially better than expected from their general combinability estimates, may be exploited extensively and repeatedly for producing superior crosses. Although formally-designed studies to obtain combining ability estimates have played a minimal role in cassava breeders' choices of parents, they have been conceptually and practically a part of the criteria. For improving efficiency of parental selection in the future, more attention will need to be given to this basic genetic parameter.

Chapter 9 gives more detail on the theoretical and applied basis of combining ability in relation to quantitative genetics

4. COMPLEMENTARITY

Practical experience in cassava breeding shows that many successful new varieties are the result of selecting parents that complement each other in the traits they express. Weaknesses in one parent are compensated by strengths in the other. Parents that are both weak in a given trait have little likelihood of producing strong progeny in that trait. An exception is for recessive traits: two parents heterozygous at the loci in question can produce the desired homozygous recessive genotype. There are some concrete examples where recessive alleles may be of practical interest in cassava, e.g. for root surface or root flesh colours.

A single recombination between two genotypes is most unlikely to result in any perfect individual genotype, no matter how many recombinants are produced. The accumulation of favourable alleles over time, using the principle of complementarity, is best done by recurrent selection (see Chapters 6 and 9).

5. HETEROSIS

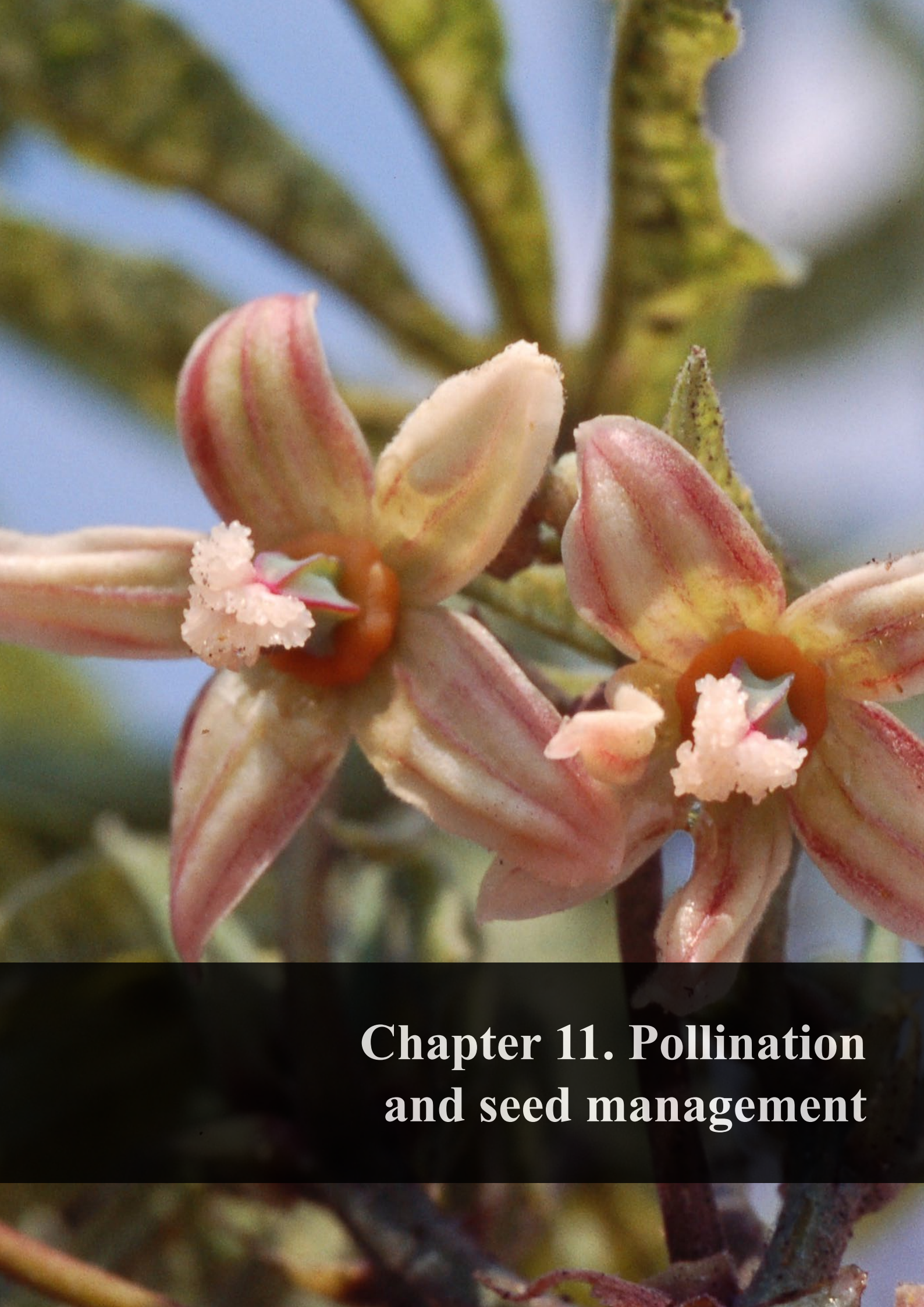
Heterosis is also defined as hybrid vigour, where an F_1 family mean exceeds the values of either parent, with respect to some quantitative character or characters. This concept may be applied to nearly any trait, but most often refers to plant vigour or yield. There appear to be two main components of heterosis: the masking of deleterious recessive alleles and the additive or complementary effects of distinct gene products that may result from two different functioning alleles at a given locus. For most practical breeding programmes, the distinction is not critical, but will become more so as molecular approaches develop.

It is unfortunate that very little is known about heterosis in cassava, though it is not surprising that this has been a relatively lower priority for breeders' research attention. Heterosis in cassava can be easily exploited by vegetatively propagating, indefinitely, any individual plant showing high heterotic effects. Breeders could improve efficiency of parental selection, or design of breeding methods to exploit heterosis better, if it were better understood. To the extent that plant performance is limited by the presence of recessive deleterious genes (this is currently unknown), heterosis could be exploited through inbreeding to select out these genes, followed by crossing to restore heterozygocity (see related discussion in Chapter 9).

CIAT used DNA markers to compare genetic variation within and among groups of clones representing adaptation to different agro-ecosystems (CIAT, 1994). Results suggested significant differences among the groups and the possibility of using this information to design crosses that would capitalize on heterotic effects.

A breeder who is intent on applying strict plant breeding theory might despair at the meagre database of genetic parameters available for cassava, especially from standard quantitative genetics studies. Breeders need to rely heavily on empirical data to guide decision-making. This empirical evidence strongly suggests that the best parents are often those with the best *per se* performance. In other words, a clone's value as a parent is often reasonably estimated from how that clone itself performs under the target area conditions. This is because most traits of interest are quantitatively inherited, with mainly additive effects and with intermediate to high broadsense heritability. For most traits, progeny values follow nearly a normal curve, between values of the two parents. The exceptions are not unimportant, but seem to be relatively few.

Implications for a breeding programme are profound. Parental selection on *per se* performance greatly simplifies the breeder's work. Although maximum progress probably will not be obtained without more extensive inheritance studies, even a programme with very limited resources can expect to make good progress in breeding using very simple criteria as the basis for parental selection.



Chapter 11. Pollination and seed management

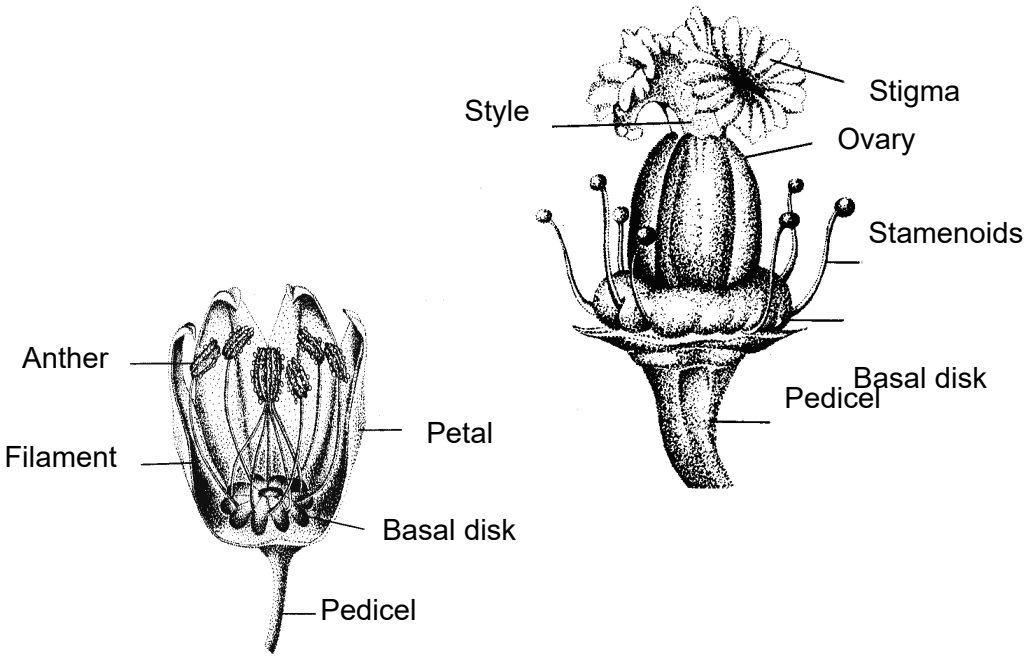
1. REPRODUCTIVE CHARACTERISTICS

Cassava is monoecious. Pistillate and staminate flowers, borne in terminal panicles, develop separately on the same plant. Transformation of terminal buds into a reproductive phase causes the branching that is characteristic of the crop. Branching habit is a simple indicator of flower initiation. Flowers at the first level of branching often abort during early development. First panicles often appear only at the second and later branching levels.

1.1 STAMINATE FLOWERS

Staminate flowers are smaller than their pistillate counterparts (10–12 mm versus 20–25 mm across the diameter of the open perianth) and occur in terminal clusters. The perianth is cup-shaped, consisting of five imbricate lobes enclosing a ten-lobed glandular disc. There are ten stamens, arising from the base of the perianth. Anthers are arranged in two levels, five have shorter filaments curved inward, while five with longer filaments curve outward. The shorter stamens are opposite the perianth lobes while the longer ones are alternate relative to these lobes. Anthers are dorsifixed and dehisce by longitudinal slits (Figure 11.1). Pollen grains are large and spherical (120–190 µ diameter). Each staminate flower produces only about 1 600 grains. Dried pollen remains viable for approximately six days.

Figure 11.1 Parts of the male and female cassava flowers



Source: CIAT (1981)

1.2 PISTILLATE FLOWERS AND FERTILIZATION

Pistillate flowers develop on average about two weeks earlier than the staminate counterparts of the same panicle and are fewer in number. The perianth is deeply five-lobed, sometimes with a purple border. The pistil is attached to a basal ring (Figure 11.1). The ovary is trilocular, spherical and with a capitate, three-lobed stigma. Each locule contains one pendulous, anatropous ovule, with ventral raphe and micropyle directed upwards and outwards. At the tip of the outer integument a soft tissue, the caruncle, is formed which caps the ovule. There is also an obdurator mechanism which is a peg-like growth formed from the placenta. This grows towards the ovule, curves around the caruncle and enters the nucellus through the micropyle. It is composed of thin-walled, elongated cells rich in nutrients. The pollen tube, in its passage to the embryo sac, grows through the obdurator. Fertilization requires 8-19 hours. After fertilization the obdurator disappears. The stigma remains receptive up to about 24 hours after the flower opens.

1.3 FRUITS AND SEEDS

Development of fruits, from fertilization to maturity, requires about three months. Fruits are globular, about 20–25 mm in diameter, with six thin, narrow wings. At maturity the hard capsule forcibly dehisces into three cocci, each with a seed. Seeds are elliptical, black, grey or mottled, with a thick, hard seed coat. The large endosperm encloses the embryo. Two leaf-like cotyledons of the embryo are pressed against each other by the endosperm. The radicle of the primary axis is directed towards the micropyle.

During germination the radicle pushes itself out through the micropyle and develops branch roots at its tip. The hypocotyl is curved, and by its further elongation the cotyledons are pulled out of the seed. They then expand, become green and contribute to early photosynthesis.

1.4 FACTORS INFLUENCING FLOWERING

A wide range of factors appears to influence initiation of flowering. Most of them are poorly understood. Among factors reported are: genotype, soil moisture level, soil nutrient status, photoperiod, solar radiation, temperature, growth regulators and girdling of the phloem near the base of the plant. The interactions among these influences can be very complex and have made interpretation of experiments difficult.

The breeder's interest in understanding flowering may be from at least three different perspectives. If a clone is to be used as a parent in a traditional crossing scheme, firstly, it must produce flowers, either staminate, pistillate, or both. A non-flowering clone cannot participate as a parent. Secondly, even if one is working solely with clones that flower, there may be wide variation in their time to flowering. In order to make specific cross combinations it may be necessary to synchronize flowering by manipulating planting date or by other experimental treatments. Thirdly, because flowering is closely associated with branching habits, and therefore with plant type, the influence either of selection or of the environment on flowering will have important implications for stability and acceptability of plant type.

Few studies have specifically looked at environmental influences on flowering, but a sizable literature exists on the effects of several variables on branching behaviour or on production of apices. Some of these results can be extrapolated to conclusions about flowering behaviour. In some cases, programmes have seen marked differences in flowering across sites, without any clear explanation. IITA greatly improved its ability to make crosses by moving clones to Ubiaja in Edo State, Nigeria.

1.4.1 Genotype

Genetic makeup of a cassava plant is one of the most important influences on flowering. Under environmental conditions favourable to flowering, some genotypes may flower as early as one or two months after planting, while others may never flower. There is continuous variation between these two extremes. About three-quarters of the accessions held by CIAT will flower between 6 and 18 months after planting under conditions of CIAT-Palmira.

1.4.2 Soil moisture

Conner and Cock (1981) reported on effects of water shortage on canopy dynamics in two varieties. Rate of branching was reduced when plants were well into the stress period, but not necessarily immediately upon presentation of the stress. In the less branching clone, MCol 22, flower initiation was completely suppressed during the stress period, while in MMex 59, branching was reduced but not completely suppressed. The rate of flower initiation was also reduced for a period after removal of the stress.

1.4.3 Soil nutrients

Cours (1951) found that under low fertility conditions, initiation of flowering may be considerably delayed, or may not occur at all. CIAT (1990) reported a tendency for the number of apices to increase with added NPK (increase in initiation of flowering), but markedly higher flower and fruit production in treatments without fertilizer. Flowers initiate better in high fertility, but are more likely to develop fully with some nutrient stress. At CIAT, the breeding programme has normally selected fields of known lower fertility for planting hybridization nurseries, because of generally more profuse flowering in these fields.

1.4.4 Photoperiod

Indira (1978) showed inconsistent results in a trial on photoperiod effects on flower initiation. Percentage of flowering plants increased with either an increase or a decrease of four hours of light, as compared with a 12-hour photoperiod. Plants did not flower under constant light. Keating *et al*. (1982) observed a concentration of flowering during the period of the year in Australia when the photoperiod is greater than 13.5 hours. This is consistent with results at CIAT (1990), and of da Cunha and da Conceição (1975) and de Bruijn (1977).

1.4.5 Temperature

Irikura *et al*. (1979) observed that flower induction was delayed as temperature either increased or decreased from 24°C. Various breeders report that flower production is sparse in lowland tropical areas and that flowering can be improved by moving clones to higher elevations (Bolhuis, 1969).

1.4.6 Growth regulators

Indira *et al*. (1977) attempted to induce flowering by application of various growth regulators. Indole acetic acid (IAA), napthalene acetic acid (NAA), ethrel, 2,3,5-tri iodo benzoic acid (TIBA) and kinetin were effective in accelerating flowering (up to 45 days earlier as compared with control plants), and also promoted flowering in a poorly flowering cassava genotype. The most effective treatments for the latter were NAA, IAA (50 ppm) and ascorbic acid at 100 ppm concentration applied at four months after planting as foliar spray, and repeated four times monthly thereafter. It appears that little use has been made of these techniques in any practical breeding programme, and more work needs to be carried out to confirm the effectiveness and practicality of these treatments. Tang *et al*. (1983) were able to induce flowering *in vitro* in vegetative growing points of meristem-derived plantlets, using both cytokinens and gibberelic acid.

1.4.7 Photosynthate partitioning

Based on the assumption of internal competition for available photosynthates between aerial parts and roots, CIAT (1990) experimented with removal of a band of phloem (girdling) near the base of the stem. The girdling treatment increased flowering and seed production by about three-fold in one clone, but had no effect on the other. Root weight was severely reduced by the treatment. This may be a practical solution for specific clones but could be difficult to manage across a broad range of genotypes.

1.4.8 Male sterility

Sterility is common in many vegetatively propagated crops, because they have been subjected to little or no selection pressure for seed production during evolution. Various kinds of male sterility are reported for cassava, including: early bud abscission (common in most clones under certain environmental conditions), non-disjunction of the microspores from the tetrad stage, non-disintegration of the tapetal cells leading to the production of non-viable pollen, non-dehiscent anthers and various degrees of

chromosomal pairing aberrations (Jos, 1978; Jos and Bai, 1981; Bai, 1987). Only about ten distinct male sterile accessions have been identified in CIAT's germplasm collection, so it does not seem to be a very common trait in cassava.

2. DYNAMICS OF NATURAL POLLINATION

Traditionally, recombination is achieved through transfer of pollen from selected plants to stigmas of plants chosen as female parents. In naturally outcrossing species, there are possibilities for using both controlled and open pollination. Usually controlled pollination is more time-consuming, but allows more control over the male and female gametes to be recombined. A breeder needs to analyse carefully which of the alternatives is most suitable to his or her objectives. Producing hybrid cassava seed through controlled pollination is relatively simple, though labour-intensive. Generally, each pollination will produce only about one seed. Far less labour input is required to collect large numbers of seeds from open pollination.

In order to utilize open pollination schemes effectively, one needs certain basic information regarding pollinating agent(s), dynamics of pollen transport by those agents, and levels of selfing or outcrossing that result.

2.1 POLLINATING AGENTS AND POLLEN MOVEMENT IN A FIELD

The majority of allogamous crop species can be classified as *anemophilous* (wind-pollinated) or *entomophilous* (insect-pollinated). The physical characteristics of cassava flowers and pollen are typical of species that are primarily insect-pollinated. Flowers produce nectar, which attracts insects. Pollen grains are large and sticky, which would seem to favour transport by insects rather than wind. Studies at CIAT demonstrated a negligible level of wind pollination in cassava (Daza and Alvarez, 1985).

At CIAT-Palmira the common honey bee (*Apis mellifera*) is the principal pollinating agent, though many species visit flowers, and could make minor contributions to pollen movement (Table 11.1). Most of the bees' activity takes place from 1130 hours to 1430 hours, which coincides with the period of anthesis. During the period immediately following anthesis, up to seven bees simultaneously visited the flowers of an individual inflorescence (Daza and Alvarez, 1985). At IITA in Nigeria, Mr Hahn (personal communication) reports that the African honey bee, a strong flier, is the principal pollinating agent. While these bees reportedly carry pollen over considerable distances, the honey bee at Palmira distributed over 90 percent of the pollen within a 10 m radius. This allows planting of separate hybridization blocks in relatively close proximity to each other, or to other sources of pollen, without high risk of contamination.

2.2 LEVELS OF SELF-POLLINATION

Cassava, like many outcrossing species, suffers severe inbreeding depression when selfed. Therefore, a pollination scheme (whether based on natural or controlled pollinations, but especially the latter) should take into account the potential level of selfing that could result. In a clonally propagated crop like cassava, selfing can occur in two ways. Firstly, pollen may fertilize pistillate flowers on the same plant. This type of selfing appears to be limited due to protogynous flowering habit. Secondly, the equivalent of selfing can occur when pollen from a given plant fertilizes a flower on a different plant of the same clone. The probabilities of this event increase as the size of a monoclonal field increases, thereby lowering the chances of pollen from another source entering the pollen population.

Table 11.1 Register of insect visitors to cassava flowers in pollination fields at CIAT-Palmira, Colombia

Order	Family	Species	Frequency of visits[a]
Hymenoptera	-	-	1
Hymenoptera	Braconidae	-	1
Hymenoptera	Vespidae	-	1
Diptera	Syrphidae	-	2
Hemiptera	Reduviidae	-	3
Coleoptera	Chrysomelidae	*Diabrotica spp.*	3
Hymenoptera	Vespidae	*Polistes spp.*	4
Diptera	Tephritidae	*Anastrepha spp.*	4
Hymenoptera	Apidae	*Apis mellifera*	5

[a] *1 = Generally not observed daily*
2 = Occasionally observed taking nectar
3 = Occasionally observed eating pollen
4 = Visit flowers daily. Polistes spp. seek nectar and Anastrepha spp. primarily look for young fruits on which to oviposit
5 = Visit flowers daily when flowering; principal pollinator

Source: Daza and Alvarez (1985)

The theoretical optimum design to maximize outcrossing would be individual plants of distinct genotypes interplanted in a polycross design – systematic designs that give all entries an equal chance of being adjacent to another entry. Daza and Alvarez (1985) addressed the question of levels of selfing of clones planted in a polycross design by using marker genes (light versus dark root surface colour), and comparing the proportion of progeny from emasculated and non-emasculated plants that were homozygous recessive (white roots). Six diverse clones ranged from 0–49 percent selfing, with a mean of 20 percent. Higher levels of selfing occurred in clones that produced more staminate flowers. These data are similar to findings of Meireles da Silva *et al*. (2003). Using isozyme markers, they estimated selfing rates of 0-31 percent among eight varieties in farmers' fields in Brazil.

3. POLLINATION DESIGNS AND METHODS

In cassava, hybridization and seed harvest are normally protracted activities, as compared with crops where flowering occurs over a short period. Pollination in a given crossing nursery may continue for six months or more, and seed collection for an equivalent period. Programmes that undertake hybridization need to plan for the resources to support a long-term, and probably year-round, set of activities.

There are three principal considerations in choosing between an open or a controlled pollination system. Firstly, production of seed by open pollination methods is far less labour intensive, and requires a somewhat lesser degree of skill, as compared with controlled pollination. Programmes with limited budgets may find it impossible to hire people to make a significant number of controlled crosses. Secondly, the primary interest in most hybridization programmes is to recombine selected parents to produce progeny containing the highest possible level of the positive features from all parents. For this purpose, it may not be a high priority to know both male and female parent. Size of F_1 populations may be more important than precise pedigree information. With highly specific selection objectives, the greater precision of gene manipulation that controlled crossing allows may be necessary. Thirdly, open pollination can inadvertently bias selection towards earlier branching and poor plant types. Without some means of regulating flowering, more profusely flowering (i.e. highly branched) types will contribute more male and female gametes to the gene pool.

3.1 CONTROLLED POLLINATION

The monoecious flowering habit of cassava makes controlled pollination relatively straightforward and easy. There is no need for emasculation, as staminate flowers on the same panicle open well after pistillate flowers are no longer receptive. Key elements of controlled pollination are to: (1) efficiently move viable pollen from selected male to selected female parents; (2) achieve the highest possible number of seeds per pollination; (3) avoid contamination from foreign pollen; (4) assure high success in development of healthy, viable seeds; and (5) maintain accurate records.

3.1.1 Planting designs

The essential features of a field planting design for a controlled pollination nursery are that: (1) adequate pollen is produced from selected male parents, and sufficient pistillate flowers are available on selected female parents to meet seed production goals; (2) the planting design allows easy access to flowers on all plants in the nursery, and that there be minimum chance for mistaken identification; and (3) collection of staminate flowers and pollination are efficient in terms of area of collection or area to cover for making pollinations.

A simple and effective field plan involves planting clones in rows of eight to ten plants, with about 2 m between rows, and alleyways between different sets of rows. This design allows better flowering by minimal competition among plants. Depending upon the number of crosses desired and the abundance of flower production for a given clone, different numbers of rows can be planted.

There are various possible arrangements of male and female parents within a field. If one set of clones is to be used exclusively as males and another exclusively as females, the two groups may be separated to facilitate pollen collection and pollination. Groups may also be defined based on breeding objectives, grouping together clones to be interpollinated.

The breeder should have information on the flowering habits of the clones he or she wishes to cross, particularly the time to first flowering, relative abundance of flowering and any anomalies such as male sterility or problems of low seed set. In order to accomplish specific crosses, it may be necessary to plant the late-flowering clones some time before the earlier flowering ones. Although this practice may help to synchronize flowering, it may be difficult from a management standpoint. In fact, simultaneous planting of clones having different periods to flower initiation may be less problematic than it seems. The early-flowering clones will normally continue to flower through various branching levels, and thus continue in flower as the later clones initiate flowering.

3.1.2 POLLINATION METHODS

Pistillate flowers of cassava normally open in the late morning or early afternoon hours. However, *Manihot* species vary in their time of anthesis from early morning to late afternoon (Asiedu *et al*, 1994). Attempts at interspecific crossing will need to take into account synchrony of flowering not only by time of year but also by time of day. Flowers to be pollinated must be covered before opening so that no contamination occurs prior to application of pollen from the chosen male parent. With a little experience it is possible to predict with a high level of accuracy those pistillate flowers that will open later on the same day. These flowers become larger, more deeply ridged, and have a yellowish hue. A more precise means of determining which flowers will open is to carefully pull open one of the sepals of the flower. Those flowers about to open, normally have a drop of nectar at the base. However, because this is a rather time-consuming procedure, this method should be used only in the process of learning to identify flowers about to open, from their external appearance.

Flowers are covered with a cloth, paper or perforated plastic bag that prevents entrance of pollen-carrying insects, but avoids any damage to the flowers due to high moisture or temperatures. One of the most convenient covers is a light fabric bag with a drawstring, measuring approximately 15 x 20 cm. This size allows an entire panicle to be covered without problems of damaging the flowers. Any pistillate flowers already open at the time of covering should be pinched off, as they may already have been pollinated by an unknown pollen source.

After flowers are covered, and an estimate made of number of flowers to be pollinated, staminate flowers are collected in small bottles or vials, with ventilated lids to prevent extreme temperatures and condensation inside the container. Vials should be cleaned with alcohol each day, or left unused for a few days to assure that all pollen adhering to the inside is not viable. Vials are identified with the male parent before collection of flowers begins.

Collecting recently-opened flowers has the advantage that nearly all flowers will be useful as pollinators. There are two disadvantages to this methodology. Firstly, open, undisturbed staminate flowers may be available only for a very short time during the day. The time of flower opening is also the time of major insect activity, and flowers must be collected quickly to assure that sufficient pollen remains. Secondly, and more critical, is that collection of flowers after opening gives opportunity for contamination. However, no estimates of this potential contamination have been made. Possibly, for purposes of routine crossing and hybrid production for varietal selection purposes, some low level of contamination is acceptable; however, a breeder normally expects that in a controlled pollination system, contamination should be near zero.

An alternative is to collect staminate flowers nearly ready to open (visual clues similar to those for pistillate flowers are applicable), and place them under conditions conducive to their opening before pollination begins in the afternoon. Staminate flowers often do not open as well after they are removed from the plant as when they remain attached. Better success is possible if only the flowers very close to opening are taken. Even then, the flowers may not open quite as fully as if they were left intact on the plant, and this can cause some difficulty in pollination.

Soon after midday, pistillate flowers are sufficiently open to begin pollination. Prior to this, the breeder makes a generalized plan for pollination to be made, based on pistillate flowers covered and staminate flowers collected. This will normally coincide with an overall plan for pollination that defines groups of crosses to be made and some order of priority.

Procedures for pollination are quite simple. The least complicated method is to use the staminate flower itself as the applicator. The flower is held by the base of the corolla, between the finger tips, and the pollen-filled anthers dabbed gently on the stigma until it is covered well with pollen (visibly yellow). The stigmas appear to be easily susceptible to damage, so it is important that the pollination be made gently, with only the anthers themselves touching the stigma, and not the basal disk. Bolhuis (1969) found that two people could make 1 250 pollinations per day, but most programmes seem to attain only about half this efficiency.

Mbahe *et al*. (1994) found an average of 337 pollen grains per stigma under natural pollination conditions, and 560 per stigma with hand pollination. With an average of 1 600 pollen grains per staminate flower, each flower will serve to pollinate three to five stigmas. This figure is also borne out in practical experience of many thousands of hand pollinations. As staminate flowers are normally produced in abundance, there is usually little reason to be excessively conservative with the use of pollen. More commonly, pistillate rather than staminate flowers will be limiting in availability.

Not all pistillate flowers in a panicle will open the same day. Those that were open prior to covering, or have not opened by the time pollination was effected, should be pinched off, as they may be pollinated later from an unknown source. If fewer than half the pistillate flowers in a panicle have opened, one might logically choose to leave the panicle to pollinate the next day.

When changing from one male parent to another, some method should be used to prevent contamination from pollen grains remaining on hands or clothing. An effective way to do this is to wipe the hands with an alcohol-soaked cloth.

Crosses should be labelled immediately after pollination. Minimum information to include is: female and male parents (female first, according to plant breeding convention), and date of pollination. In order

to keep records on efficiency of pollination, it is also useful to record the number of flowers pollinated in each panicle.

There is no general agreement among breeders as to the necessity of covering flowers after pollination. Probably there is little or no contamination by extraneous pollen if a stigma has been well covered by pollen from the desired source. However, no data appear to be available to support this assertion. For particularly valuable crosses, or crosses where it is essential to know with certainty the parents (such as in genetic studies) it is best to take no chances, and cover pistillate flowers immediately after pollination. Depending upon the type of bag used, covering recently pollinated flowers may inhibit fruit development. Flowers need to be covered for only a few days, after which the stigma is no longer receptive and contamination cannot occur.

3.2 OPEN POLLINATION

Open pollination schemes in cassava should aim to: (1) maximize intercrossing among plants in a population or gene pool, with near-equal contribution of each genotype to the gene pool; (2) minimize self-pollination; and (3) minimize contamination from other pollen sources.

3.2.1 Planting designs

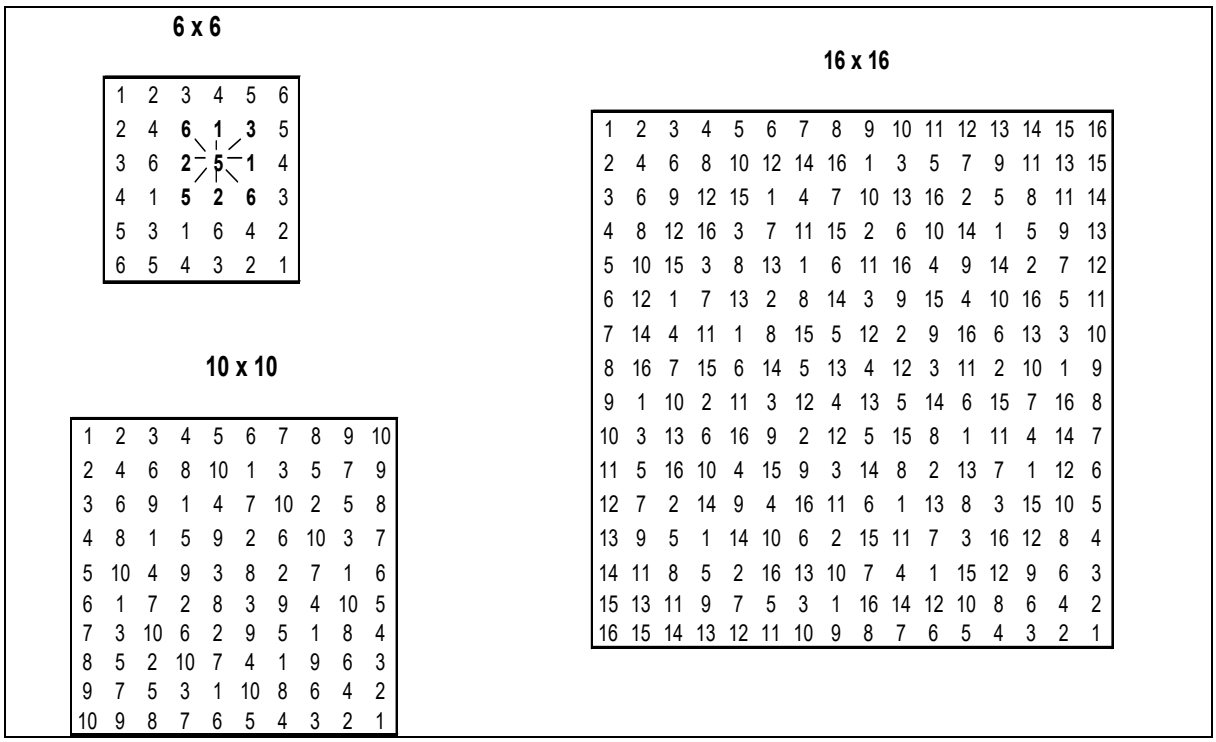
Open pollination methods may include various design options, from simply collecting seeds randomly in any cassava field, to planting selected clones in designs intended to optimize intercrossing. Control of pollen sources is usually achieved relatively easily and contributes substantially to genetic progress.

Although the monoecious flowering habit of cassava helps to reduce self-pollination, it is not uncommon to find staminate and pistillate flowers open at the same time at different branching levels on the same plant. Also, crosses between different plants of the same clone are effectively selfs; the result is genetically the same. This means that to minimize selfing in an open pollination scheme, the plot size for each clone should be as small as conveniently possible, unless some means of pollination control such as emasculation or male sterility is used.

Polycross mating designs are commonly used in vegetatively propagated, outcrossing forages to produce composite varieties. Polycross nurseries may follow any of various systematic designs, which provide the same probability of each clone being adjacent to another. Usually each plot is an individual plant, and in a given crossing block, each clone is replicated the same number of times. A simpler, alternative is to position clones randomly within each replication, which may give nearly the same degree of random intercrossing as a polycross, but these comparisons have not been made for cassava. Wright (1962) illustrated plans that allow considerable flexibility in terms of number of genotypes included (Figure 11.2). These plans have been used extensively at CIAT, and also by CNPMF in Brazil in their cassava breeding programmes.

The basic procedures for design of the Wright polycross are as follows. Plans following this procedure can only be drawn up using one less than a prime number, e.g. 6, 10, 12, 16, 18, 22, 28, 30, 36, 40, 42, 46, etc. This should not be a major constraint for any programme, since the increments between permitted designs are rather small. Referring to Figure 11.2, the procedure for design, for example, of an *n* x *n* polycross, where *n* equals the number of clones in the polycross (following the rule of one less than a prime number) can be followed.

Figure 11.2 Systematic polycross designs to optimize pollen distribution for random mating among a given number of parent clones. Each block can be repeated multiple times to achieve a desired level of seed production



6 x 6

1	2	3	4	5	6
2	4	**6**	**1**	**3**	5
3	6	**2**	**5**	**1**	4
4	1	**5**	**2**	**6**	3
5	3	1	6	4	2
6	5	4	3	2	1

10 x 10

1	2	3	4	5	6	7	8	9	10
2	4	6	8	10	1	3	5	7	9
3	6	9	1	4	7	10	2	5	8
4	8	1	5	9	2	6	10	3	7
5	10	4	9	3	8	2	7	1	6
6	1	7	2	8	3	9	4	10	5
7	3	10	6	2	9	5	1	8	4
8	5	2	10	7	4	1	9	6	3
9	7	5	3	1	10	8	6	4	2
10	9	8	7	6	5	4	3	2	1

16 x 16

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	4	6	8	10	12	14	16	1	3	5	7	9	11	13	15
3	6	9	12	15	1	4	7	10	13	16	2	5	8	11	14
4	8	12	16	3	7	11	15	2	6	10	14	1	5	9	13
5	10	15	3	8	13	1	6	11	16	4	9	14	2	7	12
6	12	1	7	13	2	8	14	3	9	15	4	10	16	5	11
7	14	4	11	1	8	15	5	12	2	9	16	6	13	3	10
8	16	7	15	6	14	5	13	4	12	3	11	2	10	1	9
9	1	10	2	11	3	12	4	13	5	14	6	15	7	16	8
10	3	13	6	16	9	2	12	5	15	8	1	11	4	14	7
11	5	16	10	4	15	9	3	14	8	2	13	7	1	12	6
12	7	2	14	9	4	16	11	6	1	13	8	3	15	10	5
13	9	5	1	14	10	6	2	15	11	7	3	16	12	8	4
14	11	8	5	2	16	13	10	7	4	1	15	12	9	6	3
15	13	11	9	7	5	3	1	16	14	12	10	8	6	4	2
16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

Source: Wright (1965)

The first row is consecutive from 1 to n.

Likewise, the first column is consecutive from 1 to n.

For rows 1 to n/2, the first number in the row indicates the interval between adjacent numbers, e.g. for row 3, the numbers follow: 3, 6, 9, 12, . . . ; for row 5, the numbers follow: 5, 10, 15 . . .

When *n* is reached (in the previous instruction), the next number equals: the next number that would follow in sequence, minus the prime number upon which the polycross is based. The plan then continues with the same interval, to the end of the row.

This plan works for rows 1 through n/2. Row (n/2)+1 is the reverse of row n/2. Row (n/2)+2 is the reverse of row (n/2)-1, and so on.

As additional checks on the design, the last row and the last column will count down in reverse sequence from n to 1.

Each clone in each replication must be identified if records of female parentage are to be maintained. Plants can be identified with their actual name, or with some simplified code when large numbers of plants are used. Seeds from each female parent are bulked across replications. The end product is a series of half-sib families; there will be as many families as there are clones from participating parents, if all clones have set seed.

If the number of parent clones the breeder wants to use does not correspond exactly to a number required by the systematic plan, two alternatives are available. First, if the breeder has access to male sterile clones, these can be used as filler, to complete the number of entries required, without contributing to the pollen population. Whether or not seeds are collected from these male sterile plants depends upon their potential value as parents. A second alternative is to make additional repetitions of some of the clones with lowest levels of flower production. This could bring the method closer to the ideal of equal contribution of all clones to the pollen pool.

The planting design should provide wide spacing between all plants, both to promote more flower production and to facilitate access to all plants for management purposes. Two to four square metres per plant is desirable, depending upon normal growth conditions at the site (wider spacing for more favourable conditions).

Even with these steps to design an effective polycross block, it is unlikely that fully random mating will be achieved, due in large part to the generally uneven levels of flower production among cassava clones. This can be corrected in part by appropriate management. Contribution of female gametes is easily regulated during or after harvest of seeds, simply by the amount of seeds collected from each genotype. Contribution of male gametes is more difficult to control. One method is to remove staminate flowers such that pollen production of all clones is reduced to the level of the least prolific clone. This is relatively easy, and can be done on a weekly or bi-weekly basis. If some clones are very poor flower producers, it may not be desirable to reduce all clones to this level of production, because it could create a deficiency of pollen to the point of leaving some flowers unfertilized. In this case, common sense discretion of the breeder would dictate what level of emasculation is reasonable.

A second, possibly less intensive alternative for regulating the contribution of specific clones to the pollen pool is to control branching, thereby controlling flowering. This simply involves removing some of the branches (pruning) of the more prolifically flowering clones, again, to avoid their excessive contribution to the pollen pool. This method indiscriminately removes both male and female flowers, but removal of some female flowers in prolific types should normally create no shortage of seed. Pruning can be done quickly and at a lower frequency (approximately once a month) than flower removal, and therefore may be the better choice for some programmes.

Even with these possibilities for regulating flowering, there is concern among some breeders about the implications of the use of open pollination with regard to long-term, inadvertent selection for more prolific branching. Without careful control and monitoring, there can be a marked selection for early and prolific branching within breeding populations, traits are generally considered undesirable.

3.2.2 Seed collection

A cassava fruit has the potential to produce up to three seeds. In controlled pollination systems, a much lower efficiency is normally achieved. Bolhuis (1969) working in Java, obtained average seed production per fruit of 2.1, 1.3 and 1.7 in the years from 1939 to 1941. At CIAT, in large part due to insect problems and abortion during high temperature periods, an average of about 0.7–1.0 seeds per fruit is obtained. Mbahe *et al*. (1994) reported that only about one-third of pollinated flowers set fruits. These averaged 2.8 seeds per fruit, or an overall efficiency of about one seed per pollination.

Just as for controlled pollinations, fruits from open pollination must be covered or collected before complete maturity; otherwise the capsules dehisce and seeds are lost. Individual fruit clusters may be covered with cloth, paper or perforated plastic bags prior to dehiscence, or alternatively, nearly-mature fruits may be collected and placed in labelled cloth bags. Covering fruit clusters has the advantage that it needs to be done at relatively infrequent intervals, as infrequently as once a month, for example. Care simply needs to be taken to ensure that all fruits that will dehisce prior to the next inspection of the nursery, be covered. A disadvantage is that many bags are required, and much labour to place them over the fruit. On the other hand, if fruits are collected prior to maturity, it is necessary to have more frequent inspections of the nursery (a minimum of once a week) because the fruits must be very nearly mature, but not yet dehisced, before they are collected. The advantages of this latter system are that no bagging

of fruit is required, and cleaning and sorting seeds after harvest is facilitated somewhat by having seeds from individual plants already bulked at harvest.

4. PESTS AFFECTING FRUIT AND SEED DEVELOPMENT

Insect, mite and pathogen problems affecting fruit and seed development vary from nearly nil to very severe, depending upon the region. In most of Africa and Asia, there are reportedly few problems with fruit development from a phytosanitary perspective. No chemical protection or other special precautions are required to produce good quantities of healthy seed. In parts of Latin America, insects may almost totally destroy developing fruits, and bacteria and fungi infest seeds. Apart from the direct importance to the breeder of obtaining healthy vigorous seed to plant nurseries, the phytosanitary status of cassava seed has important quarantine implications.

Fruit flies (*Anastrepha pickeli* and *A. manihoti* [Tephritidae]) are some of the most devastating constraints to seed production in parts of Latin America. The adult female oviposits on young fruits, and larvae bore throughout the fruit, destroying the developing seeds. Levels of infestation may be so severe during some years and seasons at CIAT headquarters in Colombia that virtually no seeds can be obtained without insecticide control or without covering fruits soon after pollination. After the capsule begins to lignify, in about six to eight weeks, larvae will not penetrate the seeds. Fenthion®, applied as a foliar systemic at seven to ten day intervals, provides good control.

5. SEED PROCESSING AND STORAGE

Mature fruit capsules should be gathered periodically from hybridization lots to reduce risk of infestation by insects or pathogens prevalent in plantations, and to minimize loss of identification tags. These should be stored in a cool dry place to inhibit further development of pests and pathogens and to maintain optimum seed viability. Storage in bags from the field should be kept to a minimum. If unprocessed fruits are stored for more than a few months, they should be treated with vaporizing insecticides/fungicides.

If fruits collected in the field are not completely dried, they should be spread out and dried thoroughly before packing into any kind of container. When they are packed for temporary storage, there should be ventilation through the bags to reduce development of micro-organisms. Packing tightly in any kind of container or packing in plastic or tight mesh bags should be avoided until seeds are completely dried.

Processing fruit capsules involves separating seeds from capsule fragments and the dried remains of the capsule. Although it may be technically feasible to mechanize this process, most breeding programmes work with a relatively small number of seeds for each cross, and mechanization is not practical.

After processing, seeds from each set of cross-parents are combined (identified by the tag made at the time of pollination, and maintained with the seed bag). Small (about 5 x 10 cm) seed envelopes are labelled with all the cross combinations that resulted, seeds are placed in these envelopes, and envelopes are ordered in trays or boxes, first by female parent, and then by male parent within female. After seeds are placed in the envelope or container in which they are to be stored or shipped, they should be treated with an insecticide/fungicide powder. Probably many different seed treatments could be effectively used, but little cassava-specific research has been carried out in this area. At CIAT, Orthocide® (captan, 50 percent a.i.) has been very effective in eliminating pathogens from the seed surface.

Germination increases significantly between harvest and about three to six months of storage at ambient (room) temperature conditions, apparently owing to some dormancy effects. Seeds begin to lose viability rapidly after two years of storage at ambient temperatures (Kawano, 1980). However, tests at IITA showed that seeds can be stored at least seven years with virtually no loss of viability at conditions of 5–10 °C and 60 percent relative humidity. These results support research of Ellis *et al.* (1981) showing that cassava seeds are orthodox, and respond to standard storage conditions of low humidity and low

temperature. Marin *et al*. (1990) and Mumford and Grout (1978) successfully recovered viable seeds from liquid nitrogen storage. This, however, would have implications more for long-term germplasm storage than for day-to-day breeding activities.

6. INFORMATION MANAGEMENT

Accurate records of pollination, seed production and seed inventories are central to a breeding programme. The core of this information is the pedigrees, which need to be kept precisely and in a highly accessible form. Accompanying this are records of nursery design, pollination records, seed production and coding of crosses.

6.1 HYBRIDIZATION

Information on nursery design includes parents, planting date and management practices. A field map is useful for reference during and after pollination in the nursery. Controlled pollination is normally carried out over extended periods. A simple and accurate system is essential. Field record-keeping should not detract substantially from the amount of time that can be dedicated to pollination itself, or require expertise beyond the level of labourer/technician, who normally manages pollination on a day-to-day basis. Further processing of these data may of course require more sophisticated techniques and expertise.

In the field, the key information management steps are the identification of vials of pollen (male parents) and of crosses. This information is placed on tags in the field and on forms for office use. Accuracy of all these procedures is greatly enhanced if the coding system for clones (hybrids and accessions) is kept very simple. Complicated nomenclature, which may have to be written and transferred thousands of times, introduces much higher possibilities of error as compared with simple codes. Even with very automated systems of data management, there are usually some steps that require hand input of data.

6.2 SEED PRODUCTION

Seed production can be registered periodically over the duration of seed harvest or after processing is finalized. The basic data include: nursery identification, female and male parents (or female parent only, in the case of open pollination), number of pollinations for a given cross combination and number of seeds produced. For simplification of information management, most programmes will want to codify cross combinations rather than directly use the two-parent codes.

There are many possibilities for codifying crosses, and again the rule of thumb is to combine simplicity with complete information. The main characteristics of an effective coding system are that the code should: (1) be unique to a particular cross combination; (2) provide some basic information about the cross; and (3) allow for efficient organization and searching for pedigree information (e.g. logical ordering by letters or numbers). The simplest system is one of consecutive numbering. This has the disadvantage of providing, of itself, virtually no information about the cross. Another simple but more informative cross code consists of two parts: first, letters or numbers indicating type of cross (e.g. controlled, open pollination, self-pollination); origin of cross (e.g. experiment station, region, or country code); or purpose of cross (e.g. disease or insect resistance, yield, quality, adaptation, etc.); and secondly, a consecutive number within each previously defined category.

As an example of a coding system, CIAT defines three basic types of crosses and assigns each a two-letter code (related to a Spanish designation for the type of cross): (1) open pollination (SM); (2) controlled pollination (CM); and (3) self-pollination (AM). The second part of the code is a consecutive number assigned to crosses of each category, beginning with the initial crosses made in the early 1970s. Examples of codes are: CM 5420 (controlled pollination; cross combination #5420 = MBra 5 x MBra 9); SM 720 (open pollination #720; female parent = MCub 74). Reciprocal combinations are assigned a single code, with the objective of increasing the number of progeny within a cross combination, and under the assumption that the reciprocal effects are unimportant. The way to manage

coding of reciprocal crosses (i.e. same or different codes) is a matter of personal preference of the breeder, but may become more critical if cytoplasmically controlled characters are discovered.

For open pollination progeny, new codes are assigned for each new cross. Even if a female parent has been used previously, the blend of pollen will differ for each nursery, and a new code is required to maintain the distinctive identity of the two populations.

In a vegetatively propagated crop, as with self-pollinated species, there is the possibility to repeat crosses among identical genotypes throughout seasons or years. The breeder may choose either to repeat the previously used code for that cross, or, alternatively, assign a new consecutive code each time the cross is repeated. The former requires a search of the database for repeated crosses each time crosses are coded. If data management is efficiently computerized, this may be a simple matter. Otherwise, most programmes will probably find that it is more convenient to assign new codes to crosses each time they are made, whether previously existing or not.

Good records on pollination and seed production allow the breeder to analyse overall efficiency of hybridization and possibly detect points in the system that could be improved. They allow identification of good and poor parents for seed production, or for modifying future hybridization nurseries. Consistently poor seed producers may be discarded or used primarily as male parents, for example.

Accurate and accessible pedigree records are essential to the breeder. These can be kept manually, but there are striking advantages to managing them on computer files. Breeders can design simple spreadsheet or database programmes, or use a commercial software package.

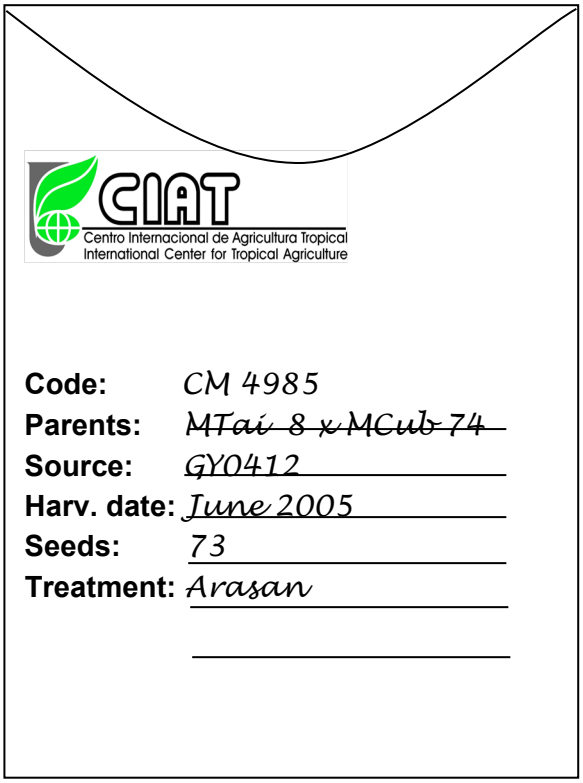
6.3 SEED STORAGE AND SHIPMENT

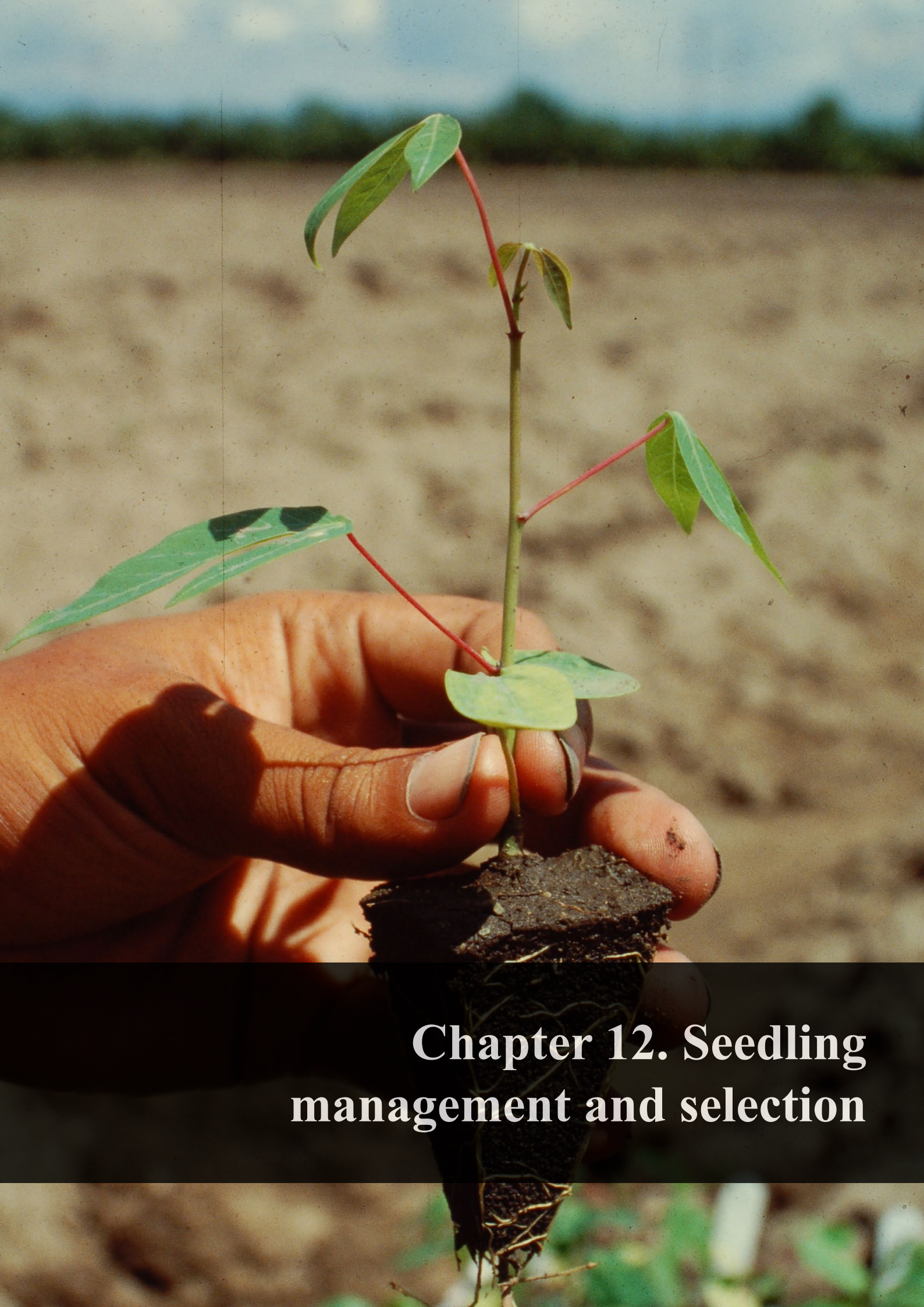
Seed produced in a breeding programme may be stored, planted as part of the selection programme, or shipped to collaborators or some combination of these. A precursor to information management is to organize seed in envelopes. These should be labelled with, at a minimum: cross code, source (code or name of the hybridization nursery from which seed was derived) and number of seeds. All other data can be derived from the appropriate databases. Nevertheless, it is often useful to also include directly on the seed package: pedigree information, seed treatment, phytosanitary tests performed and date of seed harvest (Figure 11.3). These additional details are especially useful if seeds are shipped to collaborators.

A computerized system of maintaining an up-to-date seed inventory is especially useful when large numbers of seed are being handled, when seed stocks are carried over years or when seed goes to various destinations. Keeping an inventory current and accurate is a simple matter with a database or spreadsheet programme. Information should include cross code, source of seeds, number of seeds, pedigree, seed treatment or testing. Additional information on storage conditions and viability testing can be useful.

Seed movement can be linked to the inventory, for automatic updating whenever seed is taken from stock, for planting, shipment or other purposes. A register of how seed has been used or to where it has been sent is especially important for programmes which regularly send seed to collaborators.

Figure 11.3 Example of labelling for a seed package, for temporary or long-term storage, or shipment





Chapter 12. Seedling management and selection

When a breeder initiates selection of new cassava varieties from true seed, a wide range of management decisions needs to be made. Procedures differ substantially from a selection programme that begins with existing clones. Special features of seedling management and selection relate principally to establishment procedures, developmental distinctiveness of seedling versus stake-derived plants, alternatives for evaluation and selection and information management.

As the F_1 is the only segregating generation and the only seed-propagated one, its management is substantially different from subsequent generations of selection. Figure 6.1 illustrated the generalized scheme of selection, when initiated from F_1 seeds. In the F_1, each individual is genetically distinct and F_1 populations usually show wide variation in morphological, quality and resistance traits. Individuals selected at this stage are then propagated vegetatively and pass through a series of trials of increasing precision (larger plot size, more replications) and fewer genotypes.

1. BASIC SEEDLING MANAGEMENT STRATEGIES

The F_1 generation of cassava provides the breeder with the widest range of variability of the entire selection process. The effectiveness of selection at this stage is crucial, some 80-90 percent of the total variability created is commonly eliminated before the next stage of selection. For selection to be effective, as in all stages, the materials need to be grown under conditions where the traits of interest will be expressed appropriately. The dilemma however, is that conditions defined as appropriate for selection of vegetatively propagated cassava may be rather drastic for seedlings and excessive mortality or very inferior growth can result.

Growing cassava seedlings successfully requires certain minimum facilities and conditions. The special care required for managing seedlings in the early stages can mean that one of the main criteria for choice of a site is the presence of adequate personnel and infrastructure. If these conditions can be met in an environment reasonably representative of the target area, selection can effectively be applied, probably for several traits, directly in the seedling generation. This is designated here as the F_1 selection strategy. On the other hand, if the area where seeds are planted differs markedly from the target region, selection should be minimal or null in this first generation. An alternative for this scenario is denoted here as the F_1C_1 selection strategy (first clonal generation of the F_1). Minimal selection is practised among F_1 seedlings. One or more stakes are cut from each plant, taken to a site more appropriate for evaluation and the trial managed in a way similar to the F_1. While most programmes will probably have a situation where selection directly in the F_1 is appropriate, there are several examples where the F_1C_1 selection strategy is more appropriate.

The Centro Nacional do Pesquisa da Mandioca e Fruticultura (CNPMF) in Cruz das Almas, Bahia, Brazil, has national responsibility for coordinating cassava research. It is strongly involved in germplasm introductions, through CENARGEN in Brasilia; and evaluation jointly with state and other national institutions. Brazil is a very large and geographically diverse country and to try to select varieties in the Cruz das Almas area in the northeast, for the entire country, would be futile. However, not all the target regions have programmes with resources to manage seed introductions. Until this capability is developed, the breeders at Cruz das Almas are planting seed in their station and sending stakes from all plants to the region of interest where they are planted and selection begins.

CIAT has all of Latin America, Asia and Africa as target regions. The headquarters station at Palmira, Colombia is representative of only a small fraction of world cassava growing regions, but other sites in the country cover a wide range of soil, climatic, pest and pathogen situations. Seed populations can be managed easily at the headquarters station, but it is very difficult to provide the necessary inputs and care at the other sites. All selected clones need to be maintained at CIAT headquarters for multiplication and distribution and for inclusion in crossing nurseries. Similar to the strategy in Brazil, CIAT does little selection in the seedling generation at headquarters and sends largely-unselected clonal populations to more representative environments to begin more intense selection.

Table 12.1 gives an overview of common problems and their solutions in cassava seed and seedling management and these are discussed more fully in the following sections.

Table 12.1 Common problems in cassava seed and seedling management

Observed problem	Possible causes	Corrective measures
Poor germination	Soil temperature too low	Modify environment to provide soil temperature of 30-35ºC
	Pest/pathogen infestation of seed	Treat seed during storage, e.g. Thiram®
	Non-viable seed	Discard seeds that float in water
		Determine conditions of seed storage for likely loss of viability
	Dormancy	Allow two to four months post-harvest before planting
Seedling deformities	Genetic anomalies	Discard affected seedlings; make note of parent clones
	Micronutrient deficiencies	Foliar applications
Leaf damage	Foliar pests and pathogens	Modify environment to slow pest/pathogen development; apply chemical control
Etiolation	Overcrowding	Provide adequate space for each seedling; transplant before excessive growth; clip stems and allow re-growth for two to three weeks
	Low light intensity	Provide full, or near-full sunlight
Seedling wilting and death	Damping off	Reduce watering
		Isolate affected plants or flats; treat with systemic fungicide
Poor growth or leaf discoloration	Nutrient deficiencies	Incorporate a complete fertilizer in soil mix; apply nutrient indicated by symptoms
	Herbicide residue	Use soil from a source that has no history of herbicide applications

2. IMPLICATIONS OF INTRODUCTION PROCEDURES

One of the first determinants of a seedling management strategy is whether cassava seed is to be introduced or locally produced. For introduced seed, quarantine regulations may require specific procedures, such as seed health testing or quarantine. Locally produced seeds are generally not similarly regulated. In this discussion details of those regulations are of no concern but rather only whether such regulations have implications for a strategy of seedling management and selection.

If seedlings are grown in a greenhouse or screenhouse for more than a few months, many traits of interest probably will be expressed quite differently from what could be expected of early field transplanting. Where these isolation/observation procedures are required, little or no selection for agronomic traits should be applied. One or more stakes may be taken from each individual plant, for planting in the field and initiation of selection. If planting in the screenhouse or greenhouse is for only a few months or less (for observational purposes) and seedlings are then transplanted to the field, there is a better possibility for effective selection in the seedling generation.

Some countries permit cassava seeds to be planted, or transplanted as young seedlings, directly to the field, but only in a specified, isolated quarantine area. Whether or not selection should be applied here will depend entirely upon how closely the environment of the quarantine area approximates that of the target environment. If temperature, rainfall and soil conditions are very different, one or more stakes may be cut from each plant, to be transferred to an appropriate selection site.

Most countries that permit introduction of cassava seeds do not have strict quarantine regulations concerning the area to which these seeds may be introduced. This allows the breeder flexibility to choose a suitable site for growing the seedlings, with a greater possibility of practicing selection in the F_1.

3. PRE-PLANTING PLANNING

Trial management planning must begin well before any seeds are planted. The questions of how, where, when and how many, should be well thought out in advance of any greenhouse[3] or field activities.

3.1 DIRECT SEEDING VERSUS TRANSPLANTING

Planting seeds in containers, with later transplanting to the field, is certainly more intensive than direct seeding, but most breeders choose transplanting as the more successful alternative. However, before automatically accepting the necessity for transplanting, one should study the possibility of success with direct seeding. A wide range of levels of success or failure has been reported for seed germination. Probably the most important factor influencing success is temperature of the germination medium. Ellis *et al*. (1982) demonstrated that a suitable regime for germination was an alternating temperature of 30°C (8 hours) and 38°C (16 hours) applied for a minimum of 21 days. These suggestions were intended as standards for viability tests and it is unlikely that such a precise regime will be possible in large-scale plantings in less controlled environments. The data do, however, point out that relatively high temperatures are required for germination and suggest why direct field planting is often unsuccessful. Both CIAT and IITA sow tens of thousands of seeds each year with good success (generally 80-90 percent germination). The main factors influencing success are: a soil temperature of 30–35°C, control of damping-off organisms, consistent moderate soil moisture and good weed control.

If field seeding is anticipated, a first requirement is to have data on diurnal/nocturnal and seasonal fluctuations in soil temperature at the experimental site. Black plastic or organic mulches could be used

[3] The term greenhouse is used to describe any enclosed or semi-enclosed area designed to provide partial environmental control (e.g. greenhouse, screenhouse, roofed shelter, walled shelter, etc.).

for increasing or decreasing soil temperatures. With a greenhouse it is generally possible to regulate temperatures more precisely and achieve better and more consistent germination.

3.2 LOCATION OF F_1 PLANTING

The decision on where to plant an F_1 trial should be guided in general by the overall strategy of site selection posed in Chapter 7. However, a few special considerations apply to the F_1. Seed-derived cassava plants are more delicate in the initial stages of development (up to about three months). Subjecting young seedlings to the same stress conditions of other selection trials could result in severely retarded development or even mortality. This may apply selection pressures unrelated to the genetic ability of the plant to survive and produce when vegetatively propagated. Generally, this means that the conditions for F_1 planting need to be more favourable. If sufficiently favourable conditions can be found in the principal selection site, or produced through modification of the environment, then there is good reason to have the F_1 population at this site. Where soil water availability or soil nutrients are the major stress factors, these can sometimes be adjusted to conditions appropriate for seedlings. Likewise, where mites or insects are major stress factors, these are often relatively easily controlled with pesticides. Where diseases, especially bacterial pathogens, are severe, their chemical control is often difficult and F_1 planting in a less affected site may be desirable.

3.3 PLANTING DATE

The planting date will normally be determined by the initiation of rains or, in the subtropics, arrival of summer. For transplanting schemes, sowing of seeds in the greenhouse should be four to six weeks prior to this period, such that transplanting corresponds approximately with the normal planting season.

Where the F_1C_1 option of seedling management is chosen, an alternative may be to plant seeds approximately six to eight months prior to the subsequent desired planting period. A mature single stake can be cut from each plant and planted in the selection site at the normal planting time. The reduced duration of the F_1 (about six months) could slightly reduce the time for variety development. This alternative will be possible only where climatic conditions permit off-season planting, or irrigation is available.

3.4 SEED QUANTITY

How many seeds are required to meet the selection objectives? This question can be answered only in rather general terms of probabilities and the normal strategy is to manage as much genetic variability as is practically possible, given a known level of labour and land resources and seed availability. Most breeding programmes manage a few thousand seedlings a year but a few manage up to 50 000 or 100 000.

Resources to be considered are: greenhouse space; containers for planting; management in the greenhouse (soil preparation, watering, fertilization, chemical protection); labour and purchased inputs for transplanting, land preparation, weeding, irrigation, chemical protection, harvesting and selection; and land. The following sections discuss alternative management strategies for optimal allocation of these resources.

3.5 PREPARATION FOR PLANTING IN THE GREENHOUSE

3.5.1 Basic facilities

As greenhouse facilities will be used for only a short period (to germinate seeds and establish seedlings), minimum facilities are called for. An expensive infrastructure is not justified, but certainly can be used if available. At a minimum, all that is needed for seed germination is floor space, containers with soil for planting seed and water for irrigation. Additional components that facilitate management, or provide an additional element of environmental control, are a transparent roof to keep out rain and allow near-full sunlight; benches; protection against animals, insects and/or wind; and possibly some degree of temperature control.

3.5.2 Containers for planting

Cassava seeds may be planted in a wide range of different containers, so long as they meet certain basic criteria.

Volume. For a normal transplanting situation, where seedlings are transplanted to the field between four and six weeks, a minimum soil volume of about 3 x 3 cm wide by 8 cm deep is required. Larger soil volumes make little difference in plant growth during the first four to six weeks and only add to the labour and space requirements. Larger volumes can act as a stabilizing influence to maintain more uniform soil moisture or temperature. On a hot sunny day, a larger soil volume may prevent the temperature from rising too high, or soil from drying too quickly. Normally, however, there are other simple ways to prevent this, such as shading and misting or sprinkler systems.

Ease of handling. As containers for planting seeds will need to be disinfested, filled with soil, moved to the site for germination and growth and finally moved to the field for transplanting, ease of handling is an important consideration. A first choice is something that can be moved in bulk, such as planting flats with individual compartments, or small plastic bags in a tray. This aids not only in moving plants, but helps to keep identification errors to a minimum.

Suitability for seedling growth. Many types of plastic, organic or clay pots are suitable for seedling growth. However, any container that is not specifically designed and tested for growing plants should be tested in advance for possible toxicity effects or other problems. The container should be free of pathogenic micro-organisms or be suitable for simple treatment against such organisms.

Suitability for transplanting. The container should permit transplanting with the least possible disturbance of the fibrous root system, because seedlings can ill afford damage to their already-sparse root systems. The least damage is caused by a container that is transplanted along with the seedling and the most damage by undivided flats where roots from neighbouring plants intertwine and are damaged during separation. Plastic bags, torn off from around the soil and root mass, as well as individual containers that allow roots and soil to be removed relatively intact, are both well-suited for transplanting. Most commercially available seedling containers have slanted sides to make removal of the root mass easier.

Cost and availability. Each programme must work within its resources, which may mean improvising or settling for less than the optimum situation. Lack of local availability or import restrictions may also influence choices. As many different kinds of seedling containers have been used successfully by different programmes, it is obvious that undue complication or expense need not be assumed. On the other hand, successful establishment of vigorous seedlings is basic to the success of cassava breeding and a reasonable investment to assure this success is justified.

3.5.3 Soil preparation

Soil for planting cassava seeds should have the same characteristics of soil for starting seedlings of most species: light, well-drained soil of good fertility. At CIAT, a clay-loam soil is mixed, two parts soil to one part sand, to improve the texture;. 10-20-20 (NPK) fertilizer is added at the equivalent rate of 2 tonnes/ha. Although experimental data are lacking, it may be inferred that good soil phosphorous is essential to good establishment. Seedlings have a poor fibrous root system and will lack a native mycorrhizal population if sterilized soil is used.

If soil-borne pathogens are a potential problem, soil sterilization may help. On the other hand, sterilization also destroys beneficial or neutral micro-organisms. This can result in higher aggressiveness of a pathogen if it is introduced to the soil medium, where no competing organisms limit its growth and reproduction. It has not been determined what effects mycorrhizae have on seedling establishment and vigour. One option to assure an adequate mycorrhizal population is to inoculate the soil after sterilization. An alternative is to assure phosphorus availability with high soil P levels.

At CIAT dramatic differences in susceptibility of plants to soil drying, in steam-sterilized versus unsterilized soil were observed. After almost complete drying of the soil and rewatering, nearly all the plants in the sterilized soil died, while most of those in the unsterilized soil recovered. It could not be determined if this effect was due to a structural change in the soil from the high temperatures of sterilization, an imbalance in the soil flora, or some other factor. Damping-off, one of the main problems of seedling establishment, may not be controlled by soil sterilization. Airborne spores readily reinfest the soil. Some fungicides for seed and/or soil treatment may be preferable to soil sterilization, although no literature exists on this specifically for cassava seeds.

Most cassava programmes have historically had success in germinating and growing cassava seedlings in unsterilized soil, with fungicide-treated seed. There are potential complications with sterilization that may indicate that its use is unwarranted unless individual experience shows the practice to be needed for successful seedling establishment.

4. PLANTING SEEDS

The planting design should facilitate both sowing the seeds in containers and later transplanting to the field, with a minimum risk of errors in identification. The system that best accomplishes this is probably to plant in rows within flats, from front to back. Each cross should be labelled with the appropriate code. It is normally not necessary to label each individual plant. Small plastic or wooden labels can be used, marked with lead pencil or indelible ink. Ball point pens or other water soluble markers should be avoided.

Seeds are planted to a depth of 1–2 cm. The position of the seed appears to have little effect on rate of emergence. Seed should be covered loosely with soil and not compacted, so that emergence is not inhibited. Planting the seed in the middle of the container (when individual containers for each seed are used) allows for the most uniform root distribution and ease of transplanting.

5. GERMINATION AND SEEDLING CARE

5.1 GERMINATION

Normal viable seeds, past their dormancy period and given good conditions as described above, begin to emerge after about ten days. Most seeds germinate by about 20 days. When conditions are suboptimal, the period for germination can be considerably extended, or germination can even fail completely. Therefore, it is most important that, at least during the first two weeks after planting, high soil temperatures (30-35°C) be obtained for rapid germination. Young seedlings should be observed daily for any abnormalities or conditions requiring treatment.

5.2 WATERING

During the entire period of seedling growth in containers, soil should be kept moist at moderate, but not excessive levels. If the local water supply is known to have quality problems, such as high salt concentration, rainwater should be collected and used to avoid deleterious effects on the seedlings.

5.3 FERTILIZER

If good fertile soil is used, or fertilizer added to the soil mixture in the proper amounts, there will probably be no need for further fertilization prior to transplanting. Low fertility may be indicated by leaf colour and other symptoms. Although there are no studies on nutrient deficiency or toxicity symptoms in seedlings, there is no reason to believe that these would be dramatically different from those of stake-propagated plants. The elemental levels at which these symptoms are expressed might, however, be different.

Some of the common soil chemical problems are phosphorous deficiency, salinity and zinc deficiency. Phosphorous deficiency shows up as purpling, first of the young leaves and in more severe cases, of the whole plant. Zinc deficiency is evidenced by a light and dark green mosaic on the leaves, with interveinal chlorosis. If deficiency symptoms show up prior to transplanting, foliar application can be made. The nutrients will be rapidly absorbed and the symptoms corrected. Salinity effects are shown by an overall yellowing, with white necrotic spots on the leaves.

5.4 PESTS AND DISEASES

The most common biological problems in seedlings are damping-off (due to several possible species of root-rotting pathogens), CBB, thrips and spider mites. Damping-off is difficult to control after it has begun to spread. Normally, unless soil is generally infested, the infections begin in focal points and spread outward. When these focal points are first observed, affected plants or flats should be isolated and surrounding plants treated with a systemic fungicide. CIAT has had some success with the product Aliete® (fosetyl, 80 percent a.i.), with 5 g/litre applied as a spray. Plants already infected are unlikely to recover, but the fungicide treatment may prevent spread to healthy plants. Cutting back on watering may help slow the spread of disease. Also, watering should be done with a gentle sprinkler or mist type system that does not cause splashing of the soil and movement of water and pathogen propagules from one container to another. Growth of damping-off organisms is favoured by cool, wet soils, so these conditions should be avoided.

If CBB is endemic in the area, every effort should be made to isolate seedlings from inoculum sources. The disease progresses rapidly in the succulent tissues of young seedlings. The pathogen will be carried to the field and progress systemically throughout the plant's development. Some programmes, most notably in Brazil, have developed controlled and systematic seedling screening for CBB resistance, but for most, avoiding the disease until later selection stages is probably the best strategy.

Spider mites are common on many greenhouse-grown plants. Thrips are less common and generally appear to be controlled by the normal routine of sprinkler watering a few times a day. Both problems can be controlled relatively easily with a systemic insecticide such as Sistemin®, or spider mites by a specific acaracide. The dose should be reduced somewhat as compared with that recommended for mature plants, as seedlings are more susceptible to phytotoxicity effects of chemicals. Theoretically many other pests and diseases may potentially affect seedlings, depending upon local conditions. However, to date, no other major problems have been reported.

Viruses may be cause for special concern, because they cannot be controlled chemically and may be borne in symptomless plants. To date, however, only a few minor viruses have been reported as potentially capable of transmission through cassava sexual seed (see Chapter 5).

5.5 SEEDLING ABNORMALITIES

As cassava is a vegetatively propagated, heterozygous plant, it probably conceals many deleterious recessive genes. Seedling populations may occasionally express these genes, especially where some selfing is likely, as in populations from open pollination. One of the most common of such abnormalities is *albinism,* which may be caused by a breakdown at any number of points along the pathway for chlorophyll synthesis. Albino (white) plants normally appear vigorous soon after germination, but because they rely totally on seed reserves to sustain themselves, they soon degenerate and die.

Several breeders have reported seeing seedlings with deformed leaves, sometimes with virus-like symptoms. These symptoms may disappear as the plant grows, but in other situations the symptoms continue throughout the life of the plant. Most cassava viruses are not seed-transmitted and there is no evidence of a virus causing these defects. It appears that most of these symptoms are caused either by hypersensitivity to some nutrient or toxic factor in the soil, or are genetic defects. As most of these deformed plants do not flower, it has not been possible to perform any genetic studies. If the effects are generalized across a high percentage of the seedlings, it is likely to be a generalized nutritional or toxicity factor. If it is localized within particular crosses, it may be a genetic defect (such as plants ultra-sensitive

to some environmental condition) or a virus. If there is doubt about the possibility of a virus, the affected plants should be removed and grown in isolation for further observation and testing. As the frequency of such abnormal plants is usually very low, the breeder need not be concerned about losing significant genetic variability. It is best to err on the side of caution when viruses are even slightly suspected, especially when seed has been introduced from outside the region.

Growth under insufficient light can cause etiolation, or abnormal tissue elongation. This may result either from reduced sunlight or by crowding of seedlings. Some etiolation is to be expected and will not significantly affect the seedlings' growth and vigour after transplanting. If etiolation is excessive, the stems of seedlings may be very weak and break or fall over after transplanting. So long as stems remain upright and sturdy after transplanting, the level of etiolation should not be a great concern. Etiolation can progress rapidly, so the breeder should keep close watch on seedlings as the canopy closes.

Etiolation can be reduced by appropriate spacing between plants (minimum of about 10 cm^2/plant for seedlings up to six weeks) and by having full or nearly full sunlight conditions. Allowing space between individual flats will also allow greater light penetration to the seedlings, without modification of the container size.

If transplanting is not possible and seedling growth is excessive, plants can be cut back to 4-6 cm above the soil line. Plants will re-sprout and be ready for transplanting in two to four weeks.

6. FIELD PREPARATION

Preparation of the soil for transplanting seedlings to the field follows basically the same norms as for commercial preparation. The hills may be marked prior to transplanting, or a guide such as a tape measure used to mark distances at the time of transplanting. Ridges should be used in heavy soils and even in well-drained soils a small ridge or line greatly facilitates laying out the field design.

7. TRANSPLANTING

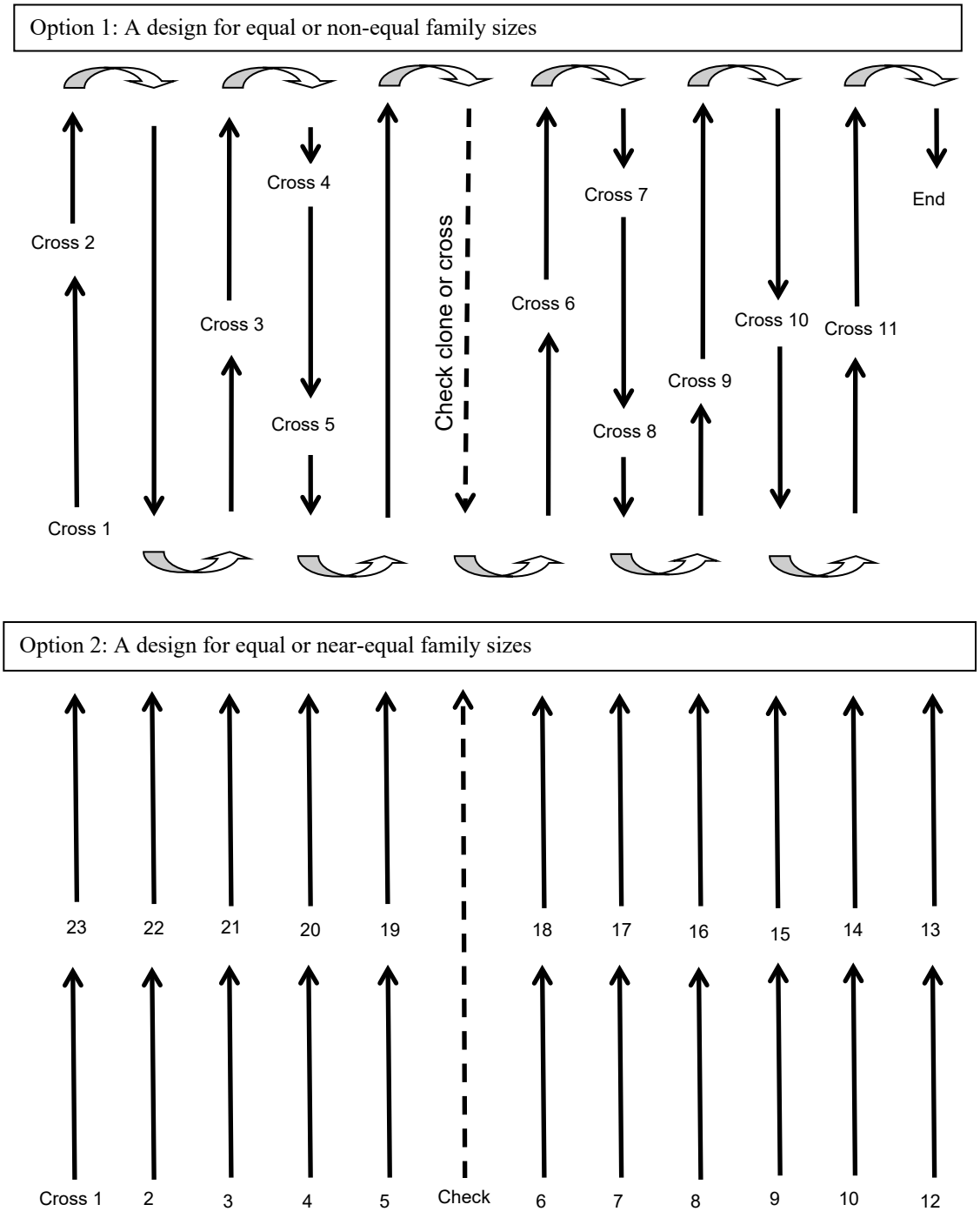
7.1 PLANTING DESIGN

The planting design chosen for transplants should satisfy several criteria. The main objectives are to facilitate efficient crop management and identification of superior genotypes. The design should:

- minimize competition among plants;
- allow efficient evaluation; i.e. permit observation of individual F_1 plants with a reasonable amount of walking (distances can be quite long for evaluation of an F_1 field, one kilometre or more for every 1 000 plants!);
- allow easy and error-free identification of crosses;
- allow comparison with a known check variety.

Two common designs are used for F_1 fields. When the numbers of progeny in each cross combination are almost equal, each set of progeny may be planted side by side in single rows (Figure 12.1, Option 1). This design is certainly satisfactory, except that it may imply some inefficiency in accessing each cross to effect evaluation, treatment and harvesting, etc.

Figure 12.1 Alternative designs for transplanting seedlings for efficient management [a]



[a] *Each arrow represents the seedlings in a single half- or full-sib family*

The second design is a zigzag type planting that is essentially a continuum from one cross to another (Figure 12.1, Option 2). This design is almost a necessity to utilize field space efficiently if the number of progeny varies widely from one cross to another. Seedlings are transplanted starting at the front of the field and the next cross combination continues directly behind the first (with a space for identification). When the end of the experimental field is reached, seedlings are transplanted in the reverse direction, from back to front. This alternation of planting front to back and back to front, continues throughout the entire field. Field operations performed on a plant by plant basis can be carried out in a continual line, thus utilizing time and energy efficiently.

7.2 PLANT SPACING

Appropriate plant spacing should be considered on the basis of: (1) minimizing competition effects between neighbouring genotypes; (2) allowing access by the breeder to all individuals in the population throughout most or all of the season for evaluation purposes; (3) land available; (4) a suitable canopy cover to aid in weed control; and (5) whether or not selection and roguing will be done in the early growth stages.

If taken individually, these criteria can imply quite distinct design options. For example, to minimize competition, spacing should be as wide as possible, while weed control through crop canopy cover implies close spacing. An appropriate compromise seems to be a spacing of about 1 m between plants within a row and 2 m between rows on fertile soils; and about 0.75 m between plants and 1.5 m between rows for low fertility soils. The wide spacing between rows allows the breeder to walk freely between rows for evaluations.

7.3 CHECKS

Comparison with known checks is standard practice in virtually any breeding programme. This is best begun as early as possible in the selection sequence. In the F_1 of cassava, use of checks is not so straightforward. One needs to be aware of differences resulting from seed- versus stake-propagated plants, as opposed to genetic differences. Nevertheless, planting check clones is still a useful practice to compare traits with similar expression across the two forms of propagation and also to obtain a general idea of the environmental variability within the experimental field. One simple design for checks is to include a check clone every five to ten rows throughout the F_1 field (see Figure 12.1). One or several checks may be used, depending upon selection objectives and availability of material.

An alternative is to plant a set of standard crosses (derived from seed) at intervals throughout the F_1 nursery. These crosses would need to be produced periodically for continual availability through the years. Over time, the characteristics of these crosses would become well-known and performance could be compared directly with the experimental crosses in the nursery, because all would be compared with the same propagation system. In Thailand, Rayong 1 x Rayong 90 crosses have been produced abundantly and have served as an informal check in F_1 nurseries.

7.4 TRANSPLANTING OPERATIONS

Seedlings are carried to the field in the same container in which they were germinated and grown for the first weeks. If the field has been prepared with ridges, planting in a slight depression in the top of the ridge will help prevent rapid soil drying around the plant. The seedling is placed in a prepared hole with as little damage to its root system as possible. Unless the soil is very wet, each plant is individually watered to settle the soil around the root system and reduce the transplanting shock. Each cross combination is clearly labelled in the field with a stake or other durable label. Leaving an empty space between crosses clearly marks the change from one cross combination to another, to facilitate evaluations and selection. Soon after transplanting, a detailed field plan is made so that loss of labels or plants causes no confusion later.

8. SEEDLING MANAGEMENT IN THE FIELD

During the first two to three months after transplanting, seed-derived cassava plants are more delicate than their stake-derived counterparts. They may require special management, especially fertilization, irrigation and pest and disease control. After this initial establishment period, management requirements are essentially identical to those of stake-propagated plants.

8.1 FERTILIZATION

Stake-derived plants have a large nutrient reserve in the stake itself. This normally gives good establishment and early vigour even in poor soils. Seedling transplants, on the other hand, need to draw immediately on the soil nutrients. Root damage and other shock effects of transplanting may reduce the plant's ability to take up soil nutrients. To counter these effects, fertilizer at about 50 percent above the normal recommended levels should be applied. A split application at time of transplanting and at about three months is best to avoid toxicity effects in sensitive young seedlings.

8.2 IRRIGATION

Transplants must be kept well-watered for at least two months after transplanting, until new roots have established and penetrated deep into the soil profile. If irrigation facilities are not available it may be necessary to water individual plants manually. Water stress can be observed by yellowing and leaf fall, rather than wilting of leaves. Even though seedlings may survive after losing all their leaves due to drought, their establishment and growth will be greatly affected and they would regain normal vigour only after a long delay. Seedlings will normally lose some leaves after transplanting owing to other shock effects, even if water is adequate.

8.3 WEED CONTROL

Weeds can compete very seriously with the slow-to-establish cassava transplants. Pre-transplant chemical control makes the post-transplant weed control easier. Post-transplant control must be done either by very careful directed applications or manually. Young seedlings are more susceptible to herbicide damage than stakes and can easily be killed by a careless application. A pre-emergent herbicide at 50-75 percent of normal rate may be used. Toxicity and efficacy will vary with soil and climatic conditions, so trials should be carried out on surplus seed prior to treating breeding nurseries.

The safest choice is to hand-weed during the first two to three months, as necessary to avoid weed competition. As with most other management aspects of cassava seedlings, weed control practices after two to three months are identical to those for stake-derived plants. Due to the generally low plant density and lower shading of an F_1 planting, weed control may have to continue throughout most of the growth cycle.

8.4 PEST AND DISEASE CONTROL

Transplants should be observed daily for a few weeks after transplanting to assess their phytosanitary status. Early seedling establishment is most affected by stem- or leaf-cutting pests. As the seedling stems are thin and succulent they may be easily damaged by chewing insects such as crickets, or by slugs.

Crickets and slugs can be controlled with an insecticide incorporated into a bait placed around each plant. Special commercial formulations are also available in some parts of the world. At CIAT, Sevin® (carbaryl) in powder form is sometimes applied around the base of each transplant and this gives relatively good control.

One of the most important variations on pest control in seedlings as compared with stake-propagated plants is the result of differences in pubescence of the apices. Mature cassava plants vary widely in apical pubescence. For plants originating from stakes, these differences are generally consistent throughout all growth stages. Pubescence on the apices appears soon after sprouting (on clones with the

genes for this trait). Seedlings, on the other hand, appear to be uniformly non-pubescent in the first several weeks after germination.

This lack of pubescence means that virtually all seedlings are susceptible to thrips and may be more susceptible to mites than their stake-propagated counterparts. The breeder should not consider screening for differences in resistance to these pests until after about four months. If thrips or mites persist in high populations during the early months after transplanting, they should be controlled by insecticide applications (such as Sistemin®) every 10–14 days or according to the level of damage that appears. Unprotected seedlings in heavy thrips population conditions can be seriously damaged, to the point of remaining stunted throughout their entire cycle. Even in areas where thrips are apparently not a problem in commercial plantings, they can become severe when given an environment of uniformly susceptible seedling hosts.

Even moderate thrips damage may cause loss of apical dominance and sprouting of lateral buds. The plant produces many stems and evaluation of its genetic potential is difficult.

9. COMPARISON OF SEED- VERSUS STAKE-DERIVED PLANTS

Understanding the differences between sexually and vegetatively derived plants is critical to planning selection in the F_1. Effective selection may be practised for characters that have high heritability and similar expression for the two forms of propagation. Conversely, the breeder should delay selection until later generations for characters that have low heritability or are expressed differently across the two forms of propagation (i.e. show genotype by form-of-propagation interaction).

During the first years of its programme, CIAT conducted a few detailed studies as part of breeding methodology development, comparing stake- versus seed-derived cassava plants. Comparisons are not easy to make, because propagation techniques do not yet allow for simultaneous comparison of seed and stake-propagated plants in the same year/location. They can only be compared across seasons, which also means a potential confounding of genotype x environment and genotype x environment x form-of-propagation interactions.

9.1 TOP GROWTH AND PLANT ARCHITECTURE

Early vigour of seed-derived plants is considerably lower than that of the same clone when stake-propagated, but seed-derived plants tend to catch up to stake-derived plants during a full growing cycle. CIAT measured a number of growth parameters for plants propagated from stakes and an open pollinated population of that clone's progeny grown from seed (Table 12.2). Seed-propagated plants generally branched later, i.e. they had fewer levels of branching and therefore a more erect architecture. As branching is the consequence of the initiation of flowering, it follows that seed-derived plants initiate flowering later and flower less frequently than stake-derived plants. The physiological basis is not yet understood, but the implication for breeding is that F_1 plants selected for good plant type may be excessively branched when vegetatively propagated.

Table 12.2 Comparisons between seed-derived and stake-derived plants for plant growth habit

	Seed-derived	Stake-derived
Plant height (cm)	210	186
Height of first branch (cm)	137	70
Branching levels (no.)	1.4	3.7
Source: Bolaños (1987)		

9.2 ROOT GROWTH AND YIELD

CIAT recorded extensive data on yield components in seedlings versus stake-derived plants in the mid-1970s. These data show a moderate correlation for total plant weight (r=0.80**) and harvest index (r=0.68**) and Kawano *et al*. (1978a) concluded that selection for yield via harvest index in the F_1 was effective.

There have been few controlled comparisons between transplanted and direct-seeded plants. In some situations a taproot may form in seedlings. Its presence may depend in part on soil structure or management factors, because it is not commonly observed either at CIAT or IITA (S.K. Hahn, personal communication). When it occurs, this taproot may penetrate deeply into the soil and be the principal starch storage organ. If seedlings are grown first in containers and then transplanted, the dominance of the taproot is often destroyed, either in the container itself or during transplanting. Storage root formation is then similar in pattern to that of stake-propagated plants, with no central, dominant taproot.

There is evidence that root bulking begins earlier in seedlings than in stake-derived plants (Bolaños, 1987). This has important implications from two perspectives: for selection in the F_1 plants and for the possibilities of seed-propagated commercial plantings. The earlier initiation of bulking may mean that F_1 plants can be harvested and selected earlier than their stake-derived counterparts. If production from true seed becomes commercially feasible, one of its advantages could be the potential for early harvest (see Chapter 24).

9.3 PEST AND DISEASE RESISTANCE

The differences between seed- and stake-derived plants with regard to formation of pubescence and the implications for thrips and mite resistance, have already been described. Similar comparisons for diseases, or for pests where resistance is unrelated to pubescence, have not been made. At the Instituto Agronômico de Campinas in São Paulo, Brazil and at IITA, heavy selection pressure is applied for resistance to CBB in the seedling populations. However, quantitative information does not exist on the similarities or differences in reaction to the two forms of propagation. At IITA, seedlings affected by CMD are rogued, on the assumption that susceptible seedlings will also be susceptible when propagated from stakes. Although this seems a logical assumption, the data demonstrating the efficacy of this selection are not available.

9.4 ROOT QUALITY

CIAT evaluated seedling plants semi-quantitatively for root dry matter and compared that with stake-propagated plants in the single row trial. The correlation was highly significant ($r=0.48^{**}$), although only moderate success could be expected in selection at this level (CIAT, 1980).

IITA routinely screens seedlings for cyanogenic potential and rejects those with unacceptably high levels. Low correlations between the two generations suggest this to be a strategy with, at best, only intermediate effectiveness.

9.5 CONCLUSIONS

Early experiments clearly showed that many traits of agronomic importance for cassava are expressed in seed-derived plants in a similar way to stake-propagated plants. Apparent exceptions are branching habit, apical pubescence, early root bulking and tap root formation. However, all of these, with the exception of branching habit, affect primarily the early growth stages and effective selection can be made among plants at full growth cycle. For branching habit, the interaction effects for genotype x propagation form seem to be insignificant. During selection the breeder can mentally adjust for the difference between seedling plant type and clonal plant type, in order to effectively select for this trait. Factors other than genotype x propagation method interaction place greater limits on the effectiveness of selection of the F_1. The principal of these factors will usually be low heritability due to small plot size (individual plants).

10. EVALUATION AND SELECTION IN SEGREGATING POPULATIONS

Two general classes of information are derived from a segregating population: performance of families and performance of individual seedlings. Each type of information is useful for distinct purposes, but both types of evaluation can be carried out within the same populations. These evaluations are not mutually exclusive but rather are complementary. The overall performance of families is mainly useful as a way of quantifying breeding values of parents, i.e. those parents with highest genetic value will produce the best-performing progeny. The performance of individuals is used mainly to select best plants for the next generation of evaluation (usually a single row trial), with the expectation that these selected genotypes will continue to perform better than the population mean. The evaluation of individual and family performance serves distinct purposes, but can be (and should be) accomplished within the same F_1 trial.

10.1 FAMILY EVALUATIONS

A family evaluation is a measure or an estimation of mean performance of a group of related individuals. Most often in the case of cassava breeding, this will be a half-sib or full-sib family. The purpose of such evaluations is essentially two-fold.

Firstly, family performance, because it is based on a number of individuals, has a higher confidence level than an individual plant evaluation. The evaluation of an individual (subjective or quantitative) can be reinforced by the evaluation of its half-sib or full-sib relatives. As an example, an individual plant appearing resistant to mites, if it is in a family of other plants all showing high damage levels, might be strongly suspected of being an escape rather than having genes for resistance. Lower confidence would be placed on the genetic resistance of this plant compared to one of similar damage levels found in a family of all undamaged plants (given that both families are grown under the same level of mite pressure). Thus, family evaluation produces additional information for individuals within the family by virtue of some degree of genetic relationship.

Secondly, a family evaluation provides a means of assessing parental values; i.e. good progeny come from parents that have combined well genetically. Analysis of the mean performance of various sets of progeny in which a given parent clone has participated gives an estimate of general combining ability of a clone, while the performance of progeny of one clone combined with another gives an estimate of specific combining ability. The family evaluations help the breeder to choose the parents most likely to produce the best F_1 progeny and thereby continually upgrade the parental gene pool.

A family evaluation is most accurately done by averaging measures from individual plants within the family. However, even for moderate size F_1 populations, this can be quite tedious and time-consuming, depending on the number of different characters to be evaluated. As an alternative, with a little practice one can learn to carry out *eyeball* averaging evaluations of plants in a family, for many characteristics. As a simple example, the most precise evaluation for family mean plant height will be the mean of measures from each individual. The more rapid alternative is to subjectively assign an average height based on a visual overview of the family. The same procedure can be used for most metric traits. The method would not be appropriate for root dry matter or cyanogenic potential, for example. In addition, a subjective evaluation, on a 1-5 scale (from low to high expression of a given trait), is sufficiently accurate for most traits at this stage of selection. The procedure is to try to observe a family group of progeny as a unit and assign a mean value for each character of interest. The value may be relative (as compared with other families in the nursery, or with standard checks), or on the basis of established rating scales. Rating scales work especially well for pest and disease resistance, but can even be applied to root yield.

A meaningful family evaluation requires at least several plants. The mean trait expression of the sample should be close to the mean of the population, i.e. the theoretical mean of all possible genetic combinations. Ideally one would like to have 20 or more plants to make a reliable family evaluation, but if a family has as few as five or six plants, an evaluation can still provide useful information. Fewer than five plants allows too much random variation in the sample and a family mean evaluation will have little

meaning. To be most useful as a tool for selection of parents, family evaluations should be accumulated over years. Consistent, repeated family evaluations involving a particular parent clone will give a good level of confidence of that clone's usefulness as a parent.

Some breeders may find it difficult to adequately distinguish among family means, such that this information would be useful as a breeding tool. This may be the case if the environment does not include the appropriate level and balance of stresses (either too high or too low), or the genetic variability of the populations is narrow. A more reliable means of obtaining family values is to propagate all individuals in the F_1 (apply no selection pressure) and evaluate them in a trial where each genotype is represented by several plants (such as a single row trial). While this method will allow more reliable family evaluations and hence calculation of parental breeding values, it also requires considerably more resources, especially for large F_1 populations.

10.2 INDIVIDUAL PLANT EVALUATIONS

10.2.1 Evaluation techniques

As cassava is vegetatively propagated, genetic effects are fixed over generations. Insofar as the breeder can reliably identify those genetic effects on an individual plant basis (as opposed to environmental influence), these values will be valid for that clone for all subsequent generations of vegetative propagation. The difficulty is, that on an individual plant basis, heritability for most traits will be very low. The breeder will probably not want to use time compiling large amounts of data on thousands of plants for traits of low heritability.

In fact, it can be argued that for purposes of routine selection it is probably often not worthwhile to take any quantitative data on individual plants. A subjective evaluation by the breeder and a simple *keep* or *reject* decision at harvest may be just as effective as any measurements. An example could be a type of simple, integrated and subjective evaluation that answers the question, "How much do I like this plant?" This can be based on a rating of 1 (excellent overall rating) to 5 (very poor overall rating) (or vice versa, as preferred). For somewhat more information to aid in selection, one can separate this evaluation into the top part of the plant (plant form, quality of stems for stake production, leaf retention, pest and disease damage) and roots (yield, root form and colour, root attachment). Trials at CIAT showed that these very simple, rapid, integrated evaluations actually gave a better prediction of yield in advanced trials, than did yield of the individual plant itself (see following section).

For any situation, the breeder should conduct studies to determine the efficiency of selection for various traits, across generations, so as to spend time evaluating only those traits with intermediate to high broadsense heritability. For a skilled breeder, quantitative measures on individual plants in segregating populations may be unproductive and unnecessary as a part of routine selection. If trial data are to be used for additional purposes, such as inheritance studies, then clearly it is important to take a more comprehensive set of data in the F_1.

10.2.2 Selection criteria

The goal of selection in the F_1 is to identify genetically superior plants. The criteria for selecting or rejecting individual plants will have to be adjusted to the generally low heritability of characters in the F_1 generation. The breeder's first objective should obviously be to discard inferior plants, for example, those with poor performance for intermediate to highly heritable traits.

Selection of individual F_1 plants is normally based on a combined expression of adaptation, plant type, pest resistance, root form and quality, harvest index and total biomass, with less emphasis on yield, except to eliminate those at the very low end of the scale. Higher confidence of selection can be assumed when family selection is combined with individual plant selection, where plants are selected mainly from those families whose overall performance is above average.

One of the best ways to succeed in selecting superior genotypes is to understand how trait expression in individual plants of the F_1 compares with expression in advanced trials, or better yet, in farmers' fields.

In other words, one needs to understand the broadsense heritability of the traits of interest, specifically for comparison between evaluation sites and the target environment. Probably these values will be so specific to a given set of germplasm and a given set of environments, that each programme that manages segregating populations should carry out these evaluations for their specific conditions. At the same time, it is possible to obtain some general guidelines from these types of trials carried out elsewhere.

CIAT compared single-plant performance with that of the same clone in replicated yield trials, in some of the main selection sites in Colombia (Table 12.3).

Table 12.3 Linear correlations between single plants in a segregating population and the same genotype in advanced yield trials, in three sites in Colombia

Traits in advanced yield trials	Traits in the segregating population (individual plants)		
	CIAT	Media Luna	Carimagua
Root yield	Root yield		
CIAT	0.20	0.28	0.03
Media Luna	0.21	0.44**	-0.18
Carimagua	0.09	-0.14	0.58**
Root yield	Harvest index		
CIAT	0.04	0.07	-0.06
Media Luna	0.31*	0.35**	0.11
Carimagua	-0.09	-0.16	0.18
Root DM	Root dry matter content		
CIAT	0.77**	0.45**	-0.05
Media Luna	0.46**	0.75**	0.49
Carimagua	0.21	0.16	0.19
Root yield	Subjective root evaluations		
CIAT	0.35**	0.27	0.08
Media Luna	0.30*	0.52**	0.14
Carimagua	0.06	-0.10	0.62**
Root yield	Subjective foliage evaluations		
CIAT	0.33*	0.26	-0.17
Media Luna	0.11	0.53**	0.05
Carimagua	0.06	0.30*	0.58**
Source: CIAT (1985)			

Root yield and harvest index of individual plants were moderately successful in predicting root yield in advanced trials, but mainly within a site. Neither could be used reliably to predict yield at a different site. This indicates that yield or harvest index in the F_1 can be selected with limited success, probably at a level that allows the breeder to successfully eliminate the very poorest performers.

Root dry matter was successfully selected at CIAT headquarters and in Media Luna, but not at Carimagua. As the population in question was broad-based and not selected specifically for the high disease pressure environment of Carimagua, there was too much disease pressure to allow expression of genetic differences in root dry matter. It does seem, however, that breeders could successfully select for root dry matter in the F_1 generation as long at the population in question is reasonably well-adapted to general environmental conditions.

Both the subjective foliage and root evaluations described in the previous section proved to be moderately reliable predictors of root yield in advanced trials. As with root yield and harvest index, their values were mainly *within* sites, while correlations *across* sites were mostly insignificant.

Other traits that can be somewhat effectively selected in the F_1 are: resistance to several pests and diseases, especially when uniformity of pest or inoculum pressure is high; plant height and branching habit; and cyanogenic potential. A few simply-inherited traits can be selected very effectively in the F_1, such as external root colour and root flesh colour.

10.2.3 Selection intensity

Selection intensity is determined by the confidence the breeder has in the selections he or she makes, the proportion of the population of plants expressing desirable gene combinations, the rate of genetic advance desired and the level of variability sought in the selected population.

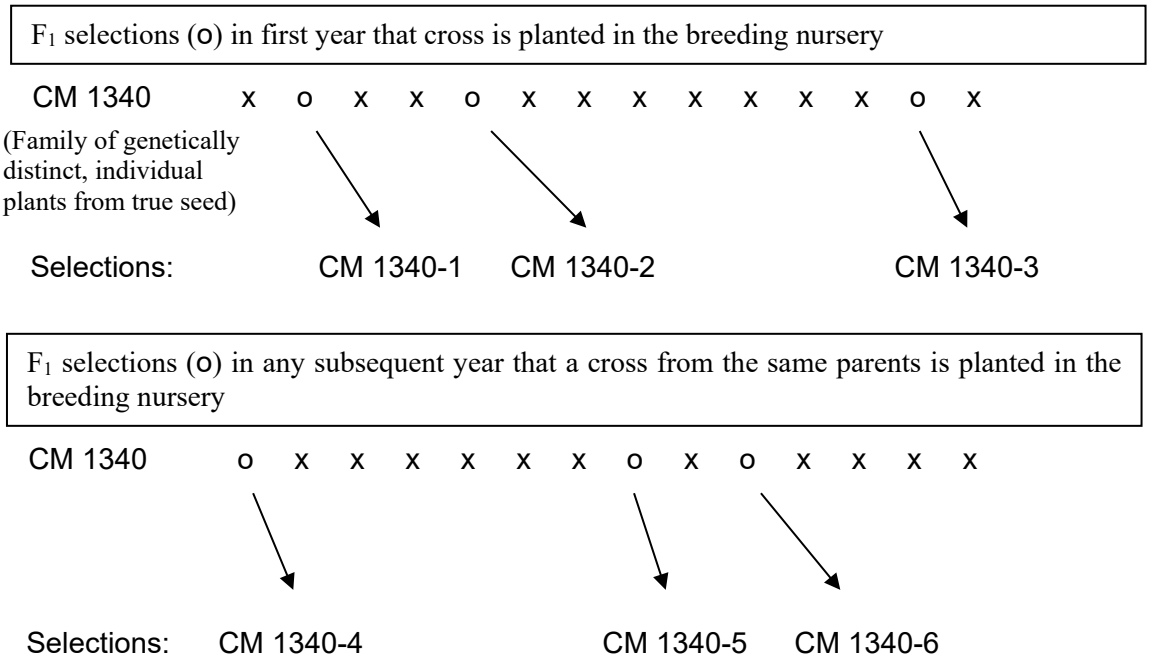
Normally, if the F_1 is planted in a site that allows good expression of the traits of interest (growth and adaptation, resistance, yield, quality) then 80–90 percent of plants can easily be discarded based on unsatisfactory performance. On the other hand, if the site is less representative of the target selection area, selection intensity will be low. If the site characteristics allow little confidence in selection, the breeder may choose simply not to make any selection in the F_1 and pass all individuals to the first clonal generation in a more representative site.

10.2.4 Procedures

If the F_1 field has been designed well, individual plant selection can be rapid and efficient, with little complication and chance for errors. The field sequence of harvest and selection should follow the same pattern as the planting or transplanting. Normally this means proceeding progressively, plant by plant, through each family in the same order as those families are planted in the field and listed on the field sheets.

For the most complete observation of each plant, it is recommended that the harvest, selection and coding of plants should proceed one row at a time. When adjacent rows are harvested, plants cover each other, making visual assessment as well as mobility difficult. As each plant is selected, it should immediately be tagged with an identifying code, which is normally the cross code plus a sequential selection number. Using the coding scheme from CIAT as an example (Figure 12.2), three plants selected from the cross CM 1340, would be labelled as follows: CM 1340-1, CM 1340-2 and CM 1340-3. This sequential assignment of selection numbers is followed regardless of the position of selected plants within a family. The number of selections from each family is noted on the field sheets for later reconfirmation of all selections made.

Figure 12.2 Method for codifying selected individuals in F_1 populations



If codes are repeated across years for the same cross combination made at different times, the assigning of codes of selected plants will need to take into account the possibility of previous selections from that cross. In this case, selection numbers will be cumulative, so that no code is ever repeated for two distinct genotypes.

Cutting stakes from selected plants may be done immediately if planting of the single-row trial is to be within a few days. Alternatively, stems from selected plants may be stored under cool, shaded conditions to preserve planting material for later.

11. INFORMATION MANAGEMENT

Information management for segregating population (F_1 and F_1C_1) management and selection is markedly distinct from later selection stages. At the F_1 stage, data are generally aggregated at the family (cross combination) level, while in subsequent stages of vegetative propagation, individual clones are identified and evaluated. These distinctions influence design of fieldbooks and files for data storage and analysis.

Fieldbooks for F_1 trials should include a general trial description (site description, planting date, objectives), pedigrees, number of seeds (F_1) or stakes (F_1C_1) in each cross, evaluations made on a family basis and selections made from each cross. Examples of fieldbook formats are given in Figure 12.3.

In the F_1 the breeder is managing a large number of newly-assigned identifying codes. The information management system must be designed to assure a high degree of accuracy and ease of use in this process, both for field operations and for fieldbooks. Simple and logical codes, a built-in system of data verification and thorough training of labourers and technicians all contribute to these objectives.

Figure 12.3. Form to manage data for harvest of F1C1.

PROJECT: _____ PROJECT CODE (1 2 3 4) YEAR (5 6) FORM NO. (7)

CROSS CODE/ PARENTS	Family mean values (semi-quantitative)						Plant number in F1C1 (top number)																	
	Branch levels (1-5)	Plant height (cm)	Root length (1-3)	Yield (1-5)	Fol. eval. (1-5)	Root eval. (1-5)	Selection Number (bottom number)																	
CM 9864	MCol 2215 X CM 4316-4						18	21	33	46	48													
	1	215	2	3	4	3	1	2	3	4	5													
CM 9865	MBra 12 X CM 6743-6						3	7	15	30	36	42												
	3	180	2	3	2	4	1	2	3	4	5	6												
CM 9866	MMex 59 X CM 6743-8						4	11	14	17	25	36	39	46	49	53	61	68						
	2	225	1	5	4	4	1	2	3	4	5	6	7	8	9	10	11							
CM 9867	MVen 125 X MBra 138						23	43																
	2	190	2	2	3	2	1	2																
CM 9868	CM 321-188 X CM 6743-6						12	14	16	21	32													
	1	180	3	3	5	3	1	2	3	4	5													
CM 9869	MCub 74 X MPar 199						4	8	11	19	21	30	37	46	48									
	2	195	2	4	4	5	1	2	3	4	5	6	7	8	9									

Chapter 13. Managing preliminary through advanced trials

In cassava, selection stages can be broadly divided into seed-propagated and vegetatively propagated generations. Although there may be several generations of selection (preliminary through advanced trials) beyond the seedling generation, their management is usually similar. Chapter 3 describes overall design of a selection scheme and Chapter 12 covers seedling-stage management and selection. This chapter covers general trial management and selection from single row trials through the final stage before regional trials, at which point the breeder may no longer have exclusive or direct control over trial management.

1. DESIGNING AN EVALUATION STRATEGY

Selection is commonly performed in a planned series of steps or stages, which begin with a large number of genotypes represented by a few plants (small plots) and progress sequentially towards a few genotypes planted in large plots. The quantity and the precision of observations increase as selection advances. There are limitless possible variations on this generalized procedure and a breeder must choose one he or she believes most appropriate for the specific situation. Figure 13.1 illustrates the generalized sequence and land requirements, from the segregating F_1 through regional trials, based on an initial population of 10 000 seeds.

The essential objective of establishing a series of selection stages is to move as quickly as possible to the final outcome of a relatively few selected genotypes, with a high level of confidence that these are in fact the best ones from within the range of available genetic variability. Design of an evaluation scheme is as vital to success in breeding as is the setting of appropriate objectives. The scheme should be well-planned from beginning to end before first selection trials are planted. New data may indicate adjustments and modifications, but the underlying philosophy and framework will be there as a guide.

At the two extremes of design, one could envision on the one hand, selection of the few superior genotypes at the F_1 stage and immediate propagation for advanced trials; and on the other hand, a long series of selection stages in many environments in order to have total confidence in the genetic potential of final selections. In virtually all cases, a strategy somewhere between these two extremes will be most effective.

Characteristics of the target area strongly influence an evaluation strategy. Some principles to consider and guidelines to develop an evaluation scheme are listed below and discussed further in those chapters concerning specific breeding objectives

- Each evaluation stage should be assigned a given set of selection objectives. These objectives will often be cumulative; that is, when a given objective enters into the scheme, it will usually continue as an objective through the remaining stages.

- The number of sites for a given selection stage should be no more than what is required to identify, with an acceptable confidence level, the genetic component of expression of the traits of interest. If the selection goal is virus resistance and high levels of confidence (repeatability) are achieved with one selection site, it is wasteful of resources to include more sites.

- In the earlier stages of selection, target characters should be highly heritable, while in later stages, multisite evaluation, larger plot size and replication aid in identifying less heritable traits.

- As a general rule, stages of selection (up to the regional trial stage), should maintain roughly equal land area within a given site. This means that the decrease in number of genotypes will be approximately proportional to the increase in number of plants represented by each genotype (i.e. plants per plot x number of replications).

- A minimum of data should be taken on discarded genotypes, except where the data are required for specific breeding studies.

- Only data having a reasonable confidence level should be taken. For traits of low heritability, it is usually a waste of time to take data on individual plants in segregating populations.

- In the early selection stages, rapid, subjective evaluations by trained personnel are often as useful as quantitative measures.

- In the early selection stages the breeder is often most interested in discarding clones that can be identified readily as deficient. Emphasis should be on identifying highly heritable characters as a basis for this elimination process.

Figure 13.1 Hypothetical illustration of land use requirements through stages of selection, for a nursery beginning with 10 000 F_1 seeds

Stage	Plot size (m^2)	Reps per site x no. of sites	No. of entries (selections)	Area occupied (ha)[a]
F_1 seeds	(in flats)	(greenhouse)	10 000	0.001
		20% mortality ↓		
F_1 seedlings	2 m^2	1 x 1	8 000	1.6
		5% selection ↓		
Clonal trial	20 m^2	1 x 2	400	1.6
		10% selection ↓		
Yield trial: yr. 1	40 m^2	2 x 3	40	1.0
		40% selection ↓		
Yield trial: yr. 2	40 m^2	3 x 3	16	0.6
		50% selection ↓		
Regional or on-farm trials: yr. 1	50 m^2	4 x 4	8	0.7
		50% selection ↓		
Regional or on-farm trials: yr. 2	50 m^2	4 x 8	4	0.7
Total area, if each stage is planted each year in overlapping generations:				6.2

[a] *Includes allowance for alleyways. Clones evaluated in years 1 and 2 for yield trials and for regional trials would normally be joined into a single trial*

The design of selection depends both on some of the inherent characteristics of the crop (e.g. rate of multiplication, complexity of objectives, the environments available for evaluation) and the genetic control of traits of interest. As criteria increase in complexity, the more selection stages and/or evaluation sites will be required for obtaining adequate confidence levels. A major limitation to moving rapidly to advanced trials is the propagation rate of cassava. This may vary considerably with both genotype and environment, but on average is about 1:10 to 1:15. However, even selection schemes based

on average multiplication rate might involve some difficulties. Below-average genotypes will not produce sufficient planting material. One should plan somewhat conservatively, also taking into account missing plants and losses during stem storage. Use of lower quality, immature stem pieces to extend available planting material should also be minimized because it will bias results on a genotype's potential.

If a system relies on 15 stakes produced from each plant and 25 percent of selected genotypes produce fewer than that, there will be a significant proportion of trial entries having either smaller plots or fewer replications. These discrepancies can be intensified through various selection cycles and make trial management very complicated and results difficult to analyse. Normally it is best to maintain uniform plot size and number of replications within a particular stage of selection, though there are certain to be occasional exceptions. For many situations, an evaluation scheme can be based on a multiplication rate of about 1:10.

Various rapid propagation schemes to increase the multiplication rate of cassava are widely used (Chapter 22). However, their greatest utility is for multiplying one or a few selected genotypes at the pre-commercial stage. No programmes currently use rapid multiplication as a means of multiplying hundreds or thousands of entries in a routine breeding programme. The resources required, when rapid propagation is applied to large numbers of genotypes, are simply prohibitive. More modest systems, however, such as slightly decreasing the average length of the stake from 20 to 15 cm, could be a viable option.

2. NOMENCLATURE

For convenience, the breeder will want to give names to the selection stages he or she establishes. These may be as simple as consecutive numbering, but some more descriptive system is generally preferred. Names should broadly indicate something about trial design or objectives. For example, the names Single Row Trial, Preliminary Yield Trial and Advanced Yield Trial are easily associated with the precision of the given stage of selection. In normal daily use of these categories, abbreviations are conveniently used (SRT, PYT and AYT in the preceding examples).

3. PRE-PLANTING PLANNING

At a minimum, the planning and preparation required prior to planting trials include: site selection, land preparation, experimental design and preparation of the materials for planting. Chapter 7 covers several points pertaining to site selection. If the field layout is not known prior to arriving for planting, it may not be possible to pre-plan trial design precisely. If fields are irregular in shape, or some areas need to be avoided because of soil abnormalities, these adjustments may need to be made just prior to planting. If possible, it is useful to inspect visually the previous crop in a field to be used for a breeding nursery. Some types of variation are best observed in the growing plants so that problem areas can be avoided. Usually, fieldbooks should not be printed until after planting, in case modifications to the planned design are required.

Management of planting material can have substantial influence on subsequent performance. Storage conditions (length of stems, ambient light, temperature and humidity), length of storage period, chemical treatment prior to storage, planting position and others may all influence performance. Breeders should provide the best possible storage conditions for experimental materials.

Plot size and arrangement are crucial for effective selection. The breeder must take into account effects of inter- and intragenotypic competition, replication to separate genetic from environmental variation and efficiency of labour and land use. The single-row trial usually includes large numbers of genotypes and the principal objective is to eliminate material that can easily be identified as inferior, based on traits of relatively high heritability. In order to reduce the confounding effects of intergenotypic competition, wide spacing between rows (1.5-2 m) should be used. Number of plants per row often ranges from five

to ten. At CIAT the advanced yield trials use a 3 x 3 harvested plot size, three replications and several sites. IITA recommends a 4-row plot (4 x 12 m; 2 x 10 m harvested) with four replications, as the most satisfactory for precision, land use and cost effectiveness (Hahn *et al*., 1977).

Advanced trials and some types of preliminary trials include border rows to eliminate the effects of intergenotypic competition in the harvested plot. A rough extrapolation to commercial yield in tonnes/ha may be made at this stage only when unharvested border rows are left between adjacent plots. In most circumstances a single border row is sufficient. Additional border rows may be used to satisfy needs for multiplication of planting material. The squarer the plot layout, the more efficient will be the use of space for the experimental area. This can be visualized in some of the possible arrangements for plots where there is one border row on all sides (Table 13.1).

Table 13.1 Comparison among plot designs for land use efficiency (percent harvested area), where each plot has a single border row on all sides

Total plants	Plot configuration	Border plants	Harvested plants	Harvested area (%)
36	3 x 12 6 x 6	26 20	10 16	28 44
48	4 x 12 6 x 8	28 24	20 24	42 50
60	4 x 15 6 x 10	34 28	26 32	43 53
64	4 x 16 8 x 8	36 28	28 36	44 56

4. TRIAL MANAGEMENT AND FIELD SUPPORT

4.1 STAKE MANAGEMENT

In a breeding programme, each successive stage of selection usually involves stake multiplication, often on a scale of 10:1, or more. This in itself may limit ability to select for optimum stake quality, especially in less vigorous clones. There may be no completely satisfactory solution to this problem. The breeding scheme should, however, plan somewhat conservatively on multiplication rate, such that stakes of good quality can be obtained from most of the selected lines, in order to advance to the next stage of selection.

Shipping stakes among trial sites, to evaluate clones across environments, involves both expense and risk. The expense results from the bulkiness and perishability of the material and the risk is primarily related to the potential transport of pests and pathogens among regions. Insofar as possible, the breeder should select a relatively clean site for producing stakes that will be distributed regionally. Additionally, plants and harvested stakes from this site should be given special care to minimize pest and pathogen infection or infestation.

On the other hand, stakes for experimental clones being tested within a region should be produced continually within that same region. This should not only be the easiest and most economical strategy, but one that also helps assure that selections have the capability to produce high quality stakes consistently under the environmental conditions for which a new variety is intended.
Stake management becomes particularly complicated when the breeder is trying to evaluate clones across distinct planting seasons (e.g. at the beginning and at the end of the rainy season) or for early maturity. There are basically two options for obtaining stakes for the interface between two cropping seasons. First is the situation where a crop is harvested and stakes from selected clones are cut directly

for the following cycle. The second possibility is where harvest of one crop precedes the subsequent planting by a lengthy period, which requires stake storage. Stake storage implies additional management questions and there are many possible alternatives. The question must be asked, first, whether the storage management systems considered have any interaction with genotype; for example, do clones respond differentially to the systems? If so, the breeder should choose a system that most closely resembles farmer practices for stake storage so that there will not be unexpected problems arising when new varieties are tested on-farm.

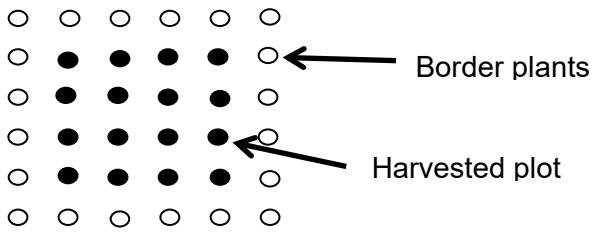
Stakes may also be stored *in vivo* by utilizing unharvested border plants, maintained up to the time of planting of the subsequent cycles. This may be the best way to maintain good stake quality, but also has some disadvantages. Often, the practice of leaving trials in the field for any length of time after harvest is not possible if the land is needed for another cropping season. In areas where planting takes place soon after the beginning of the rains, after a long dry season, starch reserves in the stems are quickly depleted by the flush of new foliage. Stakes from such plants may have sprouting problems, lower vigour and lower yield compared even with stored stakes, cut at a time of higher starch reserves. Still another drawback of utilizing border rows as a source of the subsequent season's planting material is that it reduces considerably the amount of planting material available, by eliminating the harvested plot as a source. Each breeder will have to weigh the pros and cons of each system and design one best suited to the local situation (Figure 13.2).

Experience with stake treatment is mixed: sometimes there are noticeable effects on plant health and yield and sometimes there are not. Pathologists generally recommend chemical stake treatment as a low-cost insurance policy. This makes sense in a breeding programme, where lost materials can severely disrupt germplasm flow through selection cycles. Treatment may be selectively avoided in limited cases, for example in an evaluation nursery for resistance to root rot. Most pests and pathogens can, however, still be evaluated without interaction effects of stake treatment. CIAT recommends mixtures depending upon the complex of pests and pathogens present (Table 13.2).

Table 13.2 Examples of chemical treatment for planting material (five-minute bath)

Product Name	Active Ingredient	Quantity/litre H_20
1. For sites with problems of root rot caused by *Fusarium* and *Diplodia spp*. and for general use except where cassava bacterial blight is present		
Benlate	Benomyl	6 g
Orthocide	Captan	6g
Sistemin	Dimethoate	2 cc
2. For sites with superelongation disease and/or cassava bacterial blight		
Orthocide	Captan	4 g
Difolatan	Captafol	4 g
Sistemin	Dimethoate	2 cc
-or-		
Dithane	Mancozeb	3 g
Difolatan	Captafol	4 g
Sistemin	Dimethoate	2 cc
Note: Zinc sulphate can be included in any of these mixtures, at 20 g/litre water, to correct for soil zinc deficiency in areas where is occurs		

Figure 13.2 Options for sources of planting material (stakes) in breeding trials



Strategy for harvest and stem storage	Plants available for stakes	Comments
Store stems from harvest until next planting	36	• Maximizes availability of planting material • Risk of storage losses
Leave border rows intact to use for next planting and store harvested stakes	36	• Maximizes availability of planting material • May be lack of uniformity of quality of planting material due to mix of stored and fresh stakes
Leave border rows intact to use for next planting and discard harvested stakes	16	• Reduces availability of planting material • Minimizes or eliminates risks of storage losses • Stake quality may be reduced even *in situ*, for example if rains cause flush of foliage growth and reduced stem carbohydrates
Introduce stakes from separate multiplication fields	Variable	• Simplifies procedures at harvest, since stems do not have to be prepared • Is likely to be best option for well-managed, uniform stake production • Requires additional land, management and other resources

4.2 AGRONOMIC PRACTICES

The choice of agronomic management may impose crucial selection pressures on cassava. For many years, most breeders believed that selection nurseries should be managed under optimum conditions: high fertility, good water management, complete pest and disease control and good weed control. This philosophy developed during the period when it was generally accepted that for new crop varieties to be successful, they had to be combined with luxurious agronomic conditions. While this philosophy continues to reign for breeding programmes of many crops, especially in temperate areas, it seems less appropriate as the world looks towards development of more sustainable, less input-demanding agricultural practices. It seems especially inappropriate for crops like cassava which are almost universally cultivated under stress (physical and/or biological) conditions. The debate is certain to continue and the evidence at this stage is not strong enough to recommend a given strategy

unequivocally. However, experimental results and empirical evidence suggest some appropriate guidelines. Several of these will be addressed further in later chapters covering specific breeding objectives.

Breeders are always plagued by the knowledge: (1) that at least through the intermediate stages of selection, it is very difficult to select under different sets of management conditions, and (2) that certain genotypes being discarded may in fact have been promising given different conditions. The general consensus of breeders is that agronomic practices in breeding trials should be relatively fixed, with the main variable being genotypes. In some situations, testing under variations in agronomic practices may be justified, but these should be limited to one or very few key variables. A common practice is to compare experimental materials under farmer management and under a recommended improved management package. One difficulty with this approach is that it is rarely possible to identify specific causes of genotype by management interaction when several practices are combined. A good practice is to select in a parallel or alternating system, in two sites, at the more and less favourable ends of the range for a target environment (see Chapter 14).

Defining sites as low stress or high stress is not sufficiently precise for selecting stress-tolerant varieties. Certainly, varieties adapted in a site that is high stress because of disease pressures may be very poorly adapted in a site of equally low yields, where the main constraint is drought, for example. Hence, a less-favourable selection site should be characterized by an appropriate balance of stresses common in the region, rather than a high level of only one of them.

As a broad generalization, the breeder should select under a set of agronomic practices similar to those recommended for farmer adoption. This assumes of course that these recommendations are appropriate, i.e. that they are economically within the reach of farmers, have been tested for farmer acceptance and suitability, are profitable and are environmentally sound. Improved cultural practices need not mean high input agronomy. It is contrasted however with traditional agronomic practices where productivity is limited not by variety but by nutrient, light, water or biological constraints.

4.2.1 Land preparation

Breeding trials should be conducted with good, uniform land preparation, appropriate to the region. Appropriateness is defined by factors such as slope and need for erosion control, soil structure and availability of human, animal or mechanical land preparation instruments. It is usually not necessary to duplicate regional land preparation methods. If farmers normally prepare their land with oxen, it may still be appropriate to prepare experimental fields mechanically, to gain time and save on labour costs. The important consideration is that the method adopted does not interact with genotypes in such a way as to negatively impact progress in selection.

4.2.2 Cropping system

Whether, or at what stage, typical intercrops should be included as part of the design of selection nurseries is a controversial issue. Of course, in those regions where intercropping is unimportant, the decision is straightforward. In support of selecting under intercropping is the argument that this is the only way to be certain that the appropriate traits are being selected. As many of the interactions between intercrops are poorly understood (competition for light, nutrients, water, effects on weed and pest control), they cannot be adequately simulated, or selected for, except under actual intercropping systems.

The complications of adding an intercrop to selection nurseries are no small consideration. The appropriate intercrop will usually be determined by typical cropping systems in the region of interest. If one system predominates, the decision may be relatively uncomplicated. Where several systems prevail (a common situation) it may be very difficult to include all of them. Another important consideration is the possible interaction of intercrop genotype (within a species) with cassava genotype. Thus, if a cassava breeder is faced with the need for selecting for compatibility to intercropping with specific varieties and predicting what those variety characteristics will be in ten years, the task becomes very complex.

To minimize management complications for programmes struggling to stretch resources, early and intermediate stages of selection are probably best planted to monocropped cassava. At these stages, the breeder will be able to discard the obviously inferior materials on the basis of other over-riding criteria. At advanced stages, possibly as late as multilocation regional trials, it will be useful to plant at least some locations in an intercropping situation, if that is one of the breeding objectives. Successes in several breeding programmes have clearly shown that it is possible to select varieties suited to intercropping, under monoculture conditions. This, however, does not argue against the possibility of making even better progress if selection is practised under intercropping. For most programmes, this will be only one of many criteria to consider in breeding and as such, needs to be given an appropriate weight along with other traits.

4.2.3 Soil fertility

Soil nutrient status and response expected from fertilizer inputs are prerequisite data for planning of agronomic practices that are to be applied to breeding trials. As a general rule, purchased inputs for cassava production should be planned for relatively low levels and be accessible to the low resource farmers who make up the majority of producers. Selection for a region where no fertilizer use is anticipated does not necessarily mean that breeding trials should avoid fertilizer inputs. At a minimum, nutrients should be added at a replacement level to avoid soil depletion. Even if stress tolerance is an objective, selection under conditions of continual deterioration of soil quality is counter-productive. Usually the level of nutrient stress can be adjusted such that non-tolerant types can be identified and discarded, while allowing tolerant lines to express a good level of their potential. In some programmes, evaluation under both high and low nutrient levels is appropriate as a means of selecting for broad adaptation and input-responsiveness.

While low input use is the norm for cassava, due consideration should be given to the future requirements of an industrializing cassava sector. In many areas, especially in Latin America and Asia but increasingly also in Africa, new industrial uses will demand high and efficient productivity based on purchased inputs. This transformation can take place rapidly and breeders need to anticipate varietal requirements well ahead of grower demand.

4.2.4 Soil water status

Cassava is rarely irrigated and this practice is unlikely to be an acceptable recommended practice except in very specific regions or situations such as southern India. Sometimes, especially for off-season plantings, irrigation may be needed for sprouting and early growth. During extended drought, irrigation might be applied to prevent loss of valuable breeding material. Irrigation is often needed for the early establishment of seedlings, because the young plants are not very tolerant even of short drought periods. Drought stress at different periods of growth may differentially affect cassava genotypes. Probably distinct mechanisms come into play for tolerance to early versus late season drought or for extended versus periodic stress. Therefore, selection trials should be conducted under the same rainfall regime as is normal for commercial production. If there is more than one planting season, or if this is being promoted as a new management component, varieties must be selected under the recommended set of practices.

4.2.5 Plant density

Breeders often do not give enough attention to plant density for breeding trials. Recommendations for an optimum plant density are generally based on one or a few clones. If these are traditional varieties of low harvest index, recommendations may be inappropriate for the breeder's goal of more efficient plant types. Selection under varying plant densities is usually only appropriate at later stages of evaluation, so it will usually not be possible to identify materials best adapted to distinct densities at early stages. The data available on density trials have to be evaluated from this perspective. For those programmes having efficient plant type as one breeding goal, a plant density higher than normally recommended is probably most suitable for breeding trials.

4.2.6 Disease and pest control

It is only logical that pests and diseases targeted for a host plant resistance programme should not be eliminated from selection fields. Control may be desirable or even necessary for those that are not selection targets. A more common need is for enhancement of pest or pathogen populations to achieve suitable inoculum levels, or uniformity of distribution. Chapter 16 gives more detail on these principles.

4.3 THE DILEMMA OF MISSING PLANTS

Dealing with missing plants involves both agronomic and statistical considerations. The decision on population adjustment needs to be taken early in the season. This can be in the form of over-planting with later thinning, or of replanting a few weeks after planting, when unsprouted stakes are detected. Plant death may occur at any other time throughout the growing season as well. However, replanting beyond a few weeks after planting is ineffective, in that the later-planted individuals will compete poorly with their neighbours.

Perhaps the principal criteria determining whether replanting is appropriate, are: (1) whether plant loss has been differentially influenced among genotypes, and (2) whether factors causing reduced plant population should constitute criteria for selection. Some examples will clarify these criteria. If drought stress is common near planting time and the breeder notes large differences among entries in sprouting ability which he/she suspects are related to drought, replanting to adjust stand is probably inappropriate. Likewise, early differential mortality resulting from a regionally important disease might be used as part of the resistance evaluation rather than compensating by replanting. On the other hand, early mortality by herbicide damage in some plots probably would not contribute to more effective selection and replanting missing plants could be considered.

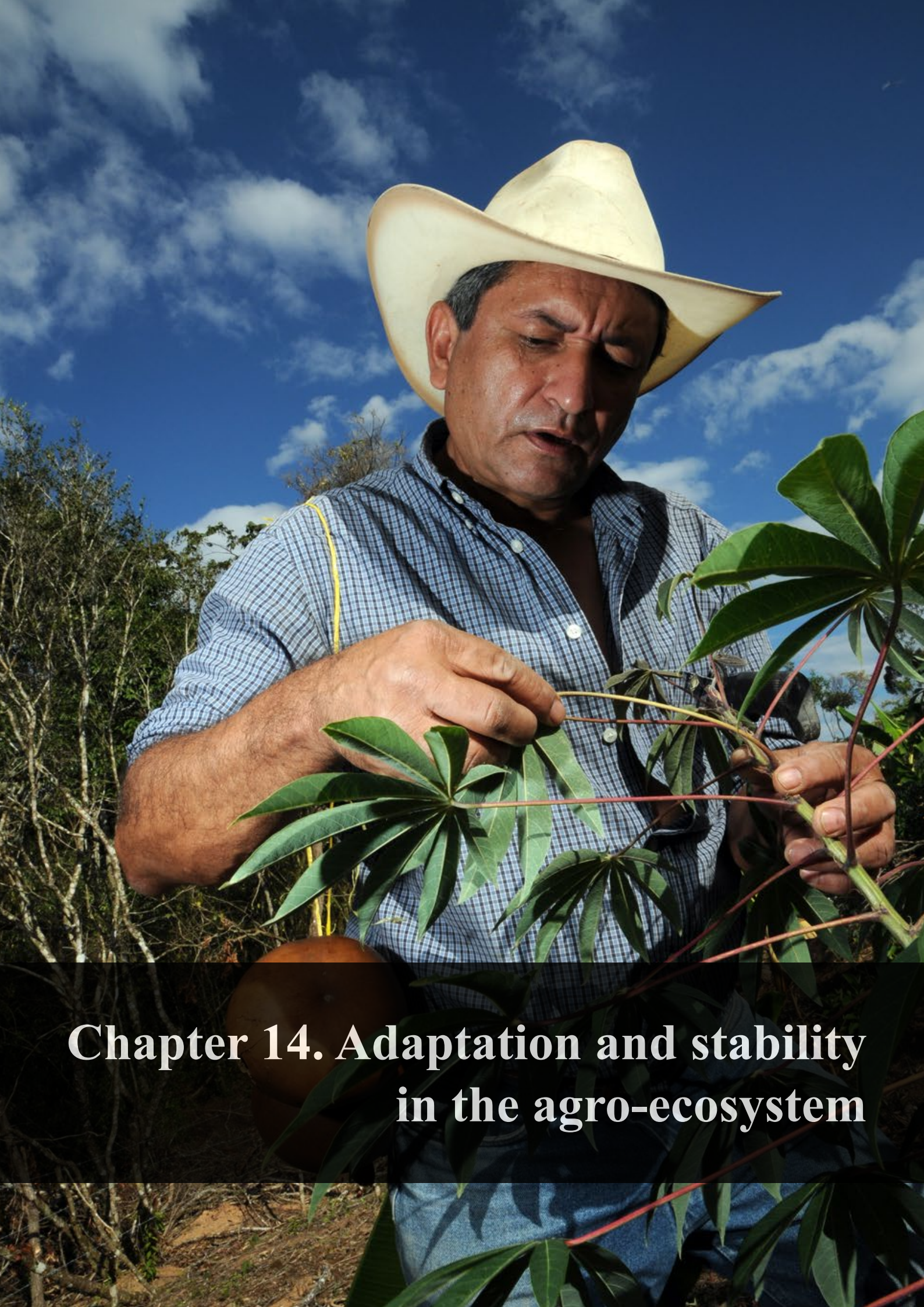
Effects of missing plants can be adjusted statistically at harvest, if appropriate. Whether or not this is appropriate is not always straightforward and the arguments follow a similar line to those for and against early stand adjustment. If the missing plants are a result of influences that are of interest in selection (e.g. common biological or physical stresses), there should be no statistical adjustment for stand. In addition, where an adjustment is made proportional to number of missing plants, estimated yields can be highly exaggerated due to compensation to lower competition among remaining plants. Using adjusted data would have just the opposite effect of that desired, with a tendency to select for plots with more missing plants. If plant loss occurs late in the growing cycle, from factors of no practical interest in selection (such as random theft), yield adjustment should be considered, especially if there has been insufficient time for neighbouring plants to compensate for yield.

In a relatively large programme with long-term continuity, adjustment for missing plants (either physically or statistically) may provide little if any advantage. This hands-off approach will help to eliminate clones with insufficient ability to vigorously establish a good stand. If a few clones are eliminated on the basis of environmental effects rather than genetic deficiencies, there is no great impact on the programme.

C. Iglesias and W. Fukuda (personal communication) used a simple and practical approach for partial compensation for missing plants, in a project for germplasm evaluation in Brazil. They developed an index based on the mean for yield calculated on a per area basis and calculated on a per plant basis. This gives some weight to the clone's ability to compensate for missing plants, but does not heavily penalize its inability to maintain a complete plant stand.

YIELD INDEX = [(yield per ha derived from yield per plant) +

(yield per ha derived from yield per plot)]÷2



Chapter 14. Adaptation and stability in the agro-ecosystem

1. AN EVOLUTIONARY PERSPECTIVE

In the evolutionary home of cassava, the Americas, landrace varieties of cassava typically have narrow geographic adaptation, and overall year-to-year stability of economic yield in their habitat of origin. Narrow adaptation has evidently come about as a result of evolution of relatively isolated gene pools within limited geographical areas. Wide separation of different cassava-growing regions, and isolation by geographical barriers such as mountains, oceans and deserts, greatly limited genetic interchange among gene pools. The gene pool within each region evolved very specific adaptation features to the combination and intensity of selection pressures in the region, but did not accumulate those genes for adaptation to factors not present. While this generalization is observed for most of CIAT's international collection, there are notable exceptions that have made significant contributions to breeding for wider adaptation.

In reality, cassava landrace varieties may be no more narrowly adapted on average than those of most other crop species. The apparent broader adaptability of individual varieties of other crops like wheat and rice, probably comes from the fact of generally more controlled and favourable growing environments. If one looks at the range of soil and climatic factors across which individual cassava genotypes are known to be adapted, it is difficult to visualize a similar range for most crops.

The nature of cassava's sensitivity to environmental variations was largely unappreciated until large-scale movement of germplasm across agro-ecosystems began to take place, especially in the late 1960s and early 1970s. It became apparent that new varieties would have to be considerably more widely adapted in order to lower cost–benefit ratios of breeding programmes. At the same time, it is desirable to maintain to a large degree, the local year-to-year stability of traditional varieties. The challenge is to achieve wider adaptation, maintain the high stability of traditional systems throughout the years without having to resort to high chemical inputs, and increase yield potential well above the modest levels of traditional varieties. The first steps in this strategy are to identify the factors causing instability and determine which are responsive to modification through breeding.

2. DEFINITIONS

General adaptation to the principal physical and biological components of the agro-ecosystem is basic for the success of any crop variety. Before the advent of plant breeding programmes with broad geographical objectives, most crops encompassed a myriad of varieties, each adapted to a relatively narrow ecosystem and often with locally preferred agronomic and quality traits. Commonly, breeders who begin their work with a local germplasm base are not fully aware of how complex a trait adaptation is, nor how many individual components need to be considered for overall good adaptation in an environment. This is often appreciated only after an introduction of exotic materials, when adaptation problems of one type or another arise. These problems were never previously considered important in the region. Lack of adaptation results in instability, fluctuations over time and/or space in the traits of interest. Usually stability is associated with yield, but may just as well refer to any other trait of interest. This chapter explores the major components of agro-ecosystem adaptation for cassava, and a philosophy and strategy for breeding for various types of stability.

Adaptation may be broad or specific. Broad adaptation refers to phenotypic plasticity, namely, the ability of a genotype to produce a phenotype, or several phenotypes, compatible with a range of environments. Pest and disease resistance is one set of factors that can contribute to broad adaptation. Specific (or narrow) adaptation describes a close adaptation of a genotype to a limited range of environmental variation. Such a genotype performs poorly outside this narrow range.

A distinction can also be made between genotypic and population adaptability. Broad adaptation of a population can result from specific and distinct subsets of genotypes in that population which are adapted to different environmental conditions. Overall population response across locations and years is similar, but in any given year some genotypes perform well and others poorly. Those that perform well or perform poorly in one year may be different from those that perform well or poorly in other

years, but the mean performance is the same. In cassava, either genotypic or population adaptation is possible in situations where clonal mixtures are used, but only genotypic adaptation occurs in monoclonal culture.

Stability describes the reaction of an adapted genotype or population across a defined range of variation. Stability of varietal performance may be classified into various logical categories, which aid the breeder in determining an appropriate strategy to achieve stability. Four types of stability are discussed here, some of which are typically interrelated: temporal, microspatial, macrospatial and system.

Seasons and years are the main categories of temporal stability. Principal elements that can affect temporal stability are temperature and photoperiod (principally related to time of planting in the subtropics), water (variations in rainfall across seasons or years), soil characteristics (especially decline of fertility over time), and diseases and pests (which may build up, or less commonly, decline over time).

Stability of performance across very diverse agro-ecosystems or geographical areas (macrospatial stability) is generally considered to be synonymous with wide adaptation. Wide adaptation is one of the most effective ways for a breeding programme to achieve economies of scale, in order to spread research and development costs over a wide area of potential impact. Nonetheless, breeding for the wide array of factors that can influence macrospatial stability is often impractical. Cassava is generally grown under low management levels and is subject to the uncertainty of natural rainfall patterns, to variation in soil fertility, and the attack of diseases and pests during its long growth cycle. Virtually every study comparing genotypes across a range of sites has found highly significant genotypes by location interaction (e.g. Tan, 1984; Dixon *et al*., 1994; Iglesias *et al*., 1994; Ngewe, 1994; Otoo *et al*., 1994; Rodriguez, 1994).

Wide adaptation is probably most feasible in Asia, where fewer diseases and pests constrain yield. On the other hand, any research institute is interested in applying its technology over as large a geographic area as possible in order to achieve a reasonable return on investment in research. There must therefore be a balance between institutional goals for wide adaptation, and the greater rate of genetic progress that can be achieved by limiting goals to more specific adaptation. Mkumbira (2002) described the situation in Africa: ". . . for most regions of Africa the terrain varies over short distances and environments are characterized by unpredictable variability in the frequency, timing and severity of a number of environmental stresses."

Defining the most appropriate range of adaptation is one of the breeder's most significant programme design challenges. Subdivision of breeding objectives by agro-ecological regions is often a practical solution. It seems logical that the cassava breeder should avoid the two extremes of either breeding for very wide adaptation (difficult to achieve in a low-input, rainfed crop), or breeding for high site specificity (high developmental costs relative to area of impact). Evolution and farmer selection have frequently taken the latter route. Breeding for stability of performance across the variability found within a few principal agro-ecological regions of a country should be a reasonable objective, both in terms of attainability within an intermediate time frame, and application of results to a justifiably large target area.

Stability across different production practices (system stability) may be important to individual farmers and certainly is important across microregions. Often, however, the longer-range goal should be to develop distinct varieties that optimize performance within specific production systems. Many of the variations between production systems preclude any single variety having both high yield and good stability across systems. Unlike pests or diseases, where resistance is generally a yield-neutral factor, plant characters associated with adaptation to different production systems may be physiologically mutually exclusive. High early vigour for competition against weeds is incompatible with low vigour to limit competition with an intercrop. Late branching for ease of field operations is incompatible with profuse branching for early canopy closure.

Finally, the farmer is likely to view stability of performance as including not only yield, but possibly also root quality, production of planting material, sprouting ability, and others that the breeder often

does not include in stability analyses. It is not enough that a variety gives consistent yield in good years and poor, but also that the product has stable commercial acceptability.

3. VARIABILITY FOR TOLERANCE TO ENVIRONMENTAL FACTORS AFFECTING STABILITY[4]

3.1 TEMPERATURE

A strong genotype by temperature interaction results from the effects of altitude within the tropics (Irikura *et al*., 1979). The available data suggest that for temperatures lower than 22°C, different genotypes are required compared with the higher temperatures of the lowland tropics (Table 14.1). For example, in Colombia few genotypes simultaneously yield reasonably at Popayan (1 800 masl), CIAT-Palmira (1 000 masl), and Media Luna, a high temperature site close to sea level. Studies on photosynthetic rate under different temperature regimes of one broadly adapted clone, *Sata Dovio,* showed lowest temperature sensitivity of any of the clones tested (El Sharkawy, unpubl. data), suggesting one possible mechanism for broad temperature adaptation. In general, photosynthetic rate at 1 800 m was about one-third that observed in warm humid sites (CIAT Annual Reports, 1987–1993).

Table 14.1 Fresh root yield (tonnes/ha) of four contrasting cassava types at 12 months after planting under three different temperature regimes

Variety	20°C	24°C	28°C
MCol 22	9.3	27.7	39.4
MMex 59	22.8	38.8	30.4
MCol 113	24.2	26.1	23.9
MCol 1522	28.9	15.7	9.4
Source: Irikura et al. (1979)			

In an attempt to broaden the temperature adaptation of cassava for the middle altitude and highland tropics, CIAT initiated a selection scheme in 1989, that involved simultaneous evaluation of the highland gene pool at a site at 1 800 masl, and another at 1 000 masl. This approach succeeded in identifying some segregants that perform well at both sites. With recurrent selection, this type of broader adaptability should be achieved, thus greatly expanding the potential impact area of the highland gene pool.

There are few data on the interaction between genotype and temperature when the latter shows seasonal fluctuation, such as in the subtropics. Data from CIAT international trials in the late 1970s suggested that certain clones (e.g. *Mantequeira*, or *CMC 40*, from IAC in Brazil) are well-adapted to the middle altitude tropics (moderate temperatures with little fluctuation throughout the year) and also do well in the subtropics, where mean temperatures may be below 10°C for one to three months of the year. Limited past evidence showed that it may be easy to move clones developed in the subtropics to the tropics, but movement in the opposite direction was less successful. More recently, varieties selected in Thailand are successfully being grown in southern China and North Viet Nam so there are certainly no strict barriers to movement in either direction between the tropics and the subtropics.

3.2 PHOTOPERIOD

Photoperiod sensitivity for seed production is an evolutionary adaptation mechanism of many plants. Cassava probably retains residual effects from its seed-propagated ancestors, and may have incorporated adaptive responses as a vegetatively propagated crop plant. The magnitude of photoperiod variations within the range of cassava's adaptation in the subtropics is certainly sufficient to affect plant development. Even the moderate seasonal changes in the photoperiod encountered in the higher-latitude

[4] This section draws heavily on the review of Cock, 1985

tropics (e.g. 15-20°N or S) may affect yields. Some varieties are more sensitive than others (CIAT, 1981, 1990; Keating, 1981). Long days especially seem to affect cassava in the first three months after planting; hence a change of photoperiod will mainly affect stability in those areas where the planting season is during or immediately preceding a long-day period. All varieties so far tested are photoperiod-sensitive in terms of such parameters as branching and dry matter distribution; nevertheless, some varieties show relatively stable yields at different photoperiods (Table 14.2).

Table 14.2 Cassava dry matter production (tonnes/ha) and distribution (harvest index) 272 days after planting, under 16 h and natural day length (approximately 12 h)

Genotype	Day length	Total DM	Storage root	Harvest index
MCol 1684	Natural	16.7	9.1	0.54
	16 h	17.3	4.6	0.27
MPtr 26	Natural	14.5	8.1	0.61
	16 h	15.9	4.9	0.42
MCol 22	Natural	15.5	9.5	0.56
	16 h	19.5	8.3	0.31
Source: Veltkamp (1985)				

3.3 LIGHT INTENSITY

Cassava is highly sensitive to shading. Major effects appear to be the result of drastically reduced leaf life and reduced photosynthetic rates (Cock *et al*., 1979; Tan, 1980; Fukai *et al*., 1984). There are some indications of varietal differences in tolerance to shading, though large scale screening has not been undertaken. Studies of 100 cultivars under coconut at CTCRI (1974) succeeded in identifying five with root yields about one-third of their usual level. This does not appear to be a very promising level of variability. Given that this sensitivity is probably a function of the fundamental characteristics of the crop's photosynthetic system, it may be difficult to achieve much higher yielding ability for cassava under shade. Nevertheless, there has never been a screening of the broad variability existing in the world germplasm collection, nor of wild species. Although most wild species would appear to be shade-sensitive, as indicated by their natural habitat, Allem (1994) also reported wild *Manihot* growing under jungle canopy in central Brazil.

3.4 WATER

While cassava's response under water stress is being continually better characterized, rapid screening methods applicable to large numbers of genotypes are not yet in use. Commonly, selection depends on overall varietal performance under natural drought conditions. This technique has the potential advantage of combining various mechanisms, as well as simultaneous evaluation for a range of other traits required for varietal acceptability. The disadvantages are the natural year-to-year variability that occurs, the length of time required for selection and the difficulty of knowing whether individual mechanisms are being optimally exploited.

Though cassava has long been recognized for its ability to tolerate drought stress, no concerted breeding efforts were specifically directed at improving this trait until recently. In 1990 institutions in Brazil, along with CIAT and IITA, initiated a major project for developing germplasm for the world's isothermic semi-arid regions. The main components of the strategy were the collection of germplasm from semi-arid regions of Latin America; evaluation in semi-arid environments in northeast Brazil; a recurrent selection programme to improve drought tolerance and combine that with other traits needed for high productivity and acceptability; and distribution of segregating populations to other regions, especially semi-arid, sub-Sahelian Africa (Fukuda, *et al*., 1992; CIAT, 1995). This project ultimately resulted in some of the most significant, directed introductions of cassava germplasm to Africa in modern times.

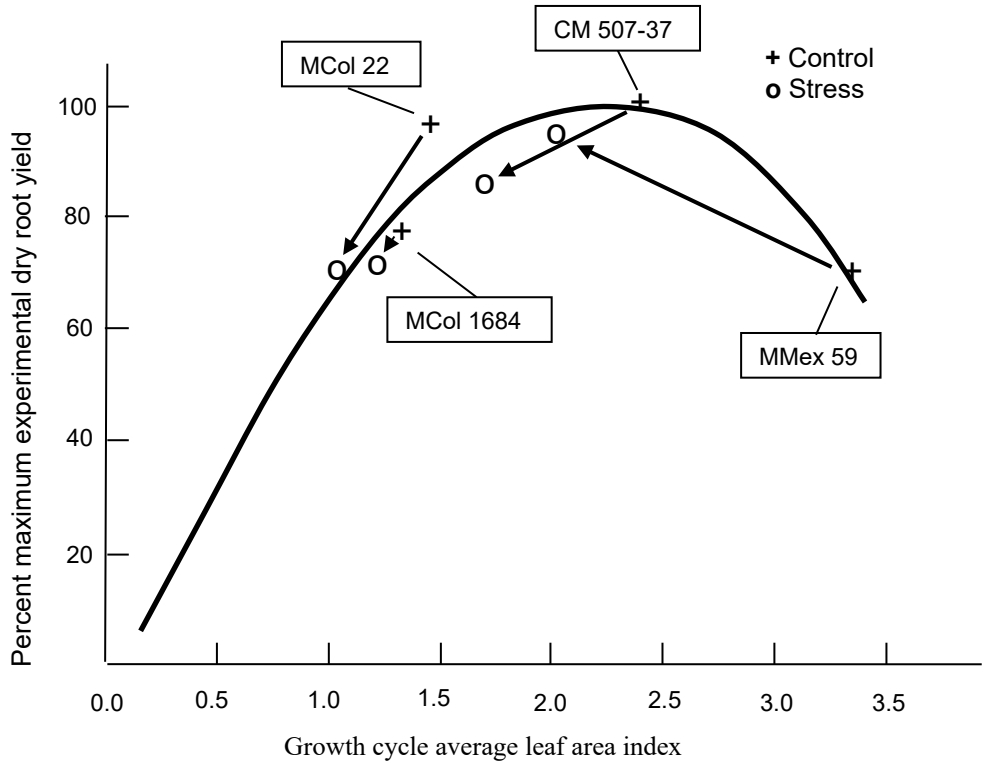
There appear to be possibilities for selecting for adaptation across a fairly broad range of water availability patterns. In a trial at Carimagua station in Colombia's eastern plains, several varieties yielded the same with or without irrigation (CIAT, 1978; 1979). In Quilichao station (Cauca Department), exclusion of rain from plots of MMex 59 actually increased yields, while decreasing yields of MCol 22. In both these trials, those clones with highest yield and high harvest index were also the most sensitive to drought stress. Certain varieties with above optimum LAI under well-watered conditions reduce the LAI to only slightly less than the optimum when a dry period occurs. These varieties have both high yield potential and good yield stability under varying conditions of water availability (Figure 14.1).

El-Sharkawy and Cock (1987a) noted differences in rooting patterns among clones, that appeared to be related to drought tolerance. The hybrid CM 507-37, compared with one of its parent clones MCol 1684, had finer roots, a greater density of root length in the upper layer of soil, and its root system penetrated deeper into the soil layers (possibly beyond the two-metre depth studied). This more extensive and denser root system is advantageous in terms of its ability to withdraw more water form larger and deeper volumes of soil. These two clones are the same ones that are described in the next chapter with regard to early root growth, giving some hope to the possibility of early rapid screening for root traits that influence drought tolerance. As deep-soil root studies are extremely difficult and time-consuming, there has been little follow-up on comparing a broader germplasm base. Certainly, breeders will not be able to screen extensive fibrous root systems unless a rapid technique with correlated characters is found to be reliable. It seems logical to expect that selection under drought conditions where deep soil moisture is available, would result in genotypes with greater ability to exploit that moisture with deep and extensive root systems.

Given the generally xerophytic adaptation of the wild *Manihot* species, one might expect a range of adaptation features within the genus with potential breeding value. In early work on mechanisms, CIAT identified several significant features. *M. rubricaulis* is native to the dry Sierra Madre of Mexico, and was reported to have the highest altitudinal range for *Manihot,* up to 2 400 m (Rogers and Appan, 1973). The species displays a double palisade layer, on the lower and upper sides of the leaf surface. This is a feature of the *Atriplex* species, which have C_4 metabolism and are also adapted to dry conditions. *M. rubricaulis* has high stomatal density on both leaf surfaces, whereas cassava generally has only a few stomata on the upper surface. Arrangement of vascular bundles, into single, double and triple aggregates, is also unique to the species (CIAT, 1994).

M. crassisepala, also from dry areas of Mexico (Morelos) has large papillae on the adaxial leaf surface (palisade side). These may create a micro-environment on the leaf surface for enhanced water use efficiency (M. El-Sharkawy, personal communication).

Figure 14.1 Dry root yield as a function of growth cycle average leaf area index under non-stress and mid-term water stress conditions



Source: El-Sharkawy and Cock (1987)

Cassava's high stomatal sensitivity to low relative humidity can be a detriment to the plant's ability to maximize yields when water is not limiting. If stomata close in dry air, while soil water is adequate, the plant will sacrifice potential yield in these non-limiting environments. El-Sharkawy and Cock (1978b) suggest that clones with stomata on both leaf surfaces (amphistomatous) would decrease stomatal sensitivity and thus maximize photosynthesis where water is not limiting. In germplasm evaluations, only about 2 percent of the 1 500 clones screened were found to have significant stomata on the adaxial surface. These few amphistomatous clones could be crossed with hypostomatous ones to reveal inheritance patterns, and to achieve a perspective on the potential of breeding for this trait.

3.5 SOIL STRUCTURE, FERTILITY AND CHEMISTRY

Many different components of soil structure and chemistry may affect the stability of performance of cassava varieties, including major or minor nutrients, pH, aluminium saturation levels, salinity and mineral toxicities.

Producers grow cassava on a very wide range of soils, but it is most commonly found on those that tend to be acid and of low fertility. Cassava is generally stable in its responses to pH *per se* over the range of pH 4.0-7.5, though some sensitive genotypes probably exist. Low pH in mineral soils is frequently associated with high levels of Al, which is toxic to many plants. Cassava is remarkably adapted to high levels of Al saturation and most genotypes show a stable reaction if Al saturation is below 80 percent

(CIAT, 1978). In highly alkaline soils, where salts are often a problem, cassava is highly sensitive to small changes in pH and salt concentrations. There may, however, be large differences among genotypes. These areas are presently of minor importance in cassava production. Nonetheless, several countries have expressed interest in growing cassava on saline soils, for example, Cuba, Mexico and Peru.

Cassava has certain inherent characteristics that make it less sensitive to fertility changes than other crops. At reduced fertility, cassava often responds by decreasing top growth disproportionally to root yield. The association with mycorrhiza reduces differences in yield or quality related to soil phosphorus levels (Edwards *et al*, 1977; Cock and Howeler, 1978).

CIAT conducted screening trials over many years for adaptation to low P and K, including some 1 600 accessions from the germplasm collection. The trials identified many clones that give similar yields under low and high levels of these elements. Though materials in the routine breeding programme are not subjected to this screen, some of the parents in breeding nurseries are selected on the basis of nutrient use efficiency. In order to select simultaneously for yield potential and low-nutrient tolerance, CIAT developed an adaptation index:

$$\text{Adaptation Index} = \frac{(\text{Yield at low K or P})(\text{Yield at high K or P})}{(\text{Mean yield at low K or P})(\text{Mean yield at high K or P})}$$

By using various hypothetical examples, one can observe that a high index will only be obtained when there is reasonable yield at both high and low nutrient levels. There are few apparent physical traits associated with good or poor adaptation to low P or K. It appears rather to be mainly the result of inherent physiological traits. Selection for higher efficiency is still possible only by evaluating performance of low and high P or K levels. Fortunately, many clones that are highest yielding at low fertility also respond well to added nutrients. Table 14.3 illustrates results of screening for adaptation to low P soils in Colombia.

In a trial of detailed crop growth analysis under different levels of P, Pellet and El-Sharkawy (1993) found that differences among varieties in P uptake were related to differences in fine root length and density, more than to infection rates with vesicular arbuscular mychorrhiza. Phosphorus use efficiency (determined as root yield per accumulated P in the whole plant) and patterns of dry matter partitioning to roots and shoots differed significantly among varieties. Along with selection under low P conditions representative of the target growing area, breeders could improve rate of progress by selecting clones with a high fine-root density, moderate shoot growth and stable harvest index.

Breeders now generally emphasize performance under low to intermediate fertility conditions, to represent the vast majority of cassava environments. As an assurance that advanced materials are responsive to improved fertility, CIAT also requires all selections to perform reasonably well in more luxurious environments, such as the headquarters station at Palmira. If environments of native high and low fertility are not available, the same might be accomplished by selecting simultaneously under fertilized and unfertilized conditions at the same site. Normally a preferred response is: good yield under low fertility with a balance between top and root growth (harvest index of about 0.5), and large response in root yield but moderate increase in top yield under higher fertility (increased harvest index, to 0.6–0.7).

Table 14.3 Root yield and low phosphorous index of clones evaluated at CIAT-Quilichao, Cauca, Colombia

	Fresh root yield (tonnes/ha		
Variety	P_0[a]	P_{75}[a]	Low P index[b]
Panameña	56.0	57.4	3.13
CM 489-1	37.3	73.5	2.60
CM 507-37	36.2	57.3	1.97
MVen 321	32.9	62.6	1.96
CM 516-7	26.4	71.3	1.79
CM 523-7	25.2	72.6	1.74
MBra 226	38.6	45.5	1.67
MBra 41	37.5	45.9	1.63
CM 305-41	34.9	48.7	1.61
CM 975-5	34.2	48.3	1.57
MEcu 68	35.6	45.8	1.55
Avg. of 77 clones	26.8	39.3	1.00

[a] *P application in kg P/ha*
[b] *Low P adaptation index = (variety yield P_0 x variety yield P_{75})/(mean yield P_0 x mean yield P_{75})*

Source: CIAT. 1992. Cassava Programme 1987–1991. Working Document No. 116, Cali, Colombia

3.6 CROPPING SYSTEM

Probably one-third to one-half of the world's cassava is intercropped with other species. For any given target region, there are predominant patterns of intercropping under which new varieties should be selected. These systems typically have greater stability of economic yield as compared with monocropping and may have additional advantages in natural resource management.

Genotype by cropping system studies often show a significant interaction, i.e. demonstrate that genotypes will be ranked differently in intercropping as compared with monocropping situations. Agronomists often then conclude that selection must be carried out under typical intercropping systems for valid results. Breeders, on the other hand, tend to shy away from further complicating their selection strategies. Indeed, incorporating cropping system variations into all stages of selection would simply be unmanageable for most programmes. A reasonable compromise may be to identify traits whose variations influence acceptability in intercropping, and select for these in the early to intermediate selection stages. At the later stages, with fewer genotypes, trials may be conducted with cropping system components.

The Africa-wide COSCA studies found that farmers generally prefer more upright, less branching varieties for intercropping with early maturing crops such as grain legumes and maize (Nweke, 1994). Similar experiences emerge from Asia and Latin America. This basic information already gives the breeder some guidance on key selection criteria that can be applied even in the absence of an intercrop.

Kawano and Thung (1982) demonstrated that the most important consideration in selecting cassava genotypes for intercropping with soybeans was a moderate (as opposed to high) leaf area index in early growth stages. Osiru and Ezumah (1994) compared a wide range of cassava genotypes under monoculture or intercropped with either maize or peanuts. They concluded that there was no need to select different genotypes of cassava for different cropping systems. At the same time, however, they note persistent complaints from farmers about the low branching and extensive canopy of a new variety because it is not suited to intercropping.

3.7 OTHER AGRONOMIC PRACTICES

Agronomic practices are generally designed to increase yield as well as to improve yield stability, but such practices can affect different genotypes in different ways. The breeder should be aware of variations in agronomic practices to which new varieties may be subjected and the stability of performance across this variation as compared with varieties being replaced.

Level of weed competition can cause very large differences in yield, especially in less vigorous varieties. These same varieties are often efficient in dry matter partitioning, and have a high harvest index, and high yield potential. In Colombia's north coast region, MMex 59, with heavier top growth, showed remarkable yield stability over a wide range of different weed management practices, whereas MCol 22 was extremely unstable. However, highest yield was obtained with MCol 22 under good weed control. This interaction, which also occurs with respect to other factors such as disease and pest resistance, might suggest that stability in some cases can only be obtained as a trade-off with yield potential. Nevertheless, results from technology validation trials have shown that moderate and stable yields can be obtained over a range of different management systems.

Although rarely taken into account in breeding programmes, plant density interacts strongly with variety. Vigorous clones with low harvest index often have the ability to yield well only at low density, while efficient, high harvest index types respond well to high density.

4. A FARMER'S PERSPECTIVE ON STABILITY

The key to a successful strategy for breeding for stability is to understand the farmers' perspectives on the issue. This can involve information on the levels of risk tolerance versus risk aversion, or the ability of farmers to counter unstable performance with management practices. Each situation is unique; there are no universal guidelines. Furthermore, farmers' ability to address instability and their perceptions of an acceptable level of risk, are constantly changing. A plant breeder needs to stay attuned to the target area characteristics, with the help of collaborators in socio-economic disciplines.

Table 14.4 gives a hypothetical example of how an individual farmer might view the selection of a variety or set of varieties on the basis of stability of yield over a five-year period. This example excludes other factors that may influence a farmer's choice, such as suitability for intercropping, ease of harvest, or root quality.

The example includes stresses mainly resulting from deficient or excessive rainfall. On average the varieties yielded 10.7 tonnes/ha during the five years, with a range of means from 9.1 to 13.6 tonnes/ha. Selecting the best variety is far from straightforward. The following are some alternative criteria, each resulting in a different outcome. For mean yield over the five-year period, *Verdecita* is best. The most stress-tolerant variety, i.e the one with highest yield in the two most stressful years, is *Venezolana*. *Consteña* has the highest yield potential, i.e. the highest yield in the two least stressful years. *Secundina* is the most stable clone, with the lowest difference in yield between the best and the worst years.

Table 14.4 Farmers' considerations in variety selection: strategies for balancing stability and performance, and implications for breeding

		Hypothetical yields, in tonnes/ha					
Yr	**Description**	**Blanca Mona**	**Secun-dina**	**Vene-zolana**	**Verde-cita**	**Cos-teña**	**Mean**
1	Normal rainy season; normal dry season	12.4	10.5	12.3	15.6	18.3	13.8
2	Excessive rainfall; severe dry season	9.3	8.3	8.4	12.3	11.8	10.0
3	Normal rainy season; severe dry season	10.8	9.4	11.0	13.6	12.1	11.4
4	Below normal rainfall year-long	8.9	9.3	10.4	10.2	7.9	9.3
5	Below normal rainfall year-long	8.6	8.9	10.2	9.6	8.3	9.1
Mean		10.0	9.3	10.5	12.3	11.7	10.7
Range		3.8	2.2	3.9	6.0	8.0	4.5
Mean in two most stressful years (yrs 4 & 5)		8.7	9.1	10.3	9.9	8.1	9.2
Mean in two least stressful years (yrs 1 & 3)		11.6	10.0	11.6	14.6	15.2	12.6
Difference (yrs 1 & 3 minus 4 & 5)		2.9	0.9	1.3	4.7	7.1	3.4
Highest yield potential: Costeña – 18.3 tonnes/ha in best year (year 1)							
Highest yield in high-stress year: Venezolana – 10.2 tonnes/ha in year 5							

Only farmers can determine what will be best for their particular growing and marketing situation, and ultimately they need to be provided with a range of choices, showing performance over time and space, that allow them to identify the best varieties. As this type of situation is so common, where different varieties each meet different needs, it is very common for farmers to select a range of varieties, and thus to be prepared for various situations during the growing season. Where the specific stability criteria of farmers can be discerned, a breeder should be able to focus on improving varieties for specific stability traits. More often, however, the situation will be less than clear-cut, and the breeder will need to combine a range of selection environments with an evaluation time frame covering several years, in order to select for the type of stability that farmers seek.

Temporal stability has special importance in cassava, because of the potential stability implications of vegetative propagation. The propagation system prevents (or at least makes very difficult) rapid change of varieties. If a variety fails due to erratic performance over years (temporal instability), a grower generally requires a few years to introduce and adequately multiply a new variety. Secondly, vegetative propagation allows the accumulation of biological constraints to a greater degree than that which occurs

in most seed propagation systems. Viruses are especially important in this regard. The possibility of latent viruses causing degeneration is reinforced by the fact that yields of apparently healthy clones can sometimes be improved by passing them through a process of *in vitro* cleaning.

Varietal decline over time can also result from a general lack of adaptation in an agro-ecosystem, where quality of planting material progressively declines because of a combination of biological and physical constraints, and resulting plants become weaker and lower yielding over time. This progressive decline can occur over several years and is not easy to distinguish from the decline caused solely by the accumulation of systemic pathogens. The distinction, however, is important, in terms of defining either a breeding or a management solution. There are no documented examples for cassava where long-term instability has been caused by pest or pathogen variation overcoming host plant resistance. Nevertheless, the possibility cannot be completely discounted. System stability is regionally important, where farmers may grow cassava under two or more significantly different systems, such as intercropped and sole-cropped. Nonetheless, in these situations farmers typically have selected for distinct varieties with optimum performance in each system.

Spatial stability is generally more critical as an issue for design of a breeding programme as opposed to having importance to individual farmers. Hardly any individual cassava farmers grow cassava across widely separated geographical areas. A grower's interest is generally the performance in a single (and usually small) farm. Nonetheless, given the kind of variable environments where cassava tends to be cultivated, individual cassava farmers may encounter a range of micro-environments on their farm, with implications for varietal adaptation.

5. ASSESSING STABILITY

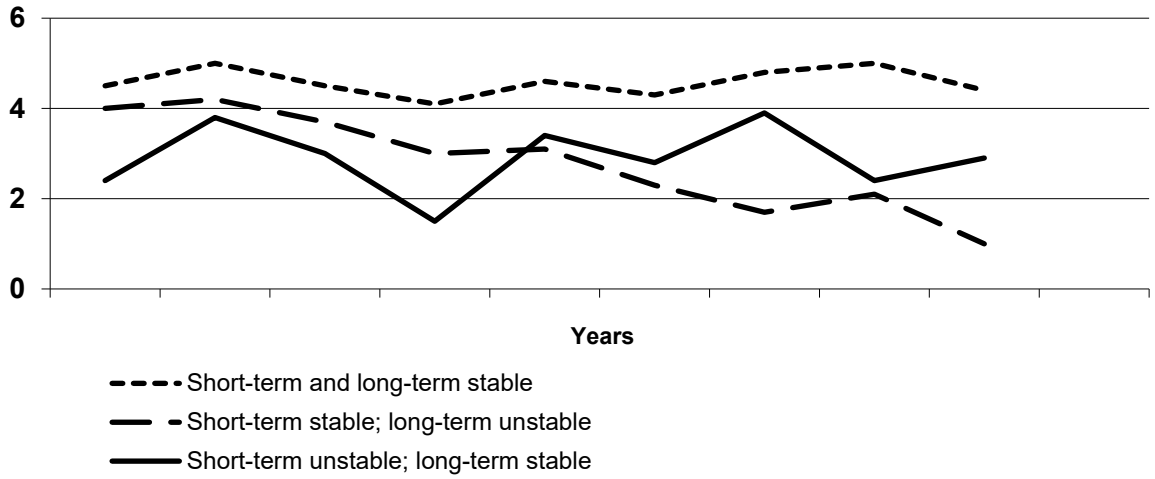
Stability analyses are generally carried out across years and locations, and less frequently across production systems. Nevertheless, for whatever parameter of stability is evaluated, similar statistical approaches may be used. Components of variance, regression analysis and principal component analysis are procedures for assessing stability of crop genotypes. There is a large volume of literature on the subject. Some of the basic treatments are Sprague and Federer (1951), Eberhart and Russell (1966), Okuno (1971), Tai (1971) and Suzuki and Kikuchi (1975).

Temporal stability can be viewed as short or long term. Short-term stability describes the level of variation from one year to another, and can be measured by the common statistical procedures for determining genotype–year interactions. Long-term stability is a subtler concept. It is not always easy to distinguish year-to-year variations from longer-term trends. Long-term decline most often occurs either as a pest/pathogen effect, or a soil fertility problem. The genetic control of stability is often poorly defined owing to complex interactions among yield-limiting factors. However, it seems logical to assume that long-term and short-term stability or instability can occur together in any of the possible combinations, i.e. short-term and long-term stable; short-term stable and long-term unstable; short-term unstable and long term-stable; and short- and long-term unstable (Figure 14.2).

6. SELECTING FOR STABILITY

Long-term stability across relevant cropping practices is important for any crop, but especially for cassava. Resource-poor farmers can ill-afford the risks of varieties that decline over time, especially with the long development time and slow turnover rate for new varieties. In a breeding programme, clones with exceptionally low temporal stability can be discarded after a few seasons, but even the eight or ten years of testing typically required for varietal development cannot guarantee long-term stability. Nevertheless, application of appropriate tools during selection can raise the level of confidence in stability. The three vital ingredients required are: an adequate germplasm base; appropriate evaluation tools (including environments that exhibit the factors creating instability); and time.

Figure 14.2 Hypothetical representation of variations in temporal stability/instability for cassava



Tan (1984) reported that in her study of 16 cassava varieties at five locations, most of the clones that were high yielding in any given site were rated as unstable, while the most stable clone was a low yielder. Tai (1971) reports similar results in his analysis of potato regional trials. He suggested that the lack of association between high tuber yield and stable performance, for clones that reached an advanced trial stage, indicates the need for further research to determine the nature of stability of tuber yield. Tan suggested that, in conjunction with stability parameters, overall mean yield (over environments) is still an important criterion in the selection for adaptability of cassava varieties. IITA and collaborating national programmes carried out extensive G–E trials in four West African countries from 1983–1989. The high G–E interaction indicated to researchers the need for different varieties in different zones (IITA, 1993a). However, they were unable to link specific environmental factors to causes of this interaction.

These few results summarize what apparently is a common experience in breeding for yield stability across environments: the most stable genotypes are rarely the highest yielding in any given environment, and may even be below average in yield in most environments. In fact, if taken to the extreme, the most stable genotype is one with zero yield in all environments. Clearly, both yield and stability of yield need to be taken into account in practical selection.

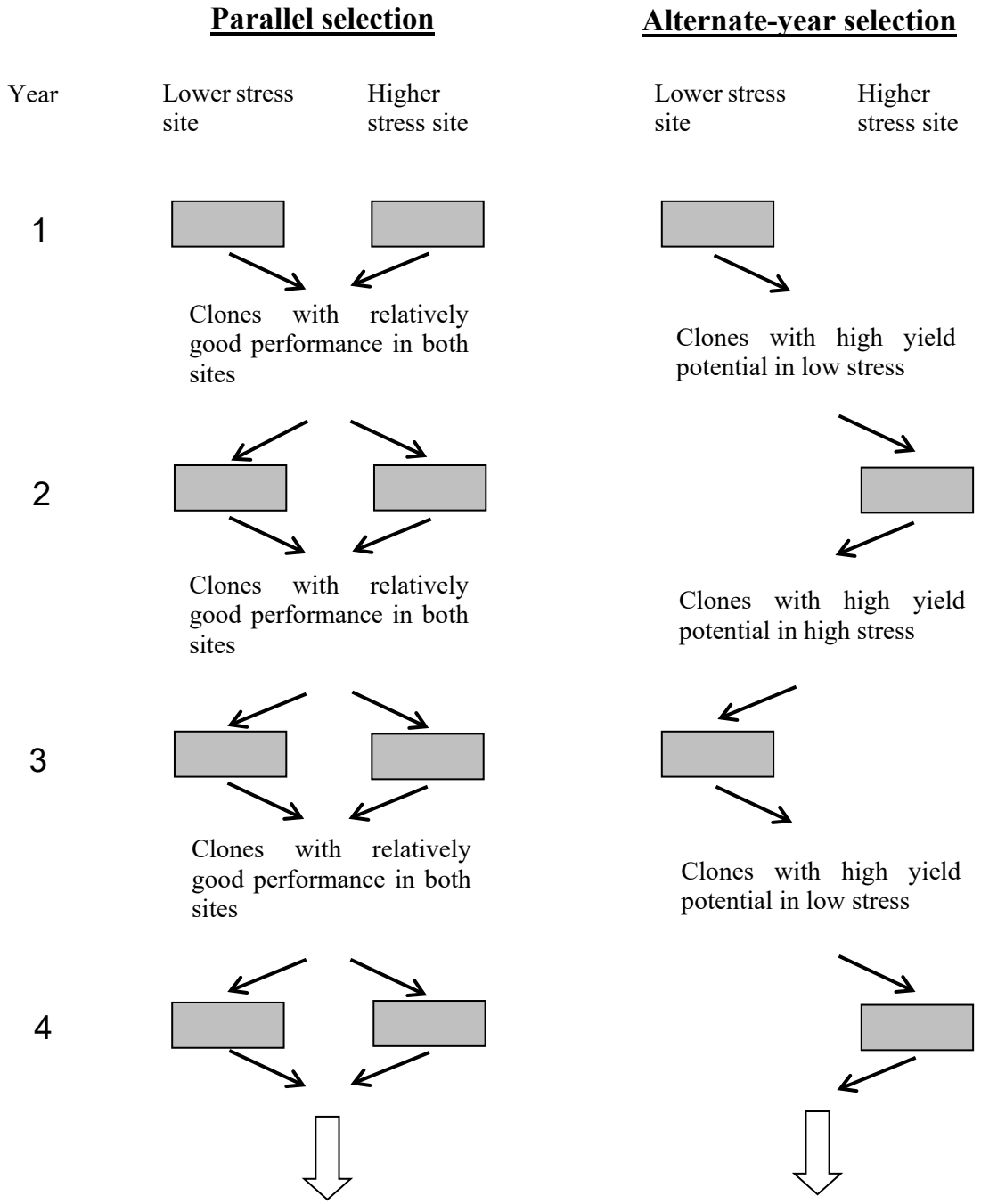
Yield stability is genetically controlled, and selection for stability can be effective. Two basic alternatives are available. Firstly, one can select for improvement of a stability index, when a set of genotypes is planted in two or more environments. Alternatively (or simultaneously), it may be possible to identify and select for the individual components that confer stability. Some common examples of such components are pest and disease resistance, or tolerance to toxic levels of minor elements in the soil.

It is sometimes possible to select for yield stability using two contrasting environments (such as locations or seasons). Oka (1967) called this disruptive seasonal selection, a method best known for its success in Mexican dwarf wheat varieties. In wheat, the main attributes incorporated through disruptive seasonal selection were insensitivity to photoperiod and resistance to several races of rust. Figure 14.3 illustrates two alternatives for disruptive selection, where contrasting sites are used either simultaneously or alternately (across years).

IITA does multilocation testing in neighbouring environments within each agro-ecological zone for at least two years. The top high yielding and stable clones are identified. These are then tested across agro-ecologies to identify adaptable varieties and to determine the ecological limits of adaptation. CIAT tests breeding materials across agro-ecologies already at the single-row stage of evaluation, in order to subject

a broad germplasm base to the criteria of wide adaptation. Breeders generally select potential new varieties based on a combination of superior performance in a particular agro-ecosystem, but also take into account broad adaptability.

Figure 14.3 Disruptive site selection alternatives for breeding for adaptation across the range of stress variations within a target region



Disruptive seasonal or site selection does not, of course, assure stability of the type desired. The choice of specific selection environments is crucial. They should include the extremes of variation that are in fact present in the target environment. For example, simultaneous selection in a very wet and a very dry environment (other factors being equal), would probably make no meaningful contribution to improved stability across the target environment that was exclusively a dry ecosystem.

There are two basic means of measuring long-term stability or decline, when pathogen races are not involved. Firstly, comparisons can be made over time, relative to constant checks. Secondly, comparisons can be made periodically with well-controlled sources of planting material for the same clone, such as plants derived from aseptic *in vitro* cultures. This second type of comparison allows separating cumulative effects of declining quality of planting material (e.g. viruses), as compared with external effects such as declining soil fertility or increasing pest pressures in the environment.

Use of a large number of testing sites certainly allows for selection of spatial stability, and may simultaneously confer a degree of temporal stability. It would seem *a priori* that the effect of different rainfall patterns across sites would simulate variation within sites across time. Spatial stability may not be related to temporal stability for the effects of pests and diseases, especially where there are regional differences in biotypes. On the other hand, a variety grown from clean and vigorous planting material may yield well under stress conditions the first year, but as it becomes infested with diseases and pests, and debilitated by low nutrient status, its performance declines. In such a case, a large number of trials in different sites might replace long-term trials to select for a temporal stability that relies on broad-based resistance.

There is no fully adequate substitute for long-term evaluation to determine stability of a variety. However, the breeder must take some risk, or no varieties would ever leave the experiment station. If a breeding programme is properly managed, the normal time between introduction of a clone and the testing of on-farm trials will involve adequate time to assess stability reasonably. Even if a clone is introduced and planted immediately in a preliminary yield trial, four to five years of testing would be involved before release. The danger comes when breeders are pressured for one reason or another to make recommendations for new varieties after just one or two years of testing. Unfortunately, this is not uncommon.

Distinct environments may require different periods of testing before confidence in a genotype's stability is possible. In relatively favourable environments in Asia, few destabilizing pests and diseases are present. Stability is more related to sensitivity to physical environmental factors, whose variations are more predictable. Chapter 22, describing procedures for varietal release, suggests further measures against developing and releasing unstable genotypes.

7. TRANSGENIC TECHNOLOGIES

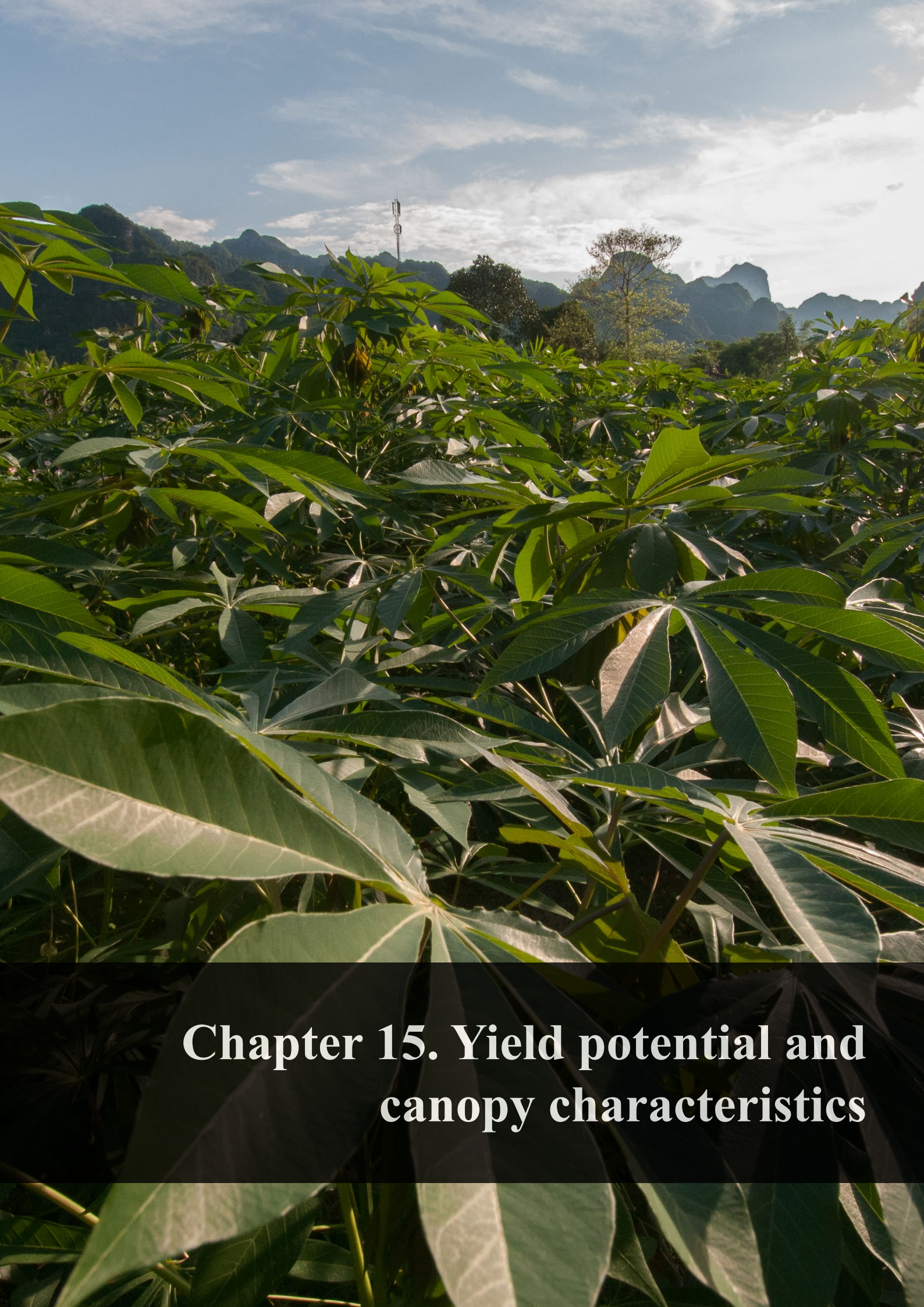
Cassava's comparative advantages in drought-prone environments and low-fertility/acid soils mean that its cultivation is highly skewed toward marginal lands. Often these are hillsides or sloping lands threatened by erosion. The crop's potential as a vehicle for rural development rests in part on farmers' ability to stabilize and improve these soils. Transgenic technologies could offer the possibility of introducing effective no-tillage or minimum tillage systems in cassava, where soil and vegetative cover are minimally disturbed, and at the same time minimizing competitive effects from weeds. Herbicide-resistant varieties would provide new options for farmers for cost-effective soil conservation, along with safe and easy weed management strategies.

Herbicide resistance was the first transgenic technology to become widely adopted for any crop. Glyphosate resistance in soybeans is still the most widely used of transgenic crops, and the same resistance has been incorporated into numerous species. Most of the soybeans planted in the United States are resistant to glyphosate. However, intellectual property rights issues have to date prevented transfer of the EPSP-synthase gene to cassava. There is little doubt that this gene could be

inserted and effectively activated in cassava. However, the intellectual property issues appear to be intransigent and cassava may need to await other gene sources or resistance to other herbicides may be sought.

The bar gene confers resistance to the herbicide Basta (ammonium glufosinate), and has been used widely as a marker for gene transfer protocols for many species, including cassava. CIAT recovered transgenic plants from the clone MPer 183, expressing the bar gene (Sarria, 1995). Transgenic plants did not tolerate the full commercial rate of 1 500 mg/litre, but these early plants demonstrated proof of principal for this strategy. The bar gene is now also available and being tested in the clone ICA-Negrita at CIAT.

The idea of herbicide resistance in cassava has always generated some controversy, with some fearing that it would lead to reliance on purchased inputs that farmers cannot afford, and also due to human and environmental safety concerns. For these reasons, as well as the intellectual property issues, interest in herbicide-resistant cassava has waned for the time-being.



Chapter 15. Yield potential and canopy characteristics

1. AN EVOLUTIONARY PERSPECTIVE

Until the latter part of the twentieth century, cassava was grown mainly in traditional, shifting agricultural systems, with low inputs and generally with intercropping of various species. Much of the area is still cultivated with practices that have changed little for centuries. Thus, the evolution of present-day varieties has resulted essentially from the pressures inherent in these types of systems. Competition effects were a major factor impinging upon the evolution of yield potential. To survive conditions of both interspecific and intraspecific competition, cassava could be neither excessively competitive (reducing yields of intercropped species), nor of very low vigour (suffering excessive competition from neighbours of either the same or different species). Where weeds were yield-limiting, high competitive ability would be important.

It seems clear that yield was (and is) only one of a wide array of traits cassava farmers considered important. Yield potential is seen by farmers as one objective among many. This history of evolution and farmer selection should mean that the variability for yield potential has not nearly been fully exploited. Unlike crops with a longer and more intensive breeding history, there should be little concern about approaching yield plateaus for cassava in the near future, at the farm level.

2. DEFINITIONS

Improved root yield is a nearly universal selection objective in both past and current cassava breeding programmes. This can readily be justified on the basis of one or more of the following situations: (1) local varieties, developed for traditional, low-input systems, do not respond well to modified agronomic practices; (2) local varieties evolved from a narrow genetic base and new genetic diversity can improve yields even without modified agronomy; or (3) cassava is a new crop in a region where no locally selected varieties exist.

The breeder's interest in the cassava plant canopy is usually directed both at its relation to yield and its importance for compatibility with cropping and management systems. Increasingly, breeders are also looking at the possibilities for improving cassava as a forage crop or a dual-purpose root/forage crop. Both theory and practical experience indicate that genetic yield improvement and canopy modification are interrelated and jointly possible.

The classical definition of yield potential used by agronomists is: yield under conditions of no constraints, i.e. with no limitations on light, water, nutrients or soil structure and free from pest or disease attack. This definition used for cassava would be relevant primarily from an experimental perspective, as a useful concept for studying physiological processes and understanding response of the plant to various environmental situations. For a breeder the concept of yield potential needs to be adapted to the reality of most cassava production conditions, where a complex of constraints affects the crop. Given this reality, a more appropriate definition is: yield under soil and climatic conditions representative of the target environment, with inputs and agronomic practices applied at levels recommended for commercial production and without constraints from pests and diseases. The definition excludes biological constraints, as these would presumably be controlled, in the long term, by host plant resistance, biological control, cultural practices, chemical control, or some combination of these in an integrated pest management programme. Allowing effects of biological constraints to be included in the definition of yield potential markedly confounds the concept and its discussion.

Yield in cassava is often defined in terms of marketable root yield, although leaves, stems or even seeds could potentially be additional economic products. Yield is most appropriately discussed in terms of dry matter yield and thus implicates root quality as well.

Any discussion of yield potential in cassava should be with reference to a given set of environmental conditions and agronomic practices, for a given plant part or product and over a defined growing period. Yield potential should be defined within and not independent of, a comprehensive set of objectives.

3. PHYSIOLOGY OF YIELD FORMATION

Wholey and Cock (1974) showed that at 24°C and where water and nutrients are not limiting, root bulking begins about two months after planting. There was little genetic variability for this trait among the clones they studied. Differences in yield among varieties, therefore, are largely due to differences in rate of root bulking, once initiated. Boerboom (1978a) showed a linear relationship of weight of roots as a function of weight of the whole plant throughout the growth cycle.

Total dry matter production is a function of rate of dry matter accumulation and time. Crop growth rate (CGR), has a parabolic relationship with leaf area index (LAI) (ratio of leaf area to land area), reaching a maximum at an LAI of approximately 3.5 (Enyi, 1972; Cock *et al*., 1979; Cock, 1983). LAI is a function of: (1) total leaf number per plant; (2) individual leaf size (area); and (3) planting density. At very high LAIs, leaf life becomes shorter and shorter owing to shading. Though the number of leaves per unit land area and total carbohydrate production may be high, the high turnover rate of leaves demands a large proportion of this carbohydrate.

Total leaf number per plant depends on the differential between rate of leaf formation (number of apices x rate of leaf formation per apex) and rate of leaf fall (or leaf life). Leaf formation rate at a given temperature appears to be relatively constant across varieties with similar levels of branching, with a gradual decline in rate over time in branched varieties (Tan and Cock, 1979). Leaf life appears to have a large genetic component, but varies as well with the age of the plant when the leaf was formed and with branching characteristics (Tan and Cock, 1979). Leaves formed in an older plant are shorter-lived. Unbranched varieties are better able to maintain a constant leaf life throughout the crop cycle. Leaf life may be shortened by shading, drought, diseases and pests and by higher temperatures. Cooler temperatures prolong leaf life (Irikura *et al*., 1979).

As cassava leaves remain photosynthetically active under prolonged water stress (more than two months) and because the stressed leaves are also capable of partially recovering from stress once water becomes available again, leaf retention may represent a significant savings in biomass invested in leaf formation (El-Sharkawy, 2004).

Branching habit can be defined by the time to first branching, the rate of subsequent branching (a constant) and the number of apices formed per branching point. These combined parameters determine the overall canopy characteristics.

Individual leaf size ranges from less than 50 to over 350 cm^2 (Tan and Cock, 1979). Plant genotype is a primary influence, but plant age and environment also have large effects. Leaf size reaches a maximum at three to six months and then declines. This decrease in leaf size is much more pronounced in branched as compared with unbranched varieties. Environmental factors, such as drought, pests and diseases and nutritional disorders can also reduce leaf size.

Planting density in commercial production is normally kept at about 8 000–10 000 plants/ha under monocropping and favourable growing conditions. Spacing is often increased with intercropping. Under less favourable conditions, spacing may be increased as a means of allowing individual plants to draw on more resources, or decreased because plants are less vigorous and require less space. Which of these strategies is more appropriate will depend on experience of farmers in a region and experimental results. As yet, there are no clear criteria for predicting optimum planting density based on physiological or morphological traits. This is determined only by density trials under specific conditions with specific varieties.

Photosynthetic efficiency, by definition, must be one of the key determinants of total dry matter production. Nevertheless, physiologists working with many species have generally had little success in demonstrating that the photosynthetic rate of single attached leaves is related to total dry matter production. This is probably because the relationship is confounded by the total number of leaves in the canopy, the degree of their intershading and hence the differences in the photosynthetic rate of various

levels within the canopy. Nonetheless, in cassava trials in water-stressed sites in Colombia, El-Sharkawy *et al*. (1990) showed good correlations between photosynthetic rate at various growing periods and final root yield. These data give some cause for optimism about the eventual efficacy of using photosynthetic rate as a criterion in breeding for yield potential in cassava. CIAT made an initial assessment of diversity for photosynthetic rates by screening part of the core collection under field conditions. Values ranged from 21-27.6 m $CO_2/m^2/s$. The methodology may be too expensive and time-consuming to apply to thousands of individuals in early selection stages, but it is practical to select genotypes with high rates to use as parents.

Cassava is capable of maintaining high photosynthetic rates under hydric and high temperature stress. Understanding the basis for this could lead to assay procedures to select for higher photosynthetic rates and biomass accumulation. Compartmentalization of photosynthetic enzymes is central to the role that improved photosynthesis may have for a species. CIAT studied compartmentalization of gene expression through *in situ* hybridization with labelled sense and antisense RNA (CIAT, 1994).

Wild *Manihot* species appear to represent a valuable resource for physiological traits, although their study has been very limited. Studies at CIAT revealed that *M. rubricaulis* and *M. grahami* have amphistomatous leaves (stomata distributed on both the upper and lower surface). These species also had high photosynthetic rates and an elevated activity in leaf extracts of the C_4 photosynthesis enzyme PEP carboxylase (as compared with typical C_3 species) (CIAT, 1994). The combined characteristics of leaf anatomy, high photosynthetic rates, low photorespiration and elevated PEP carboxylase might indicate that cassava and some of the wild *Manihot* species represent an intermediate photosynthesis between the typical C_3 and C_4 species (El-Sharkawy and Cock, 1987b). Since cassava lacks the Kranz leaf anatomy typical of C_4 species (essential for the separation and compartmentalization of the C_3 and C_4 main enzymes), there is continuing debate about the meaning of the elevated C_4 enzymes. The fact that the family *Euphorbeaceae* contains both C_3 and C_4 species opens the possibility that cassava represents an evolutionary step towards C_4 photosynthesis (El-Sharkawy, 2004).

The implications or the possibilities of this apparently unique photosynthetic system for cassava breeding are still unclear. There needs to be considerably more basic research before any practical applications can be possible. One of the steps should be to look at a wider range of the wild species to see whether there is an evolutionary pattern whereby some species may have acquired different aspects or levels of anatomical or molecular components of the C_4 species. Crosses among species with varying photosynthetic characteristics could give clues to the inheritance patterns and the possibility of improvement through breeding.

4. BIOMASS AND DRY MATTER DISTRIBUTION

Total biological yield is usually not of primary interest to farmers, who are concerned mainly with the distribution of that yield to commercial products. The proportion of total yield which is economic yield is called harvest index. Harvest index measures the efficiency of the plant in partitioning dry matter to the desired plant parts. Where the economic yield comes from a vegetative part (e.g. roots), harvest index is generally larger than from a crop whose economic yield results from fruits or seeds.

Structurally, a large harvest index is less problematic for a root crop than for a plant required to physically support a heavy aerial yield. Dry matter storage in the roots results from any surplus over dry matter requirements for the production of new leaves, maintenance of existing ones, maintenance of tissues in stems and branches, as well as weight increase in these organs. This suggests that in a heavily branched clone with profuse top growth, there will be proportionately less dry matter left over for root storage than would be the case for a less branched clone. Experiments where branching was controlled to various levels in a profusely branching clone, confirm this hypothesis (Tan and Cock, 1979).

Although root yield is generally a high selection priority, there are biological limits to the proportion of dry matter that a cassava plant can partition to the roots. Adequate top growth must be maintained for

sustained production. Too high a harvest index could result in insufficient photosynthetic area (too low an LAI) for continued root bulking over an extended time period. A certain minimal top growth is needed to provide good quality stakes for propagation of the subsequent cycle. If a plant is too efficient, it may end the growing season with very good yield, but is unable to produce good quality stakes. Thus, the subsequent cycle would suffer in yield. This is particularly important in stressful environments where many factors can affect plant growth. This phenomenon has been frequently observed in the high harvest index clone, MCol 1684, which often yields quite well while producing so little top growth that few good quality stakes can be obtained.

Finally, selection for maximum harvest index may be counter-productive where defoliating or photosynthesis-reducing pest or disease attacks can be expected. An efficient plant type, where foliage development is just adequate for top growth maintenance at the optimum LAI, may be especially sensitive to defoliation. Less efficient varieties have a buffering effect against yield loss from defoliation.

Harvest index is an easily measured parameter of efficiency in dry matter distribution. Although technically, harvest index should be expressed on a dry matter basis, for practical selection purposes, simple fresh weights are sufficiently precise. Harvest index is the weight of roots divided by total plant weight (roots plus stems, petioles, leaves and planting stake). The model for the ideal cassava plant (Cock *et al*., 1979) shows a harvest index of about 0.6 to be most efficient. A range of about 0.5–0.7 is reasonable, depending upon specific environmental conditions.

5. YIELD COMPONENTS

Analysis of yield components can pinpoint specific aspects of yield on which to focus for selection. Root yield is a function of the number of storage roots, individual root size and percentage of dry matter of the roots.

Total storage root number has shown high correlations with root yield (Tan, 1981). When the root number was reduced to less than seven or eight per plant, root yield declined (Cock *et al*., 1979). In most clones, the number of storage roots is fixed quite early during the plant's growth (Wholey and Cock, 1974) and can therefore be used as a selection criterion even at an early harvest date. Root size must be considered not only in terms of maximizing yield but, perhaps more importantly, for management and market requirements. Very large roots may be more difficult to harvest and prone to breakage. Many processing techniques are designed to function best within a certain range of variation for root size and form. For the fresh market, intermediate-sized roots are normally preferred.

Root dry matter content is a component both of yield and root quality. The quality considerations often predominate in weighing any trade-offs between yield and quality (see Chapter 17). There is no clear evidence that selection for high yield will limit variability for dry matter content, or vice versa. However, physiologically, there must be some trade-off when both components are taken to very high levels.

The moderately high broadsense heritability of yield, when measured in replicated yield trials, indicates that selection for yield itself, rather than its components, can be an efficient approach for yield breeding. However, yield selection in early generations, where intergenotypic competition effects play a large role, may not be effective. Selection for yield at these stages (F_1 and single row trials) will often be more effective by selection for components (especially root number) and harvest index. Both of these traits are often more closely correlated with yield in later selection stages than is yield itself.

6. CHARACTERISTICS OF HIGH-YIELDING CLONES: MODELS AND FIELD RESULTS

Cock *et al*. (1979) developed their model for the ideal cassava plant for high yield under non-limiting conditions. Later data, however, suggest that the model is valid for a wide range of conditions. The same

general plant phenotype is apparently also appropriate for less favourable conditions, but a distinct genotype may be required to produce this same phenotype, in contrasting environments.

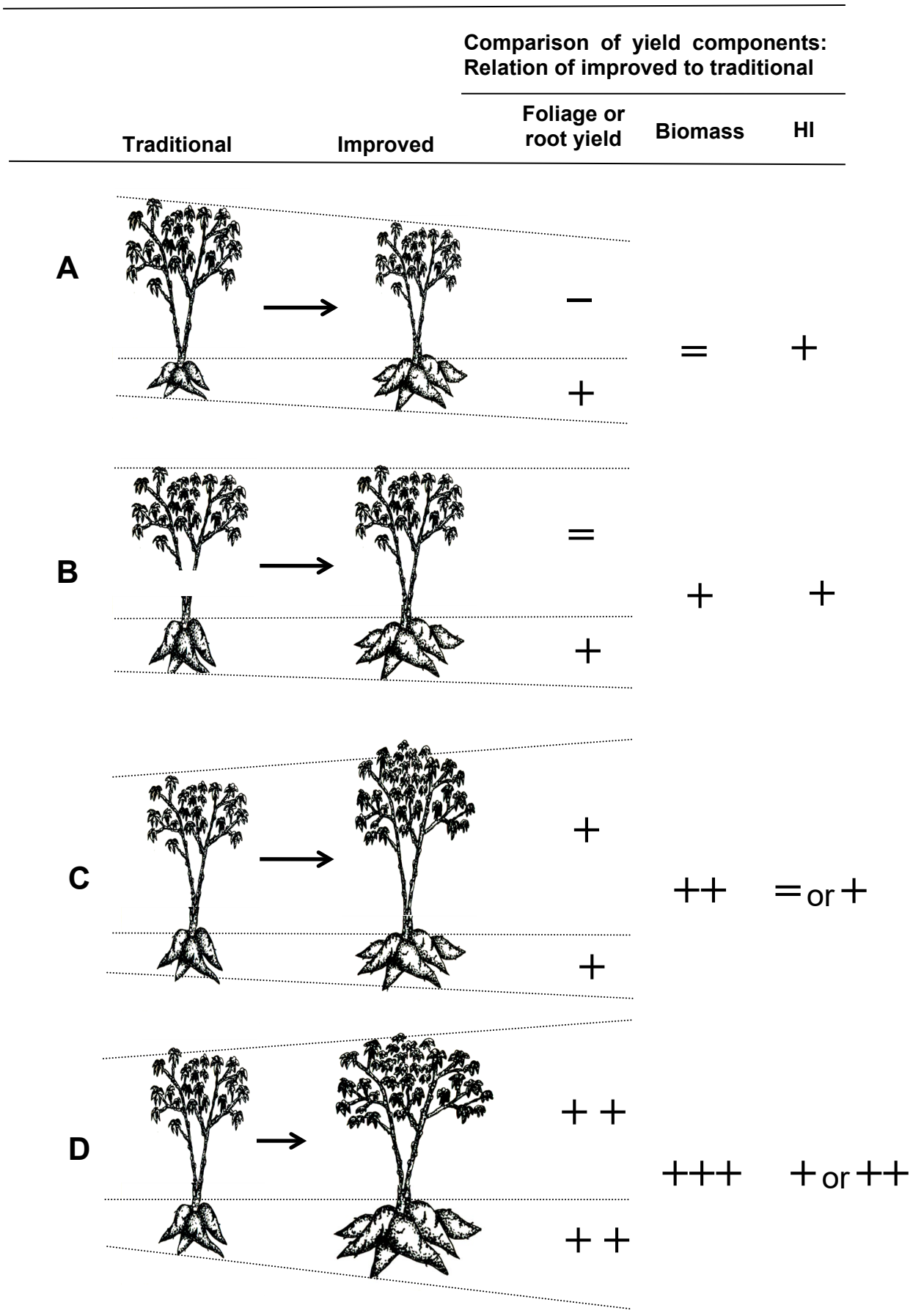
Various theories have been proposed on the modification of biomass production and carbohydrate distribution that will give maximum yield. Figure 15.1 illustrates several options. Holmes and Wilson (1977) observed a significant correlation between root yield and harvest index only among low-yielding varieties, which may indicate considerable flexibility for the breeder to modify both top growth and root yield. Kawano and Jennings (1983) suggested that best gains could be made by maintaining equal total biomass production, but improving harvest index (Figure 15.1, scenario A). Kawano (1987) showed evidence in several locations in Asia that yield gains were the result of maintaining constant top growth (and similar to local checks), while improving root yield. In this model, both total biomass and harvest index are increased (Figure 15.1, scenario B). Nonetheless, as breeding programmes advanced in the region, yield gains tended to come mostly from gains in total biomass, rather than harvest index (Kawano *et al*., 2003). Data from trials in Colombia showed that highest yielding clones had foliage yields of 20–24 percent greater than trial means and a harvest index 15–18 percent more. This trend held irrespective of the productivity level of the environment (Figure 15.1, scenario C).

As one more breeding goal, illustrated in Figure 15.1, scenario D, could be proposed, where root and top yield are both considerably higher than that of traditional clones. This ideotype may present problems of excessive top growth in terms of a sensitivity to planting density (plants per hectare). It could be an ideotype for a dual-purpose variety for harvest of both tops and roots, but this has yet to be investigated.

Part of the difficulty of interpreting results on the relationship between harvest index and yield is that most authors have calculated linear correlations, while the relationship is probably more often curvilinear, as defined in the model of Cock *et al*. (1979). That is, HI reaches an optimum at about 0.5–0.6 and very high HI is usually associated with low total biomass and moderate to low yields. Also, the data may reflect differences in the base gene pool involved in the various studies rather than suggesting conflicting selection strategies. Maintaining constant total biomass, while improving harvest index, could be the best strategy where the gene pool consists of very vigorous clones with luxurious top growth. Increasing efficiency of distribution via lower top growth and higher root yield has the highest potential for improving yield. When the gene pool has a generally good level of top growth, but has low yield, the strategy should aim at higher root yield and unchanged top yield. Finally, when distribution of dry matter between tops and roots is optimum, the only way to increase yield yet further may be to increase both top and root yields. This strategy is most likely to be applicable to already-improved breeding populations.

Ramanujan and Biradar (1987) correlated a wide range of traits with yield in an experiment extending over two years. They concluded that yielding ability is largely governed by total biomass production and balanced partitioning. Perhaps somewhat surprisingly, the two components most closely associated with yield were petiole length (0.88**) and specific leaf weight (0.86**). The concept of appropriate balance in partitioning was also supported by Ramanujam (1985). Low-yielding varieties maintained either suboptimal (<2) or supra-optimal (>4) leaf area indices during the major part of the growth period. A leaf area index of 2.3–3.5 was optimum for light interception and utilization. Profusely branching types tended to accumulate more dry matter in the tops than the roots (i.e. below optimum harvest index).

Figure 15.1 Alternatives for genetic improvement of yields in cassava by way of changing total biomass and dry matter distribution (harvest index)



It is clear that no single strategy for breeding for improved yield potential is universally applicable. The appropriate strategy appears to depend both on the local environment and on the characteristics of the gene pool used as the base for selection. As the breeding gene pool changes with selection, a strategy needs to evolve accordingly.

7. COMPETITION EFFECTS AND SELECTION EFFICIENCY

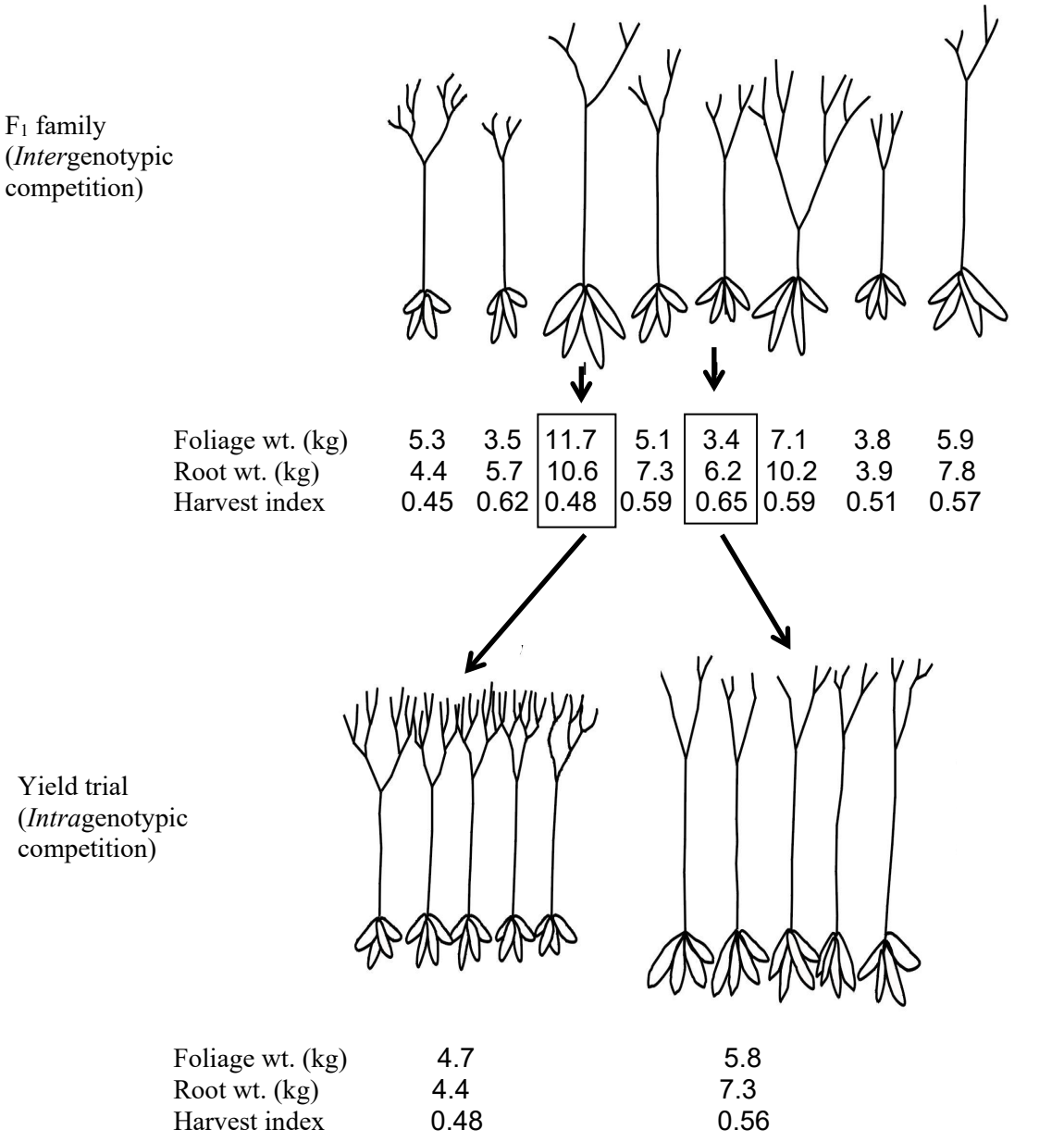
Competition effects impact nearly universally upon the selection efficiency of crop species. The breeder's task is to understand and manipulate these effects in such a way that selection objectives are efficiently achieved. Competition, in the broad sense, is the influence of a plant utilizing resources that could otherwise be captured and utilized by its neighbour. The most evident effects are the result of competition for solar energy, for water and for soil nutrients. A plant has a competitive advantage over its neighbours when it utilizes a disproportionately large share of resources on a unit land area basis. Competitive advantage normally results in greater total biomass production and usually, but not always, greater yield of roots.

Competition effects may be inter- or intraspecific and within the latter, as inter- or intragenotypic. Interspecific and intergenotypic competition refer, respectively, to competition between neighbouring plants of different species and between neighbouring plants of the same species, but of distinct genetic constitution. Intraspecific and intragenotypic competition denote competition between neighbouring plants of the same species and between neighbouring plants of the same genotype. Each of these categories of competition impinges upon the breeder's selection efficiency in a different way. Under favourable growing conditions, where neither water nor nutrients are limiting, light is likely to be the main factor for which plants compete. It is the one environmental component for which it is virtually impossible to increase the supply in a commercial field situation. In less favourable environments typical of much of cassava cultivation, nutrients and water are also frequently limiting and this will exacerbate competition effects.

For practical purposes, the breeder need not be very concerned precisely with the combination of environmental factors for which plants are competing, nor the physiological responses. Competition effects are more critical in terms of their effect on the breeder's ability to predict a genotype's performance under commercial conditions based on its performance at any given stage of evaluation. This in turn may mean predicting what yield will be in solely intragenotypic competition (commercial, monoclonal production), from trials having intergenotypic competition effects.

The initial two stages of selection usually involve plots of individual plants and single rows of individual genotypes. At these stages, intergenotypic competition effects substantially influence plant growth and yield. At later stages, these effects are removed by use of border rows. Figure 15.2 shows a simple, but common, example of how intergenotypic competition effects (F_1 family) influence dry matter distribution when compared with a situation of intragenotypic competition (bordered yield plots). The vigorous plant no. 3, with 11.7 kg of leaf and stem weight, gets a disproportional share of resources (light/water) and thereby produces a high root yield (10.6 kg). Plant no. 5 in the row has a leaf and stem weight of only 3.4 kg and is deprived of its fair share of resources by more vigorous neighbours. Nonetheless, this same plant appears to be efficient at allocating dry matter to the roots, with a harvest index of 0.65. Figure 15.2 shows a typical response of these two genotypes when planted in plots where only intragenotypic competition occurs. The highly competitive genotype will continue to be highly competitive, but since it is competing against neighbours of the same genotype which are also highly competitive, no plant has a particular advantage. All produce only average top growth. At the same time, they retain their inherent inefficiency at dry matter distribution to the roots, with the final result that this genotype that grew so luxuriously in the F_1 is now a mediocre performer in yield trials. Plant no. 5, selected and multiplied into a yield trial, now has a chance to compete against less aggressive neighbours (of the same genotype) and thereby achieves moderate plant growth. With this better top growth, combined with an efficient dry matter distribution, this genotype

Figure 15.2 Intergenotypic and intragenotypic competition effects on selection efficiency for root yield



now yields much higher than when in intergenotypic competition and higher than the highly vigorous plant in the F_1. Bordered plots should be more representative of commercial production conditions. The breeder will be required to make selection decisions at one or more of the selection stages where competition effects are quite different from those to be expected of the same genotypes if planted under commercial conditions. The strategy is to make a predictive judgment about how a genotype competing with its neighbours will perform when competing only with other plants of the same genotype. The simplest form of prediction is by regression of yield in the yield trial (bordered plots), on measures expected to have predictive value in the situation of intergenotypic competition (F_1 or single row trial).

Some of the first breeding trials established at CIAT in the early 1970s were designed to establish a methodology for selection for yield potential (Kawano *et al*., 1982). These trials provided the basis for methods used in many national cassava programmes today. One of the key trials included three selection stages (F_1, single row trial, yield trial) and two plant densities for both the F_1 and the single row trial (1 x 1 and 2 x 1 m). Yield parameters were compared across generations and across planting densities. Root yield was not significantly correlated either between the F_1 or the single row trial and the advanced yield trial. On the other hand, harvest index of both the F_1 and single row trials was significantly correlated with yield in the advanced yield trial. In the selection stages where intergenotypic competition effects were at play, the balance between root and top growth was more important as an indicator of potential yield, than was yield itself.

In a situation of intergenotypic competition, plants with highest competitive ability (usually those with the most vigorous top growth) are also likely to have highest root yield. These same genotypes, when competing against neighbours of the same clone will be competing strongly and equally against each other, resulting in lower overall yields.

These intergenotypic competition effects were most pronounced when the F_1 or single row trials were planted at the closer spacing (1 x 1 m). Lower correlations were realized at the higher density. The resulting recommendation of these studies was to plant at wide spacing those selection stages where intergenotypic competition effects may be significant and to use harvest index as the main criterion for yield selection.

This concept of using harvest index as a selection criterion has frequently been misinterpreted to imply that harvest index is more important than yield in cassava production. Rather, the suggestion is that to select for yield in commercial plantings, harvest index may be a better selection criterion than yield itself in the early selection stages. At the later stages of selection, when only intragenotypic effects exist, yield *per se* can receive more emphasis than harvest index.

These competition studies were carried out under highly favourable growing conditions at the CIAT-Palmira station, where yields of 50–60 tonnes/ha are easily obtained. As most cassava breeding programmes are selecting under considerably less favourable conditions, the question arises as to whether the same or different selection criteria are appropriate under stress. The CIAT breeding programme then designed experiments to try to answer this question (Table 15.1). Two broad conclusions were possible: (1) yield selection of individual plants in a segregating population was effective primarily within sites. The only exception was a significant correlation between harvest index at CIAT and yield in the advanced yield trial at Media Luna; (2) within sites, selection for yield *per se* in the segregating populations was more closely correlated than was harvest index with root yield in the advanced yield trials.

Characteristics of both the germplasm base and the target environment influence the appropriate emphasis on yield or on harvest index in trials where intergenotypic competition is present. For a given plant spacing, competition effects will be greater for more vigorous plants. In less favourable environments, or for gene pools with generally less vigour, yield *per se* in the F_1 or single-row trials may be as good as or better than harvest index as a criterion for yield selection. Conversely, in highly favourable environments, less vigorous plants will be at a severe disadvantage and harvest index may be the only indicator of yield potential. However, nearly all results show that any attention to yield at

the F_1 stage should be modest, whether in the form of harvest index or root yield, owing to its low broadsense heritability.

Table 15.1 Linear correlations between single plant traits (F_1 seedlings or F_1C_1 cloned seedlings) and root yield in advanced yield trials (AYT)

	F_1 - CIAT-Palmira		F_1C_1 - Harvest index			F_1C_1 - Root yield		
Site of AYT	Harvest index	Root yield	CIAT	Media Luna	Carima-gua	CIAT	Media Luna	Cari-magua
CIAT	0.20	0.14	0.04	-0.07	-0.06	0.20	0.28	0.03
Media Luna	0.20	0.29*	0.31*	0.35*	0.11	0.21	0.44**	-0.18
Carimagua	0.11	0.02	-0.09	-0.16	0.18	0.09	-0.14	0.58**
Source: CIAT Cassava Programme, 1985 Annual Report								

8. YIELD AS AN INTEGRATING SELECTION CRITERION

The importance of integrated selection objectives – overall plant improvement to produce a variety acceptable at the farm and market levels – has been stressed throughout this publication. Yield is certainly one measure that naturally integrates a whole array of traits that ultimately determine acceptability: sprouting ability, temperature adaptation, vigour, plant type, disease and insect resistance and others. Although it is probably important to monitor the effect of yield selection on other traits, selection for yield alone is often a simple and powerful means of modifying component traits. Not only does this integrate various traits, but it does so in a manner that can optimize the level of expression of each component trait for high yield expression.

9. YIELD AND INPUTS – PLANNING FOR THE FUTURE

In the past few decades, most cassava breeders have turned away from selecting under highly favourable environmental conditions such as high fertility, irrigation, or intensive pesticide use. In the short term, most cassava farmers will not adopt these practices and would reject varieties that depended on luxurious use of purchased inputs for good performance. Will this change in the future? Market forces will gradually move many cassava cultivators in the direction of more inputs for higher productivity and profitability. Research results and experience will allow them to make wise use of inputs to sustain and improve soil fertility. The rate at which these processes occur and the course they take will vary regionally. Market diversification in Asia, and small holding size, will be major forces for increased adoption of inputs. In Africa, the incentives may be primarily the pressing demand to keep up with food demand for increasing populations. In Latin America, rising incomes will increase industrial demand for cassava, especially for starch and animal feed. These markets will drive the intensification of production.

Breeders need to be planning for 10–15 years into the future and that almost certainly means taking into account input responsiveness. In the transition phase between low and high input use, breeding can be more complicated. One logical strategy is to select in favourable and unfavourable environments simultaneously (or with low and moderate or high inputs) and identify genotypes that perform well across this range. Priority can be adjusted according to the rate at which changes in agronomic practices occur in the region.

10. SELECTION FOR EARLY MATURITY

Farmers frequently cite early maturity as a high priority interest, but a common understanding about the concept as it applies to cassava does not exist. Physiologists do not recognize critical stages of development in cassava, as are commonly defined for crops where seeds are the commercial product. Throughout most of the plant's life, foliage and storage roots develop simultaneously. No distinct period can be defined at which a cassava plant attains maturity.

Figure 15.3 illustrates a definition of maturity based on yield formation over time. Yield ranking of the varieties depends upon time of harvest. A different variety attains highest yield for harvest at 6, 12 or 18 months after planting. Some CIAT data suggest that clones with highest yield at an early harvest tend also to be highest yielding clones at later stages; early maturity and high yield are often similar concepts. IITA, however, found that performance of genotypes at the early stage of the growth cycle may not necessarily predict the performance at a later stage. This generally depended on the agro-ecological zone and time of planting. IITA suggested selection for 6 or 12-month harvest as independent objectives (IITA, 1993a). MM92, a variety selected in Malaysia, shows high yield at six months, levelling off to average yields at 12 months (K. Kawano, personal communication). CIAT and CNPMF in Brazil found that, while selection for early yielding ability appears not to be so difficult in semi-arid environments, achieving acceptable quality (especially dry matter content) is much more difficult (CIAT, 1994).

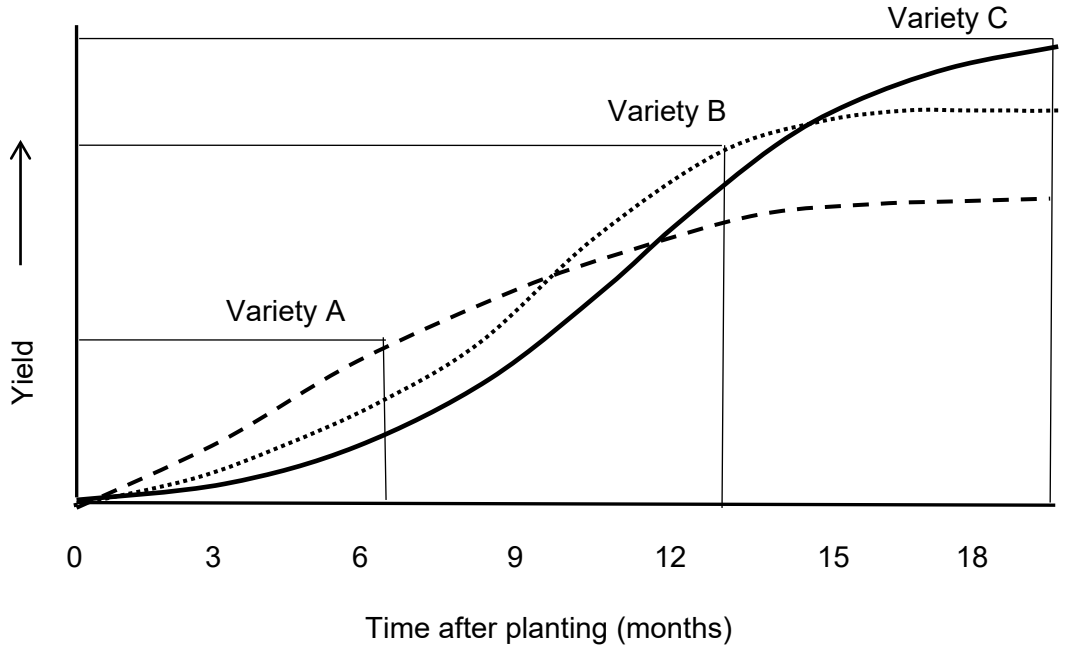
Root quality may be a primary indicator of maturity for farmers, because some clones appear to reach a certain starch content or quality earlier than others. Late varieties may maintain high quality levels for a longer period, irrespective of yield.

Another definition of maturity relates to root shape. Clones with short roots will generally produce storage roots of commercially useful diameter, earlier than those with long roots. Yield development of both clones may be the same but the longer roots do not thicken to commercially acceptable levels until later than the short ones. *Mantequeira*, or *CMC 40*, from southern Brazil is widely known as an early variety and its earliness appears to be, at least in part, a result of short roots that thicken quickly.

Breeders have not adequately screened germplasm for the various components that may define maturity. The main constraints are firstly, limited understanding of which components are locally important; and secondly, maturity evaluation is costly due to the need for multiple harvest dates. Early maturity is most important: (1) in situations where pressure on land is increasing and farmers need to intensify production; (2) in semi-arid regions where early root bulking may allow harvest after only one cycle of rain; (3) in the subtropics, where early maturity could allow harvest in one growing season, as opposed to the two seasons typically required; and (4) in the highlands, where low temperatures normally extend harvest to 15–18 months. Studies in Africa (Nweke *et al*., 1994) showed that farmers in sub-Saharan Africa generally consider sweet varieties to be earlier maturing. The length of the bulking period was cited as the single most important reason why farmers abandon one variety in favour of another.

With earlier maturing varieties, farmers may be able to intensify their farming systems to include another crop during the cycle that cassava alone previously occupied. This is most likely to be a goal where the crop normally covers two growing seasons, such as in the highlands, subtropics or semiarid regions. However, to justify the change from two- to one-growing season(s), various additional expenses need to be covered by improved performance. The costs of land preparation, weed control and harvest, on the basis of a tonne of harvested roots, are likely to all be substantially higher with a single-season as compared with a double-season crop. Soil erosion may be exacerbated by longer periods without vegetative cover. The complete agronomic and economic implications of an early maturity breeding strategy need to be understood before committing resources.

Figure 15.3 Alternative yield responses in cassava over time and relationship to maturity definitions



11. CANOPY CHARACTERISTICS

Farmers typically consider architecture to be a critical varietal characteristics for cassava. Breeders have often given inadequate attention to plant type. As a rather broad range of plant types can result in high yield potential, breeders have often ignored the importance that plant type has for other aspects of varietal acceptability. Architecture directly or indirectly influences yield potential, adaptation to cropping systems, weed control through canopy cover, lodging resistance and quality of planting material. Desired canopy characteristics may vary from one region or cropping system to another. For example, surveys in East and West Africa (Nweke *et al*., 2002) noted a markedly higher preference for non-branched types in the savanna region (33 percent) as compared with the forests (9 percent).

11.1 AN IDEOTYPE FOR HIGH YIELD

The model developed by Cock *et al*. (1979) enabled them to pinpoint characters associated with increased yield and to estimate quantitatively how much yield improvement may be expected by a known change in any one of these characters. Plant architecture is a crucial part of the model. To reach the optimum leaf area index quickly and maintain it at that level, a plant with moderate branching, beginning at about 30 weeks, is desirable. Such a plant gives an optimum balance between leaf area and root growth when planted at a population that gives 20 000 shoots per hectare.

The two direct determinants of root yield (rate and duration of root bulking) are closely influenced by the availability of carbohydrates in excess of the requirements for aerial growth. As the branching pattern plays a major role in determining the potential for aerial growth (through its influence on apex number increase and leaf and stem production), it has an important effect on root yield (Tan and Cock, 1979).

Leaf area index can be manipulated by modifying branching patterns, leaf longevity and leaf production rate per apex. Even with long leaf life, an unbranched plant form has difficulty in maintaining a high enough level of LAI, especially late in the season when leaf fall is substantial. Plants with early initiation

of branching can quickly develop an excessive LAI. Carbohydrate reserves are used for new leaf production rather than root bulking. Tan and Cock (1979) recommended selection for a moderate rate of LAI development, either by early branching with a low number of apices per branch point or late branching with a slow increase in leaf number. Later branching has additional advantages of facilitating interrow cultivation and less competition with intercrops. An earlier branched variety may be better for weed suppression and erosion control in a monocropping situation. The priorities among the various effects will determine an appropriate selection strategy.

11.2 PLANT ARCHITECTURE IN MULTIPLE CROPPING SYSTEMS

Multiple cropping includes an array of cropping sequences that all produce more than one crop within a year. In a broad sense, multiple cropping could refer to: (1) culture of two or more different crops where one follows the other; (2) mixed planting of two or more crops in the same field sown at about the same time (intercropping); or (3) interplanting of different crops in the same field in a relay pattern. In cassava systems the most common form of multiple cropping is intercropping.

Considerably more work needs to be carried out on defining cassava canopy characteristics required for specific intercropping systems. As a generalized conclusion for many systems, the ideal plant type for monoculture seems to be roughly similar to that for intercropping, i.e. plants of intermediate early vigour (which will neither compete excessively with the intercrop, nor suffer excessive competition from neighbours) and late branching (again, to avoid excessive shading of the intercrop). Late branching may, however, not be such a critical characteristic, because most short-season intercrops would be harvested before moderately branching varieties close the canopy. Most research on intercropping with cassava has been carried out with very limited genetic variability, so conclusions must be interpreted with caution.

11.3 EARLY CANOPY COVER TO REDUCE WEED GROWTH AND SOIL EROSION

Shading by the crop canopy is the principal means of weed control in most cropping systems, except in the early growth period. The degree of shading achieved is a function of planting arrangement and leaf area of individual plants. Clones that rapidly increase leaf area index provide better shading and better weed control.

CIAT breeders routinely make subjective evaluations of early vigour about three months after planting. Not only is early vigour an indicator of the level of competitiveness with weeds, but this rating is frequently positively correlated with root yield at harvest. Rapid canopy development early in the growing season need not result in excessive leaf area index during the latter parts of the growing season. This balance is achieved by having two or three main stems developing from the planting piece, but with late branching.

Weed control through canopy shading, at commercial planting densities, is normally relatively good after three or four months, until the end of the growing season, if the growing season is one year or less. Problems of weed control may again develop if the crop is defoliated during a dry season and then continues growth into another rainy season. Weeds may again gain a competitive advantage before canopy shading can exert effective control.

There are now solid data illustrating the beneficial effects of early canopy cover on reduced soil erosion. Trials in Colombia and in various sites in Asia showed that canopy growth, as influenced either by soil fertility or inherent varietal traits, is among the best ways to reduce erosion. It is unlikely, however, that farmers would give consideration to a variety's benefits in erosion control unless it also combined all other desired traits. In most situations, variety characteristics will be secondary to cultural practices to limit erosion. Nonetheless, the variety effects are sufficiently important at least to warrant monitoring the impact that any new variety might have in erosion-prone target areas.

11.4 LODGING RESISTANCE

Lodging in cassava is generally the result of partial dislodging or breakage of the root system. Unlike the grain crops, lodging in cassava usually is not associated with stem breakage. Exceptions are the damage from very high winds such as typhoons and hurricanes. Although few data exist on plant factors related to lodging in cassava, it seems logical to assume that it is a function of both canopy and root characteristics. Tall, heavy plants create more torque on the root system in heavy winds. Longer roots may logically be assumed to confer some resistance to lodging, but data to confirm this are lacking. Silva and Schmidt (1967) found a range from 12–54 percent lodging in a variety trial during a growing season with heavy winds. They were unable to relate specific plant characteristics to these differences.

CIAT normally evaluates lodging at the end of the growing season, just before harvest. In most years lodging is not a major problem at any of the CIAT test sites. For several trials where lodging was moderate to high, correlations between lodging (subjective evaluation) and various canopy and root characters were calculated. The highest and most consistent correlations (negative) were for harvest index and lodging. Root weight also showed generally significant negative correlations with lodging. Correlation with plant height, number of branching levels and top weight were inconsistent.

These data do not imply cause and effect relationships. In fact, two very different hypotheses might be proposed. One could be that lodging reduces root yield proportionally more than top yield and thus harvest index shows a fairly consistent negative correlation with lodging. An alternative hypothesis might be that lodging is reduced in plants where there is a good balance between top and root growth.

11.5 LEAVES AS A COMMERCIAL PRODUCT

Leaves are a source of protein and vitamins in human diets in several countries, especially Brazil, the Democratic Republic of the Congo and Indonesia. Cassava is used for grazing goats and cattle and leaves and young stems are sometimes harvested for feeding animals in more controlled systems. Breeders have done relatively little to understand or exploit the potential of developing cassava as a forage crop or as a dual purpose crop for forage and roots. Lutaladio (1984) evaluated 30 clones in the Democratic Republic of the Congo for leaf production, with harvests at four, six and eight months. There was considerable variation among clones, with the local variety *Mpelolongi* having the highest yield of 10.2 tonnes/ha. In this experiment, effect of leaf harvests on root yield was not reported.

CIAT screened clones for both forage and root production on the acid soils of the Carimagua and Quilichao experiment stations (CIAT, 1985). The trials included four varieties and four plant densities (20.4, 27.8, 40 and 62.5 thousand plants/ha). With multiple cuttings, up to 24 tonnes/ha dry forage was harvested in Quilichao. Varieties less productive in forage had higher root yields. For example, the local hybrid HMC 2 produced as much as 17 tonnes dry roots and 16 tonnes dry forage in two years. The best population at both sites was 27 800 plants/ha. Although the experiments were not continued beyond two years, CIAT believed that a forage harvest system could continue without replanting for several years.

Interest in the use of cassava foliage for animal feed expanded rapidly in the early 2000s, especially in Asia. Most of the current work focuses on agronomic practices to optimize foliage production, comparing characteristics of existing varieties, mechanization of harvest and post-harvest management (drying, ensiling). The accumulation of information in these areas will soon lead to breeding programmes designing new varieties for optimum foliage production, or dual-purpose foliage and root production (Proceedings of the 7th cassava workshop for Asia; available on compact disk only).

11.6 EVALUATION TECHNIQUES

Except for growth habit (mainly branching), canopy characteristics have not been widely used as criteria for selection. In part this is because of the difficulty of measurement and in part because there are many characters for which it is not clear what should be the direction of selection. Branching habit is possibly the easiest. A plant's branching habit can be roughly described by just two numbers: height to first branching point and number of levels of branching. Both can be measured very quickly in the field.

Branching angle is also a factor in determining architecture, but there is little information available describing its influence on yield or other traits. Insofar as a more closed branching angle results in more erect plant types, this might be suggested as the preferred trait. Measurement is easy, but time-consuming. A subjective evaluation, for example on a 1–5 scale is also possible. For a more detailed description of branching habit, time to each branching level can be measured, but this entails considerable investment time.

Leaf life is one of the key canopy traits influencing yield potential. This trait is easy to measure, but doing so is very time-consuming. For this reason, it has not been incorporated into mass screening. Firstly, one has to determine the best stage of growth at which to measure leaf life; this stage may vary from one environment to another. Secondly, pest and disease attacks may profoundly influence leaf life, masking genetic differences. Generally, it would not be practical to protect against attack by these pests and pathogens, because host plant resistance is being sought. Separating breeding material into pesticide-protected and non-protected plots is one alternative, but greatly complicates the breeding programme. Also, efficiency is usually lost in achieving balanced multiple trait selection by this approach. There needs to be further research to develop rapid methods for assessing leaf life for mass screening.

Leaf area index was once also very time-consuming and for precise methods, a destructive procedure. Measures of leaf area were taken on automatic area metres or estimated from grids. Portable sensors are now available (albeit, rather expensive) that measure leaf area on intact plants in the field. This technology should contribute also to developing rapid screening methods through visual, subjective evaluations.

Given the lack of information on inheritance of most of the canopy characteristics and lack of mass screening methods, Tan (1987) suggested that a subjective evaluation of the condition of the canopy throughout the crop cycle may be used as an index of its adequacy. While the canopy must not be excessive (as in highly branched forms where leaf life is reduced drastically by intershading) enough foliage should be maintained throughout the cropping season to ensure a net production of dry matter for root storage.

The breeder must also keep in mind that in the seedling population, that branching tends to begin later than for the same genotype propagated from stem cuttings. Branching angle, leaf life, leaf size or other canopy traits have not been compared between seed- and stake-derived material, for the same genotypes.

12. PRODUCTION OF PLANTING MATERIAL

As for any crop, good quality of planting material in cassava is fundamental for successful establishment, vigorous plant growth and high yields. The majority of farmers may underestimate the importance of this feature of production. In many cassava plantations, plant stand is lower than the number of cuttings originally planted, there is little uniformity in plant vigour, production per plant varies considerably and root rot is found at harvest. All these conditions can be related to quality of the propagating material. In addition, the use of infected or infested propagating material can disseminate systemic pathogens (viruses or virus-like organisms, mycoplasmas, bacteria and fungi), as well as mites and insects that attack the cassava stem. This is the most frequent means of pest introduction into plantations, regions, countries or continents where they did not previously exist (Lozano, 1985).

Production of high quality planting material from cassava has generally been considered a management factor rather than something that is subject to influence by breeding. However, when individual factors influencing stake quality are considered, it becomes evident that at least some of them are under genetic control.
Five categories of breeding objectives related to quality of planting material are discussed: (1) plant architecture; (2) storability; (3) sprouting ability; (4) pre-harvest sprouting; and (5) pest resistance. Some of these are interrelated.

12.1 PLANT ARCHITECTURE

Highest quality stakes are about 20–25 cm long, 2–3 cm in diameter and have five to eight viable nodes. Plant architecture is a key determinant of the number and quality of stakes produced. These are a function of number of main stems, levels of branching and length of individual stems. A large total length of stems in a plant does not necessarily produce a high number of good planting stakes. Branching pattern, particularly affects stake quantity. Unbranched stems are generally much more uniform in diameter throughout the plant canopy than highly branched stems. In highly branched clones, the recommended stake diameter might be available only at one or a few branching levels, while at higher levels, stems are too thin. Branching pattern also affects the capability of the scientist or grower to cut long, straight stems for convenient storage or shipping. Highly branched types can produce only short stakes or non-uniform stakes which are difficult to pack in bundles and ship or store.

These architectural traits influencing quality of planting material are highly heritable and relatively easily managed by breeding. Naturally, any objectives related to modification of architecture for improving planting material need to take into account the influence on other breeding objectives.

12.2 STORABILITY

Cut cassava stems may be stored for up to 90 days with no significant decline in sprouting ability, although some yield decrease occurs (Leihner, 1986). However, there is a real lack of information on the components of storability or their genetic variation. Observations at CIAT suggest substantial varietal differences. If stake storage is currently, or projected to be, a common practice in the target region, this may be an objective to consider in a breeding programme.

The breeder first needs to define the storage conditions under which to make evaluations. This would probably be determined by the most common method of stake storage in the region, but also taking into account new technology recommendations. After conditions are defined, it should be feasible to carry out selection for storability as a normal, routine part of the breeding programme. If harvesting and planting of breeding nurseries coincide with those of commercial plantings, length of storage should also be representative of commercial conditions. After a pre-defined storage period, those clones appearing excessively dehydrated, or otherwise having suffered from storage, could be discarded prior to planting. After planting, further selection for the ability to store well would be integrated into other selection parameters such as sprouting ability, vigour and yield.

While few cassava breeding programmes make a conscious effort to genetically improve storability of planting material, there may be inadvertent and unconscious selection occurring through evaluation of secondary effects. Continuing studies on various environmental effects on stake storage are likely to shed some light on genetic differences.

12.3 SPROUTING ABILITY

As sprouting ability is so closely related to yield on a unit area basis (normally, beyond a certain minimum level of failure at which neighbouring plants compensate for losses), selection for sprouting is probably unconsciously a part of any breeding programme working in an area where genotypic differences are expressed. However, correcting yields to a per plant basis rather than a per area basis can eliminate the positive selection for good sprouting ability that normally results from selection for unit area yield.

Reduced sprouting may be the result of soil water stress, temperature stress (either above or below optimum), nutrient or water status of the stake, pest attack or other physical damage. Genetic variation for tolerance to some of these are well known and could potentially exist for others.

Most studies on sprouting ability have concentrated on environmental effects rather than varietal differences. Selection efficiency may be improved by identifying the causes of good or bad sprouting (e.g. desiccation, low nutrient status, pathogens) and creating an environment to detect genetic

differences for those constraints. Alternatively, if the mechanisms of good (or poor) sprouting ability are identified, rapid screening techniques might be developed for some of these. For example, Mitsunori *et al*. (1990) found that stem bulk density was the main determinant of successful sprouting in the varieties they studied.

Although they worked with a only a few varieties, El-Sharkawy and Cock (1987a) showed large differences in capacity for sprouting and rootlet formation. These differences tended to persist throughout the growing season, leading to the suggestion that breeders could effectively select for ability to establish well by mass screening in semi-controlled conditions.

The Thai National Cassava Programme found dramatic differences in sprouting ability in a year when extreme drought stress affected the cassava experiment station. However, this screening was reliant on a chance environmental event and has not been repeatable (K. Kawano, personal communication). This experience and others seem to show that observed differences in any given trial can be the result of a very specific combination of circumstances. When selection pressure is inconsistent, progress will only come through sustained selection over many years. More steady progress can only be accomplished either by finding or creating the appropriate environment to provide known stress on stake sprouting, or identifying characters associated with sprouting ability which are expressed and can be evaluated in either the presence or absence of the stress. Akoroda *et al*. (1997) developed screening methods (involving sprouting trials in a water trough and in the screenhouse) and were able to find significant differences among clones in establishment ability.

12.4 PRE-HARVEST SPROUTING

Cassava growers normally expect planting stakes to sprout only after they have been planted. Some genotypes have a propensity for sprouting while still growing in the field. Although this is commonly caused in most clones when apical dominance is broken and lateral buds begin to grow, some clones sprout even when apices are undamaged. Although effects of such sprouting have not been studied thoroughly, presumably this growth could draw on nutrient reserves in the stake and thereby be undesirable for good storability. Sprouted stakes can also complicate the application of pre-emergent herbicides. It is also not known whether there might be any relationship between pre-harvest sprouting and good sprouting ability after planting in the field.

Until further evidence is brought to light, it is generally believed that preharvest sprouting is an undesirable trait which should be selected against. No reports exist on improved methodology for evaluation of this trait, as compared with opportunistic field evaluations when the trait appears.

12.5 ROLE OF DISEASE AND PEST RESISTANCE

The stem of the cassava plant may be attacked by a wide range of insects, mites or pathogens, and many of which can reduce sprouting ability. Insofar as resistance exists to these pests, there is potential for improving sprouting ability by incorporating resistance to the corresponding pests.

For many pests, the resistance may be conferred in some other plant part and thereby reduce incidence of the pest on or in the stems. Bacterial blight, superelongation disease and CMD could be included in this category. Foliar resistance to these pathogens reduces levels of the pathogen systemically in the stems and thereby reduces losses from poor sprouting or low vigour.

A second category of pests are those that locally affect the stem, or planting piece. These include non-systemic pathogens that invade only part of the stem, and insects and mites affecting the buds or other superficial parts of the stem. Scale insects, shoot flies and stem borers are important in this category. Virtually no work has been carried out to study the resistance of the cassava stem itself to these pests. Stake treatments often contribute to effective control, especially for those that are superficial, such as scale insects. Host plant resistance is usually not a priority for these pests.

Another major category of pests affecting sprouting are the soil-borne pathogens or insects. Many of these are also best controlled through cultural practices or stake treatment. However, there does seem to be some promise of resistance to at least two of these, namely, *Fusarium* and *Diplodia* root rotting pathogens. Good levels of resistance will markedly improve production of quality planting material in disease-endemic areas.

12.6 AN INTEGRATED SYSTEM OF SELECTION

Most programmes will choose not to include breeding for quality of planting material as a high priority objective. However, for regions where stake storage is sufficiently long to pose risks of substantial decline in quality, genetic improvement options should be explored. If the breeder adopts a system of managing planting material similar to common local practices, there is a good probability of identifying potential problem varieties before they reach commercial production. By exposing planting material of experimental lines to the stresses of storage, those with defects should be eliminated by poor performance in subsequent cycles.

Unless there is a specific serious constraint related to planting material, this passive integrated approach to eliminating defective clones is adequate for most programmes. On the other hand, if planting material for each cycle is introduced from optimum storage conditions, selected varieties may not show their inherent defects until well into a commercialization phase.

Chapter 16. Pest and disease resistance

Insects, mites and pathogens often reduce yield and quality in cassava, especially where natural control systems have been disrupted (Table 16.1). A cassava breeder contemplating a pest management programme needs to make critical early decisions whether any control at all is necessary and if so, whether to employ host plant resistance or some other means of management. If host resistance is chosen for some of the constraints, the approach will include: developing evaluation techniques, identifying sources of resistance, understanding mode of inheritance, defining a breeding strategy, deploying new materials to growers and measuring genetic and economic gains.

Table 16.1 Expected yield gain (percent) from complete alleviation of principal pest and disease constraints of cassava

	Africa	Asia	Latin America
Diseases	19.6	4.0	14.7
Cassava mosaic disease	10.2	0.2	0.0
Cassava bacterial blight	5.9	1.1	3.9
Root rot (var. species)	1.0	0.3	4.0
Anthracnose	1.4	0.3	2.3
Other leaf/stem pathogens	1.1	2.1	2.4
Pests	12.5	3.0	8.9
Green spider mite	8.0	1.9	2.8
Mealybug	2.7	0.0	0.6
Hornworm	0.0	0.0	1.5
Termites	0.5	0.1	0.0
Foraging mammals	1.1	0.1	0.3
Source: Unpublished CIAT Cassava Programme documents; based on CIAT programme staff estimates			

1. CONCEPTS OF RESISTANCE AND IMPLICATIONS FOR BREEDING STRATEGY

As breeders frequently have to deal simultaneously with both micro-organisms and arthropod pests, it should be advantageous to find philosophically similar and integrated breeding approaches. There is no completely satisfactory term to encompass pathogens, insects and mites and even more difficult to find one that extends to larger animals such as wild pigs or deer. For convenience, throughout this chapter the term ‘pests’ will be used for this purpose, even though in the literature it is frequently applied only to arthropod pests. Disease is the resulting injury in a plant due to pathogen establishment and growth. There is no equivalent term for insect and mite damage feeding, so pest damage is defined to include effects of pathogens, insects, mites or larger animals.

Host plant resistance is the heritable property that enables a plant to avoid establishment of a pest, to inhibit the growth of the pest once established on the plant, or to tolerate or recover from injury by pest populations that cause greater yield loss to other plants of the same species under similar environmental conditions. Resistance may involve any combination of these mechanisms.

Escape is distinct from resistance; it describes the situation when inherently susceptible plants do not become infested or infected because of factors unrelated to plant genotype. This can be the result of planting in a region or at a time when the pests are not present, or are at low levels. Escape may occur differentially within a field as a result of pest population variations.
The literature on host plant resistance is replete with terminology reflecting different schools of thought. The terms horizontal, vertical, stable, field, rate-reducing, gene-for-gene are some of the common terms describing types of resistance. This chapter gives only brief descriptions of some of these concepts,

because the fine points of their definitions are not of critical importance to most resistance breeding efforts in cassava.

Non-rate-reducing resistance is often called vertical, or race-specific resistance and usually is under the control of one or a few genes. If there is a compatible reaction between host and pathogen, the rate of disease progression will be equal on different host genotypes. Horizontal resistance reduces the rate of disease progression and is often under polygenic control. These concepts were developed on the basis of a few classical cases of clear-cut differences in host-pathogen interactions. In reality nature presents a much more complex range of possibilities.

In a review of numerous cases of crop–disease interaction, Robinson (1976) concluded that both horizontal and vertical resistance are stable in balanced natural systems. Vertical resistance is frequently present in sexually propagated annual species and often evolves from a stable to an unstable system when plant breeders and farmers disturb the natural balance. Breeders of most crops concentrated on those resistance genes most easily manipulated, i.e. resistance under the control of a few genes. In combination with monocropping systems of large contiguous areas of single genotypes, an ideal situation is set up for the pathogen to evolve new races that rapidly overcome resistance.

Relative to most other major crops, cassava has been less influenced by modern breeding, in that most countries still grow a wide array of landrace varieties. There has been less opportunity for loss of the low to moderate level, multigenic horizontal resistance that evolved in primitive varieties, or for breeders to concentrate on major-gene or unstable vertical resistance. This perhaps represents one of the great opportunities in plant breeding history, to develop broad-based, stable resistance with a germplasm base of currently used, adapted landrace varieties as gene sources. To develop multiple resistance, cassava breeders often need not resort to the long-range and difficult option of extracting genes from an agronomically inferior genetic background. Many presently grown commercial varieties appear to be good sources of stable, multigenic resistance.

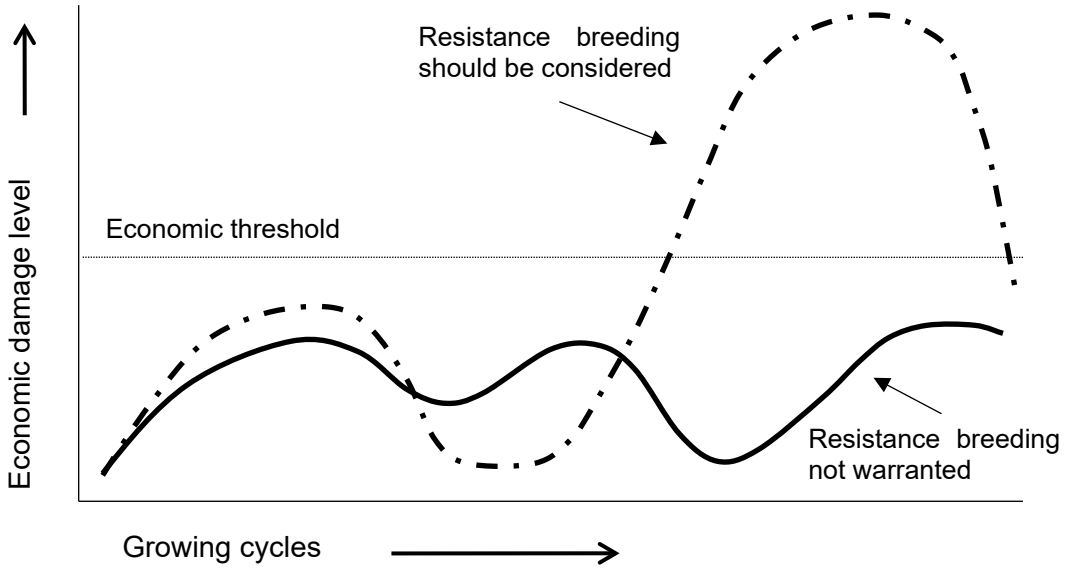
2. ECONOMIC THRESHOLDS

Visible plant damage or the demonstration of economic losses from pests or diseases are not in themselves a justification to apply a means of control. The concept of economic thresholds is broadly used by plant protection specialists to determine the appropriate level of investment in control measures (Figure 16.1). The economic threshold is the population level, or damage level, at which the economic losses exceed the costs of control. In general, the economic threshold is lower for host plant resistance as compared with other chemical and other means of control that require purchased inputs. Often resistant varieties do not imply additional cost to the farmer, but this is not necessarily so. There can be a hidden cost if resistant varieties have a lower yield potential (yield drag), such that a grower would obtain lower yields, compared with a susceptible variety, when insect or disease pressure is low. For privately developed varieties (not yet a reality in cassava breeding), it is common to charge a premium for resistant varieties, especially those developed through transformation events. In general, the economic threshold that justifies a resistance breeding programme for cassava is well below the threshold for other means. Nonetheless, breeders and plant protection scientists need to work together to understand both the biological and economic priorities.

3. CROP AND PEST CO-EVOLUTION: IMPLICATIONS FOR BREEDING

Virtually all crop plants evolved under pressures from various organisms (other than humans), which utilized these plants as food. Among the most important of these were insects, mites and various micro-organisms. A combination of isolation, cultural practices and host plant resistance normally maintained them at subeconomic and stable levels and few would be considered pests. Rotation, intercropping, burning of residue and low density planting, undoubtedly kept pest pressures under some control. Genetic resistance, probably accumulated over centuries through a combination of natural evolution and conscious farmer selection, provided further control.

Figure 16.1 Economic thresholds as a decision tool for implementing resistance breeding



The array of pests in any given location depends, firstly, upon suitability of the environment for their establishment and secondly, on an initial introduction. The length of time after introduction has substantial influence on the level of resistance that evolves. In Latin America, where cassava has a long history, there has been a long co-evolution of the crop and its pests. Low to intermediate levels of resistance to prevalent pests are common here. In Africa and Asia there have been more cases of new encounters of the crop with its pests. Most notable of these are the examples of introduction of the mealybug and green mite from Latin America to Africa. Pests can also arise as mutations of organisms that allow their feeding or infection of cassava, when previously they did not. This appears to be the case for CMD, which is unknown in the Americas.

Cassava plantings were often isolated in space by jungle or by mountain ranges and distinct pest complexes developed by regions or even quite locally. Cassava varieties evolved with resistance to distinct combinations of both biological and physical conditions. Although genes for pest resistance were common in most cassava growing environments, high levels of resistance would normally not have been necessary to keep damage levels low; other control measures also came into play. It seems to be the normal (though not universal) evolutionary trend that resistance genes do not accumulate in excess of what is needed for reasonable survival and reproduction of the host species.

In modern times, humans have moved cassava clones all over the world, to occupy completely new environments, often with different combinations and different levels of pest pressures. The result is often what appears to be a breakdown of resistance. In fact, there may have been no genetic change in the pests at all, but simply higher population pressures created by a more favourable environment, or exposure of a clone to a pest to which it had not previously been exposed and to which it did not evolve resistance.

Movement of varieties out of their evolutionary homelands, monocropping, high density planting and continuous cropping all seem to have exacerbated pest problems. Host plant resistance is now one of the

most common and highest priority breeding objectives of cassava programmes around the world and especially in Latin America and Africa.

4. PEST MANAGEMENT ALTERNATIVES

Plant breeders tend to think immediately of host plant resistance as the ideal form of pest management, which it often is. However, if there are several or many pests requiring some level of control, it is virtually impossible to develop resistance to all of them, especially if other traits require simultaneous improvement. As a broad cassava improvement strategy, pest management goals should consider each of the four basic approaches: (1) host resistance; (2) biological control; (3) cultural practices; and (4) chemical control. While strategies need to be optimized and balanced, emphasis here is on host plant resistance because it is by far the principal one with which the breeder needs to be concerned.

4.1 HOST PLANT RESISTANCE

Host plant resistance often offers the most economical and environmentally sound means of controlling cassava pests. Once resistance is incorporated into a variety, the producer has no recurring costs. Disadvantages are the length of time required for breeding, lack of adequate genetic variability for some pests and the possibility of pests evolving to overcome resistance.

Bellotti and Schoonhoven (1985) suggested several criteria to consider before embarking on a programme of host plant resistance for a given mite or insect problem. These criteria are modified slightly here to include resistance to diseases as well.

- The level of economic damage caused by a particular pest should be significant, or potentially significant in the future.
- Resistance should be sought only for those pests for which adequate genetic variability is demonstrated. With the tools of precision gene transfer, this variability need not necessarily be within species that can be crossed conventionally with the target crop species.
- The availability of adequate, low-cost, environmentally sound alternatives for control of certain pests could negate the need for entering into an extensive resistance breeding programme.
- The levels of resistance needed to reduce pest populations below an economic injury level should be considered. As some cassava varieties have a high economic threshold for damage, high levels of resistance may not be necessary.
- Low levels of resistance can be combined with other control methods (i.e. biological control or cultural practices) to maintain pest populations below economic damage levels.
- Multiple cropping systems may require lower levels of resistance because these systems in themselves can have suppressive effects on certain pest populations.

Before initiating a resistance breeding programme, basic background studies should focus on: (1) determining yield losses and levels of economic injury for the major pests or combinations of pests; (2) the role of the environment and the influence of plant age on pest incidence and severity of damage; (3) biology and ecology of all important pests; (4) potential pest problems that could occur if the cassava area increases and/or management practices change significantly (e.g. from rotational schemes to continuous cassava, or from varietal mixtures to single-variety plantings); (5) potential for minor or secondary pests becoming increasingly important as high-yielding varieties are released; (6) alternative novel control practices, such as attractants, pheromones, or insect growth regulators; (7) pest problems during the storage of planting material and the establishment phase of the plant; and (8) the production of pest-free planting material.

4.2 BIOLOGICAL CONTROL

In natural ecosystems, biological agents play a major role in keeping pests at low levels. Modern agricultural practices, especially pesticide use, have seriously affected the balance between pests and their natural control agents. Mites and insects have been targets of biological control research for many

years. More recently it is becoming clear that beneficial bacteria and possibly fungi, also play a part in control, especially of cassava foliar and root diseases.

One of agriculture's most successful and significant examples of ecologically sound pest control is cassava mealybug in Africa, through the release of parasites introduced from South America. From its introduction into West Africa in the early 1970s, the mealybug spread rapidly, eventually causing devastating losses in 30 countries. CIAT and IITA jointly introduced the wasp parasite, *Epidinocarsis lopezi*, from Paraguay to Nigeria. Here it was locally tested, mass reared and released extensively in the cassava belt. Norgaard (1988) calculated yield increases averaging 2 tonnes/ha in affected areas.

Successful commercial use of biological control against the cassava hornworm (*Erinnyis ello*) has been practised in many parts of Latin America, combining egg and larval parasitism, larval predation and larval diseases. There are also several parasites or predators of scale insects, whiteflies, the gall midge and fruit flies. The cassava green mite populations are kept in check in the Americas by many predators and parasites, several of which have been introduced to Africa. Overall, there is an excellent potential for implementing biological control as a low-cost, environmentally sound component of a cassava pest management programme. The highest potential is likely to be outside the centre of origin for cassava, where biocontrol agents may not have been introduced previously.

4.3 CULTURAL PRACTICES

To be practical for commercial use by farmers, cultural practices used for pest control should be compatible with cultural practices for high economic yield and natural resource conservation. Some of these recommended practices include the use of pest-free planting material, the destruction of plant parts containing evidence of pests, planting on ridges to reduce root rot in soils prone to water logging, the planting of several varieties in a single plantation, intercropping and crop rotation. Cultural practices as a control method have the advantage that they can normally be implemented quickly. Practices that involve substantial trade-offs in yield or quality, or introduce excessive inconvenience in management, should be avoided because they have little chance of adoption.

4.4 CHEMICAL CONTROL

Insecticides, acaracides and fungicides often offer the most immediate form of reducing pest populations over a short period. However, it is generally conceded that most pest management programmes for cassava should not depend upon pesticides. An exception to this is the treatment of planting material, which is often economical and effective, has little chance of creating selection pressures for genetic changes in the pests to overcome resistance, and presents minimum negative environmental impact.

Chemical applications to cassava foliage may temporarily reduce pest populations, but they are often ineffective over a long period. In the case of mites and insects, pesticides may reduce parasite and predator populations, leading to post-application rapid buildups of target pests, or allow secondary pests to become more destructive. Chemical control of foliar cassava pathogens is rarely economical because repeated applications often need to be made over extended periods to have significant impact on yield. In many cassava growing areas, farmers cannot afford pesticides or they are not reliably available.

Human and environmental safety are also considerations for pesticide use, especially for developing countries where farmers may not be educated in the safe use of chemicals. Nonetheless, as cassava in specific regions moves to more intensive industrial production, use of chemical pest control is likely to increase. Growers will need to be prepared with technology to prevent economic losses and emergency chemical controls may be inevitable. As chemical pest control technology becomes more target-specific and compatible with environmental and resource management goals, there are likely to be more safe and effective options.

5. PRINCIPAL PEST PROBLEMS WITH POTENTIAL FOR CONTROL THROUGH HOST PLANT RESISTANCE

More than 25 pathogens affect cassava, including fungi, bacteria, viruses, virus-like organisms and mycoplasma (Lozano and Booth, 1974). More than 90 species of insects and six species of mites are pests of cassava (Montaldo, 1967). Bellotti *et al*. (1987) considered that 17 species of mites or insects warranted control in cassava. Several nematode species are parasitic on cassava, although literature on these is sparse (Table 16.2).

Sixteen pest species, or groups of species causing similar damage, are considered as candidates for control through host plant resistance. Each of these is an important yield-limiting constraint in some major geographical area and in all cases there is some evidence that genetic variability for resistance is present in cassava. In some specific breeding programmes, other pests not covered here could warrant a resistance breeding effort. Those pests not described here are not necessarily less important, but appear to have less potential at this time for a host plant resistance approach to control.

Most of these candidate species attack the foliage of cassava; exceptions are the root rotting organisms and subterranean sucking insects. Some of the diseases, such as anthracnose, concentric ring leaf spot, superelongation disease and bacterial blight, attack stems in the advanced stages of infection, but infection begins on the leaves, where resistance would usually be most effective. Without exception these pests have the potential of causing continued attack over an extended period of time. Resistance, to be effective, must likewise be expressed over extended periods of plant growth.

In Africa, two serious pests affect cassava roots; study of the potential for control through host plant resistance has only recently been initiated. The African root and tuber scale (*Stictococcus vayssierei*) is indigenous to the humid forest zone of Central Africa. Its severity may be on the rise due to shortened fallows and general degradation of forest soils (Dixon *et al*., 2003). The root knot nematode (*Meloidogyne spp*.) appears to cause widespread yield losses, although there is considerable basic research to be carried out before effective resistance breeding can be effective.

Except for *Cercosporidium henningsii* and *Cercospora vicosae*, which have been observed in almost all lowland to middle altitude tropical and subtropical cassava-growing areas of the world, cassava pathogens tend to be associated with fairly specific agroclimatic zones, i.e. continents or ecological regions within the continents. Mites, as a group, are a universal pest of cassava, although different species have different geographic ranges. The red spider mite (*Tetranychus urticae*) is a pest in Africa, Asia and the Americas, while the green mite (*Mononychellus spp*.) affects cassava in the Americas and Africa (Table 16.2).

Table 16.2 Major pests of cassava, regional importance and possibility of breeding for resistance

		Distribution[a]			Possibility of breeding for resistance
Common Name	**Principal species**	**Americas**	**Africa**	**Asia**	
Key pests[b]					
Mites	*Mononychellus* spp.	X	X		High
	Tetranychus spp.	X		X	Intermediate
Mealybugs	*Phenacoccus manihoti*		X		Intermediate
	Penhacoccus herreni	X			Intermediate
Whiteflies	*Bemisia tabaci*	X	X	X	Unknown
	Aleurotrachelus socials	X	X		High
Thrips	*Aleurotrachelus socials*	X			Intermediate
	Frankliniella williamsi	X	X		High
Key diseases[b]					
Bacterial blight	*Xanthomonas axonopodis*	X	X	X	High
Cassava mosaic disease	Geminivirus		X		High
Indian cassava mosaic disease	Geminivirus			X	High
Concentric-ring leaf spot	*Phyllosticta* spp.	X			High
Superelongation disease	*Sphaceloma manihoticola*	X			High
Anthracnose	*Glomerella* spp.	X	X	X	Moderate
	Colletotrichum spp.	X	X	X	Moderate
Occasional pests[a]					
Lace bugs	*Vatiga spp*.	X			Intermediate
Whiteflies	*Bemisia tabaci*	X	X	X	Intermediate
	Aleurotrachelus sociales	X			Intermediate
Grasshoppers	*Zonocerus* spp.	X	X		Low
Leaf-cutter ants	*Atta* spp.	X			Low
Subterranean sucking insects	*Cyrtomenus bergi*	X			Intermediate
Cassava hornworm	*Erinnyis* spp.	X			Intermediate
Occasional diseases[c]					
Bacterial stem rot	*Erwinia carotovora*	X	X	X	Low
Bacterial stem gall	*Agrobacterium tumefaciens*	X	X	X	Low
Witches broom	*Phytoplasma*	X			Intermediate
Frogskin disease	Suspected geminivirus	X			Intermediate

Common Name	Principal species	Distribution[a]			Possibility of breeding for resistance
		Americas	Africa	Asia	
Dry stem and root rot	*Rigidoporous* spp.	X	X	X	Low
	Roselinia & Verticillium spp.	X			Low
	Glomerella cingulata	X		X	Intermediate
Root smallpox disease	Several (vector: *Cynidae* spp. insects)	X			Intermediate (to vector)
Incidental pests[d]					
Scales	*Aonidomytilus albus*	X	X	X	Low
	Saissetia spp.	X	X	X	Low
Shootflies	*Neosilba parezi*	X			Intermediate
Fruitflies	*Anastrepha* spp.	X			Low
Stemborers	*Coelosternus* spp.	X			Low
	Chilomina clarkei	X			Low
	Lagochirus spp.	X			Low
Gall midges	*Jatrophobia brasiliensis*	X			Low
Incidental diseases					
Cassava common mosaic disease	Potexvirus	X			Intermediate
Leaf vein mosaic disease	Unknown	X			Unknown
Brown leaf spot	Cercosporidium henningsii	X	X	X	High
Blight leaf spot	*Cercospora vicosae*				
White leaf spot	*Phaeoramuaria manihotis*	X	X	X	High
Cassava ash	*Oidium manihotis*	X	X	X	Intermediate
Cassava rust	*Uromyces* spp.	X			Low

[a] Many of the minor pests and pathogens have not been thoroughly studied with regard to distribution
[b]Pests that regularly limit crop production in broad areas
[c]Pests that occur at infrequent intervals or in localized areas but can cause severe damage when present
[d]Pests that are infrequently damaging, even when constantly present

Sources: Adapted from Bellotti et al. (1987); Hillocks et al. (2001); J.C. Lozano and E. Alvarez, personal communication

The viruses are among the most devastating pests of cassava, especially CMD in Africa. Cassava Brown Streak Disease (CBSD) is a rising problem. While once apparently limited to coastal East Africa, it caused serious epidemics in Mozambique in the 1990s and is also reported in the Democratic Republic of the Congo (Dixon *et al*., 2003).

Both CIAT and IITA have published colour field guides for identification and management of cassava pests.

6. PEST AND PATHOGEN VARIATION AND HOST RESISTANCE

One of the greatest concerns in breeding for host plant resistance is generally the durability of that resistance. Often in the literature it is called breakdown of resistance when a pest undergoes genetic changes that overcome resistance. This is actually a very misleading term. What in fact happens is not any change in the genetic structure of the host, but rather in the pest. The same resistance genes in the plant are no longer as effective in limiting damage by the pest. This loss of effectiveness may be the result of many causes. The one most frequently of concern to breeders, pathologists and entomologists, is the change in the genetic structure of the pest population such that a larger proportion of individuals have the genes to overcome the resistance genes of the host. This type of change can be the result of mutation of genes in the pest, changes in gene frequency in the pest population due to selection pressures, or introduction of new biotypes from outside populations. In cassava the type of gene-for-gene relationship that commonly results in pests overcoming host plant resistance is apparently rare, if it exists at all. No cases have been documented where resistance has been overcome through change in the genetic structure of the pathogen.

The anthracnose-inducing pathogens are notorious for race-specific reactions on host genotypes in many crops. This has not been reported in cassava and suggests that the species has evolved mainly horizontal (rate-reducing) resistance. Viruses and bacteria generally do not develop the type of gene-for-gene relationships with their host that would result in overcoming resistance. The same is true for low-mobility pathogens, such as root rot organisms. Physiological specialization of mites or insects for gene-for-gene interactions with host plants is relatively uncommon and no cases are reported in cassava.

Current evidence suggests that overcoming host plant resistance through genetic changes in the pest need not be a major worry to the cassava breeder. However, inappropriate breeding methodology could increase the risk of gene-for-gene specialization. Practices to avoid are: (1) selection under artificial inoculation conditions where single isolates are used as the source of inoculum. This could lead to development of high resistance to a specific race, with uncertain effects when resistant genotypes are challenged by other isolates; (2) selection of only the most resistant clones as parents for crossing. This strategy has the risk of extracting only genes of major effect, while eliminating an array of genes of moderate effect. This can both limit the long-term progress in resistance breeding and increase the risk of selecting for vertical resistance; and (3) testing in a single site, which could bias selection toward resistance to specific pest genotypes.

7. MODE OF INHERITANCE

Broadsense and/or narrowsense heritabilities are reported for resistance to *Mononychellus* mites, thrips, bacterial blight, CMD and superelongation disease. Studies by parent-progeny regressions indicate that for all these pests, resistance is primarily additive in nature and narrowsense heritability is relatively high. Although similar studies have not been done for other pests, empirical observations in CIAT breeding trials suggest a similar mode of inheritance for resistance to whitefly, lacebug, anthracnose and concentric ring leaf spot. Major genes have been identified for CMD resistance and this is a principal basis for expecting rapid progress with molecular assisted selection (see Chapter 19). It is also likely that specific genes will be identified for resistance to other pests, as molecular tools are more broadly applied in cassava breeding.

8. EVALUATING RESISTANCE

Effective detection of resistance in the laboratory, screenhouse/greenhouse or field is fundamental for successful resistance breeding for cassava. No matter what genetic variation exists, or how high the heritability, if experiments are not appropriately designed, the evaluation of genetically controlled resistance may be inefficient or even completely ineffective. Much of the success in detection of resistance depends on high and uniform pest populations. Many techniques have been developed for various crops, using both natural and artificial conditions. For a few pests, it is possible to detect resistance through evaluation of linked markers in saturated molecular maps (see Chapter 19).

Resistance may be measured by the effects of the pest on the plant (damage levels) or effects of the plant on the pest (developmental, reproductive and behavioural responses), or both. Plant breeders have generally found the former to be most practical for selection purposes. Damage levels are often easily quantified or estimated as a practical measure of resistance. Plant damage is usually more closely related to yield or quality loss than measures on the pest itself. The support of entomologists and pathologists to define and interpret pest response is essential for developing effective and efficient selection for resistance.

8.1 FIELD LEVEL

Natural field infestation has many advantages for screening. It often combines simplicity of management, ability to select under pest and environmental conditions similar to those encountered in commercial production and ability to select simultaneously for a multitude of other traits that are best expressed at the field level.

Common disadvantages are: the unreliability of pest population levels from one year to another; lack of uniformity of pest pressure within a selection field; lack of control over other interacting influences (e.g. other pests, environmental variations); and lack of control of the genetic composition of pest populations.

All these problems can often be avoided or reduced. Choice of a selection site known to have moderate to high and uniform levels of the pest(s) in question is a basic requirement. Uniformity should be high both across years and across plots within an experimental field for any given year. Other considerations, however, may override the breeder's ability to move trials to the most desirable site in terms of selection for pest resistance; for example, an ideal site for selection for resistance may be inappropriate in other environmental characteristics, or may be logistically difficult to manage.

Natural field infestation is most likely to be successful for pests that spread quickly from one plant to another from isolated loci and for rapidly multiplying organisms. These conditions are most commonly met for wind-borne organisms and apply more frequently to pathogens than to mites or insects.

Natural field infestation is the most commonly used technique for detection of resistance, but reliability of detection could often be improved. Many techniques have been successfully used to enhance field infestation, by increasing pest population levels or uniformity of distribution in the field. This approach combines the advantages of having a selection environment which permits the appropriate expression of many different traits, with the advantages of higher or more uniform pest populations than encountered naturally.

8.1.1 Agronomic practices

Several modifications of agronomic practices will increase pest populations, but these must be used with discretion. The breeder must be certain that the practice being used to improve effectiveness of selection for resistance does not impose some other undesired selection pressure.

Planting date can be a very effective way to enhance field infestations. Many of the diseases of cassava are most severe during the rainy season, while many of the insects and mites are at their highest levels during the dry season. Planting date can be adjusted so that peak pest populations coincide with the

appropriate stage of plant development for evaluation. For example, in the Llanos of Colombia, cassava planted early in the rainy season is heavily attacked by bacterial blight, superelongation disease and anthracnose. Resistant varieties enter the dry season with a well-developed canopy and with root bulking already advanced. Susceptible varieties are severely affected, even killed, by the disease complex. During the dry season, mites, thrips, lacebug and/or mealybugs may build up. However, there is little possibility of evaluating mite/insect resistance of these disease-susceptible clones planted at the beginning of the rainy season, because they will have suffered extensive defoliation and even stem dieback from effects of disease.

Clones planted near the end of the rainy season escape much of the disease pressure and most clones can be reliably evaluated for insect and mite resistance in the dry season. The mite- or insect-susceptible clones may suffer extensive damage by the end of the dry season and therefore cannot be appropriately evaluated for disease resistance during the rainy season. Use of planting dates different from those used commercially should normally only be considered when the resistance identified is not dependent on the planting date; that is, the resistance should be functional as well in varieties planted at normal periods in order to have farmer acceptance.

Planting on the flat could be a means of increasing incidence of root rot in some poorly drained areas, where ridging is normally practised. In fields ridged as recommended for commercial production, root rot may be minimal. Lozano and Fukuda (1993) used this technique in the *varzeas* (flood plains) of northern Brazil to identify resistant clones successfully.

Variations in planting density, fertility levels and irrigation can reduce or increase pest pressures, according to the pest involved and the specific situation.

8.1.2 Susceptible spreaders

Susceptible spreader rows are one of the most common and effective means for enhancing pest populations. The basic idea is to plant a known susceptible clone, or several clones, in a systematic design throughout the selection trial. These rows or plots may be left to intensify and spread natural inoculation, or may be artificially inoculated if necessary. Being susceptible and uniformly distributed throughout the field, these spreaders will act to increase pest populations quickly and uniformly, rather than in scattered focal points.

The design for planting spreader rows will depend on the behaviour of the target pest and convenience of management. For less mobile pests, spreader rows should be planted every few metres among the experimental material. For highly mobile pests, spreader rows may only need to be planted in rows every 10 or 20 m throughout the field. If the pest is wind-carried, spreader rows should be planted up-wind of the target plots. Spreader rows can also be planted as head rows along the ends of plots, such that every plot in a trial is in contact with the spreader.

The general level of resistance of the material being evaluated strongly influences pest dynamics. The breeder may need to take this into account in designing a trial to achieve optimum pest levels. For example, if the breeding material under evaluation consists of advanced clones known to be generally resistant, this will act to depress pest populations and moderately susceptible clones may escape damage. In this situation spreader rows should be planted at a high frequency throughout the field. If the material being evaluated is generally susceptible, spreader rows may not be needed at all, or used at a lower frequency. The susceptible experimental material itself would act as a spreader.

Where the breeding programme's objectives include resistance to various pests, spreader rows may be a mixture of clones, with each one having susceptibility to different pests. Some experience is necessary to achieve a balanced infestation that allows expression of the different types of resistance.

8.1.3 Selective pesticides

Under field conditions there are often complicating infestations of non-target pests that may mask resistance to the target pest. Although it is normally recommendable to integrate selection for the entire

complex of key pests, there may be times when it is desirable to keep some pest at low levels in the evaluation nurseries. Use of selective insecticides or fungicides can accomplish this. Another important case for use of selective insecticides could be to reduce parasite or predator populations, which would otherwise keep the target pest population below optimum levels for resistance evaluations. This technique is, of course, most effective for those pests under effective biological control. Mites and mealybugs will fit this category in some regions. Studies have demonstrated differences among cassava genotype for suitability as a host for *Mononychellus* mite predators (Braun *et al*. 1989). Probably for most situations natural biological control will not unduly complicate selection for resistance, but where precise evaluations are needed, biocontrol agents can be eliminated from selection fields.

8.1.4 Artificial inoculations

Artificial inoculations can effectively enhance field populations. The organisms for such inoculations may be reared artificially in the field, laboratory, or greenhouse, or collected from natural populations in the field. Any of these procedures is generally an intensive operation requiring good knowledge of the pest biology.

The breeder should consider artificial inoculation especially where pest populations are unpredictable from one planting season to another. However, insect rearing or pathogen culture generally requires a considerable lead time, often before it is possible to predict probable levels of a pest in a given site and year. Thus, if artificial inoculations are to be used, it is usually an ongoing project and not something that can be started in any particular year when pest population levels happen to be low.

Cassava breeding programmes use artificial inoculation infrequently. This probably attests to the significant time and human resources required for success and possibly also to the success achieved by other means to obtain high and uniform pest populations. Mealybug infestations are especially variable, both in time and space and some programmes have employed artificial infestation. Often an efficient means of using artificial inoculations is to inoculate only susceptible spreader rows and rely on secondary infestations of the experimental material for the resistance evaluations.

8.2 Evaluations under controlled conditions

Rapid screening techniques under controlled growing conditions (e.g. growth chamber, greenhouse or screenhouse) abound in many crop species and for many pests. Usually it is possible to observe differences among genotypes for whatever test is being applied. The scientist will then report this as convincing evidence of an effective methodology for selecting for resistance. However, this is not a sufficient prerequisite to define an effective methodology. There is one basic rule to follow with regard to artificial screening procedures: the results of the screening under artificial conditions must be significantly correlated with results in the field. Otherwise, no matter how rapid or how consistent the results are under artificial conditions, they will have no positive effect on selection at the field level.

For screening under artificial conditions to be justified, it should confer some significant advantages over field screening. Often these advantages include convenience of evaluation, space savings, speed of evaluation, ability to control level and uniformity of infestation and control of factors that might interact with pest damage to complicate the evaluation. These advantages should be balanced against the reality that screening under artificial conditions is frequently not well correlated with field results and it is difficult to achieve balanced, integrated improvement of several characters when each is selected separately and independently.

8.3 Simulated damage

Resistance to some pests may be detected by artificially simulating damage and observing plant reaction. One possible example would be to defoliate artificially to simulate damage by leaf-feeders. Damage by the hornworm or by leafcutter ants can probably be simulated fairly accurately. Damage by sucking insects or mites, or diseases, is often of the type that does not directly cause loss of leaf area, but rather reduces photosynthetically active area, photosynthetic rates or translocation of photosynthate. These effects are more difficult to simulate. The type of resistance that can be detected by defoliation is mainly

tolerance – the ability to recover from damage. There are no reports in the literature on using this technique for large-scale varietal screening in cassava, but the potential is indicated by both physiological and entomological studies comparing reaction of different genotypes to defoliation.

Another type of simulated damage is application of a chemical identical to, or which simulates, that produced by a pest. CIAT (1989) reported on attempts to simulate the apical deformation caused by mealybugs, by injecting an extract of mealybugs into young cassava plants. If a specific toxin can be isolated, this opens the possibility of screening for resistance to the toxin itself.

Zeigler *et al*. (1984) identified Gibberellin A_4 as the hormone produced by the fungus *Elsinöe brasiliensis*, causal agent of the superelongation disease and the direct cause of stem elongation. They attempted to screen various cassava clones for resistance to the hormone, but all clones showed a very similar elongation reaction. As this study was carried out with a limited number of clones, it cannot be unequivocally concluded that no variation exists for resistance to elongation; however, research was not continued.

9. THE QUANTIFICATION OF RESISTANCE

Resistance involves an interaction between the plant and the pest and can be studied in either of these two dimensions: the response of the cassava plant due to pest attack, and/or the response of the pest population as a result of its feeding on the host. Appropriate design of an evaluation scheme should make it possible to quantify these variations. Normally, the reaction of the plant is easier to quantify than the reaction of the pest and is far more commonly used to assess resistance.

A review of the literature indicates that scientists have used numerous criteria to evaluate pest resistance in cassava. These include level of damage, expression of resistance mechanisms, pest growth and development parameters and correlated traits. Some specific criteria are:

- visual evaluation of damage to plant parts, with observation of leaf speckling, discoloration and distortion, retarded plant growth, stem distortion and reduced length of internodes (most pests, except those causing symptomless infections);
- determination of the difference in yield between infested and non-infested plots (all pests);
- determination of the number of insects or mites attracted to a variety when given a free choice (whiteflies, stem borers, mites, mealybugs and lacebugs);
- measurement of proportion of root surface discoloured owing to insect feeding (subterranean sucking insects);
- observation of the comparative effects of forced insect feeding (in confinement) on plants by measuring length of insect life cycle, mortality or reproductive rate (mites, mealybugs, whiteflies, stem borers and lacebugs);
- weight of insects after a defined feeding period on different varieties (mealybugs);
- determination of number of eggs laid (mites, hornworm, lacebugs, fruit flies and whiteflies);
- determination of number of surviving insects and progeny produced (mites, mealybugs, whiteflies and lacebugs);
- correlation of level of expression of morphological factors with level of injury (thrips, mites, mealybugs and superelongation disease); and
- measurement of amount of food utilized by the pest (mites, hornworm and grasshoppers).

In spite of this rather long list of alternatives, a large majority of resistance evaluations by breeders is based on only one of these – visual evaluation of damage to the plant part(s) most affected by the pest. This type of evaluation is generally the quickest and also comes closer than most of the others to the measure of resistance of most interest to producers, i.e. improved yield or quality attributable to resistance.

Researchers have developed visual rating scales for classifying level of damage by most of the cassava pests that might be considered in a resistance breeding programme. The most commonly used rating scales range from one to five. Use of five categories strikes a balance between precision on the one hand

and on the other hand, the number of classifications that most people can conveniently remember and quickly apply in the field.

The rating scales should be thought of as damage rating scales and not as resistance scales. The difference may appear at first to be one of semantics, but it is necessary for the breeder to keep this distinction in mind. The resistance level of a clone is a given, genetically fixed attribute. The damage level is a reflection of genetic resistance, interacting with components of the environment. Thus, when one suspects that many clones in a trial were escapes, there is no problem thinking of these as having low damage ratings, but it does not make sense to think of them as having high resistance, at least not until further confirmed.

Damage rating scales, to be valid, should generally be correlated with some more exact quantitative damage assessment, such as yield loss. If the damage rating does not reflect economic damage, then improvements in that rating by breeding are also unlikely to be reflected in concrete benefit to the producer.

There is some disagreement among host plant resistance practitioners regarding the use of the value zero in rating scales. To most people, zero indicates no damage at all, i.e. either total immunity or complete escape. To give a rating of zero, one would theoretically have to do a very complete study of each plant in the plot to confirm that no pest damage exists. On the other hand, a rating of one, which may include the range from zero to very low damage, could be assigned relatively quickly, with a rapid evaluation. Some entomologists and pathologists prefer to include the zero evaluation in their rating scale, for studies where it is necessary to distinguish between very small differences in damage levels. On the other hand, breeders can generally dispense with its use.

It is useful to have the rating scales for all the various insects, mites and diseases, in the same range, for example, from one to five, rather than some being one to five, others being zero to five and others one to nine. While it is easy to standardize rating scales by simple computer programmes, the use of uniform scales during evaluations is helpful in conceptualizing levels of plant damage.

The quantification of resistance through measurement of the expression of resistance mechanisms has been little used so far in cassava. This has to do largely with the fact that resistance mechanisms are unknown for all but a few pests. The best known example is resistance to thrips conferred by pubescence of the apices: non- or low-pubescence types are susceptible to thrips and highly pubescent types are resistant. This appears to be a simple mechanical deterrent to establishment and feeding. It is a highly heritable trait and of course, can be evaluated either in the presence or absence of the insect. Pubescence has also been implicated in resistance to *Mononychellus* mites and the mealybug. For mites it is known that other mechanisms are acting as well and the relation between pubescence and resistance is not nearly so strong as with thrips. Pubescence appears to impart a small degree of resistance to mealybug, but not enough to keep populations below damaging levels.

Pest or pathogen growth parameters are generally not practical criteria for large-scale resistance evaluations. These may or may not be related to damage levels caused to the plant, which is the primary interest in resistance breeding. Secondly, these parameters are usually time-consuming and tedious to measure and therefore impractical where large numbers of genotypes need to be evaluated. Entomologists or pathologists most often conduct these types of studies to determine resistance mechanisms, or the effects of resistance on the pest biology and population dynamics.

The use of correlated traits for quantification of resistance has the potential advantage of permitting the evaluation of resistance in the absence of the pest, or when the pest population is low or variable. Correlated traits are not directly related to the resistance mechanisms themselves, but show consistent correlation with resistance, either because of genetic linkage or other association. This is distinct from pubescence, for example, which is directly implicated as a mechanism of thrips resistance. To date, no such traits have been reported for potential use in cassava.

10. SOURCES OF RESISTANCE

Inappropriate use of sources of resistance is one of the principal reasons for development and delivery of varieties that are unacceptable to growers. Breeders typically screen a broad-based set of germplasm to identify the most resistant genotypes. These resistant, but agronomically poor sources are crossed with susceptible but agronomically superior genotypes to combine resistance with good agronomic traits. One risk of this approach, for many crops, lies in transferring only one or a few genes with major resistance effects to the new varieties. These genes then have some probability of being overcome by an evolving pest. Another risk is the difficulty of eliminating all the genes that confer poor agronomic acceptability, within a reasonable time frame.

An alternative that often appears to be a better long-term strategy, is the accumulation of several or many resistance genes, each with minor effects. Clones having only moderate resistance levels, but which are also reasonably acceptable in most other traits of interest, can be intermated to combine and accumulate resistance genes. Not only does this approach allow a greater probability of stable resistance, but increases the potential for simultaneously maintaining or improving other characters. Experience in several breeding programmes demonstrates that, with recurrent selection methods, pest resistance can be developed to high levels using only clones with low to moderate levels of resistance, as original parental material.

Currently there is good possibility of determining whether resistance derived from different sources results from the same or from different genes, when the same type of resistance is involved. Usually the best means of increasing chances of bringing together distinct genes, is the use of parents of diverse origins and of apparent diverse genetic background. The technology for gene tagging with highly saturated molecular linkage maps is rapidly improving the prospects for positive identification of distinct gene sources, especially if resistance is governed by a small number of genes (see Chapter 19).

Cassava breeders can consider three basic categories of germplasm in which to search for pest resistance, each with particular advantages and disadvantages: local varieties, introduced germplasm and related wild species.

10.1 LOCAL VARIETIES

The first place to look for resistance is in local, farmer-selected varieties. These clones will have a wide range of genes for local adaptation, which will accompany the resistance genes in any recombination. One common difficulty, especially outside the area of origin of cassava, is that the variability available within the local clones may be quite limited and the potential for genetic advance using only these clones will likewise be limited. Resistance genes at a higher frequency are more likely if the pest has been associated with cassava in the area for a long time. In this case, it is likely that farmers will have selected for some level of resistance.

10.2 INTRODUCED GERMPLASM

Introduced, or exotic germplasm may be selected to have specific resistance traits at high levels of expression, but is likely to be deficient in some aspects of local adaptation. Further breeding is often necessary to improve adaptation and in this process, it may be difficult to maintain all the resistance genes that the introduced germplasm originally carried. The more carefully that introduced germplasm is selected to fit the entire range of local needs, along with carrying appropriate resistance levels, the better the possibility of obtaining good locally adapted and pest-resistant clones. Practical results from breeding programmes amply demonstrate that combining exotic with local germplasm can be a highly successful strategy.

10.3 RELATED WILD SPECIES

It is certainly appropriate to collect wild *Manihot* species and evaluate them for resistance to a wide range of pests. However, as a rule, resorting to wild species is justified only when very restricted

variability for resistance exists within cultivated cassava. Crossing with wild species achieves not only transfer of resistance genes, but also of a wide range of undesirable genes. Eliminating these will demand many generations of backcrossing to the cultivated species. In the process it is difficult to maintain resistance levels as high as previously expressed in the wild species itself, unless resistance is the result of only a few genes. Another risk is that resistance may be closely associated, by genetic linkage or physiologically, with some undesirable characters of the wild species. The ability to tag resistance genes precisely will, however, greatly facilitate extracting specific genes from exotic or wild species backgrounds.

Transfer of resistance to CMD from *M. glaziovii* is the most successful example of a wild *Manihot* as a source of pest resistance. H.H Storey made the original cross in 1937 in Kenya and only after some 40 years of concerted breeding efforts both in East Africa and Nigeria, were clones developed having good resistance levels combined with good agronomic traits. Although there is still disagreement among breeders about the potential range of resistance to CMD within cultivated cassava, the wide range of Latin American germplasm has never been tested against this virus. Currently available marker genes and certainly new ones identified in the future, will allow screening of a broad germplasm base, but this will not necessarily detect all available resistance genes.

Some variation for resistance exists within cultivated cassava for virtually all pests that have been evaluated. Even if the level of resistance is quite low, accumulating these low levels of resistance, as opposed to turning to crossing with wild species, will often be the most efficient strategy. If resistance genes are rare, the only way of discovering them may be with massive germplasm screenings. Too many past conclusions about the lack of potential for resistance are based on limited germplasm evaluations.

11. BREEDING METHODS

Evaluation should permit selection of genotypes with durable integrated resistance to key constraints in the target production area. Selection under appropriate field conditions provides opportunities for integrated resistance along with improvement of other agronomic traits. Artificial inoculations or special cultural practices may be useful to increase intensity or uniformity of given constraints. Conversely, decreasing the intensity may be appropriate if a stress factor appears to have the potential of masking important genetic differences due to high levels of plant damage.

Some integrated measure of adaptation/resistance in the target region is often more useful in making a selection decision than the individual disease or insect evaluations. The integrated measure may be a general evaluation of plant health, combined with root yield and quality. Including standard local and other selected checks permits rational decisions on relative performance of new materials.

On the basis of segregation patterns and empirical breeding results, resistance appears to be multigenic for all pests studied to date, though definitive genetic studies have not yet been carried out. Both genetic theory and many years of practical results suggest that a population improvement scheme will be most effective in breeding for resistance to most pests. Crosses between genotypes with resistance genes at different loci, each having additive effects, should result in higher resistance levels in some proportion of the progeny. A recurrent selection scheme which allows accumulation of these additive effects appears to be the most effective strategy.

12. ADVANCES AND LONG-TERM PERSPECTIVES

The following sections describe some selected examples of pests targeted for resistance breeding.

12.1 CASSAVA BACTERIAL BLIGHT (CBB)

CBB, caused by *Xanthomonas axonopodis* pv. *manihotis* is the most important of several bacterial diseases reported on cassava. It was first reported in Brazil in 1912. This disease has caused severe

losses in several Latin American countries and is widespread in Africa, where epidemics can occur in the most important cassava growing areas. The disease is present in much of Asia, but generally causes less severe damage than on the other continents. It has only been reported to infect species of the genus *Manihot*, but the pathogen can survive several months epiphytotically on weed species in and around cassava fields. This makes eradication a difficult option as a control strategy.

The disease is characterized by angular leaf spotting and blight, wilting, die-back, gum exudation and in advanced stages, stem and root vascular necrosis. This combination of symptoms is unique among the known diseases induced by plant pathogenic bacteria (Lozano, 1985). The bacterium normally penetrates the host via stomatal openings or through epidermal wounds. Following penetration, the organism first invades and destroys the spongy mesophyll and then enters the vascular tissues. Once inside the vascular system, the bacterial cells are able to move systemically throughout the plant.

Next to yield and quality, this disease is probably the single factor receiving the most widespread attention of breeders in that it is a global problem, although more resources are given to CMD. Attempts to breed for resistance to CBB are, or once were, important components of many breeding programmes, including: VISCA, the Philippines; CRTCRI, Indonesia; PRONAM, the Democratic Republic of the Congo ; INIVIT, Cuba; INIFAP, Mexico; CNPMF, EMPASC and IAC, Brazil; SCATC, Hainan Island, China; as well as the two international centres, CIAT and IITA. All these programmes have selection sites where CBB is consistently at high levels in the field, so most selection is carried out at the field level. In addition, at the IAC, seedling selection is carried out on some 60 000 individuals annually, through artificial inoculation in a seedbed. Other programmes rely mainly on evaluations throughout the growing season at the field level.

In spite of the widespread presence of programmes breeding for CBB resistance, it is difficult to quantify progress made based on published information. This is in part because CBB resistance is only one of several selection criteria for most programmes and separation of gains in CBB resistance from other gains is difficult. At CIAT the principal selection sites for CBB resistance are in the acid soil, eastern plains region (Llanos Orientales). When breeding at Carimagua station first began in the mid-1970s, nearly all the material was susceptible to CBB. Within ten years, most of the clones entering the advanced yield trials were intermediate or highly resistant (Umemura and Kawano, 1983; Hershey *et al*., 1988).

While genetic variability for resistance within *M. esculenta* appears adequate for sustained progress in breeding, other species could contribute to combating new biotypes or to further increase resistance levels. CIAT evaluated 11 species under high CBB pressure in the Colombian Llanos in 1994 and found a wide range of resistance. *M. flabellifolia* and *M. pseudoglaziovii* in particular showed less disease development (CIAT, 1994).

Since most African landraces are susceptible to the disease, cassava breeders have drawn on a fairly narrow genetic base as a source for resistance genes. The genetic variations in pathogen populations have been extensively studied in both Latin America and Benin (Africa) (Boher and Verdier, 1994; Restrepo and Verdier, 1997; Restrepo *et al*., 2000, Verdier *et al*., 2000). From a host plant resistance standpoint, this has given some understanding to the potential variations in response of resistant varieties across sites, as well as the basis for an approach to challenging screening populations with the appropriate mix of pathotypes. Nonetheless, current breeding programmes still base their resistance selection primarily on field evaluations under a natural mix of pathotypes. Apparent genotype by environment interactions have been observed both in Latin America and Africa, but the breeding methods to integrate this information are still under development. Some varieties, such as MVen 77 from CIAT's germplasm collection, have shown consistent resistance across sites for more than 30 years.

12.2 CASSAVA MOSAIC DISEASE (CMD)

CMD was first reported in East Africa by Walburg in 1884 and in Nigeria in 1926 (Beck, 1982). It now occurs in all parts of East, West and Central Africa and adjacent islands. A variation also occurs in India. The virus has not been reported in the Americas. The causal agent was not definitively described until the 1980s. It is a geminivirus (family Geminiviridae, genus *Begomovirus*) composed of paired virus particles, 20 x 30 nm in size, containing a circular single-stranded DNA genome. So far, eight distinct species of these viruses are reported to infect cassava in Africa and in India (Fauquet and Stanley, 2003). The different species can interact synergistically when they simultaneously infect the same plant. Alone or in combination, the CMD-causing virus species are responsible for a 30–40 percent yield loss in sub-Saharan Africa as a whole.

The virus is transmitted by the whitefly, *Bemisia tabaci*. A curious phenomenon is that the same species of whitefly is widespread on many crops in the Americas, but for many years had not been reported as feeding on cassava under natural conditions. In 1990 a biotype of *B. tabaci* that feeds on cassava appeared in the southern United States and the Caribbean. This raised new concerns about the potential for spread of CMD if it were introduced, or if it already existed, in the Americas. However, to date, this biotype appears only sporadically on cassava and has not been reported as a pest.

The symptoms of CMD in cassava are characteristic of a mosaic disease. Early in the development of the leaf, chlorotic areas can be observed and leaf lobes are frequently distorted. Although the planting of clean material can be effective in areas where the reinfection rate is slow, the best control strategy is generally through resistant varieties.

Germplasm derived from the former East African breeding programme is still the main source of resistance. This programme (Nichols, 1947; Jennings, 1957) began in 1935 with an international search for clones resistant to the virus. Varieties of only moderate resistance were found, though selections with higher levels segregated when these were intercrossed. These selections were very successful in Uganda (Jameson, 1964) but higher levels of resistance were needed elsewhere. In 1937 H.H. Storey began a programme to transfer resistance to cassava from three tree species of *Manihot*, namely, *M. glaziovii* (Ceara rubber), *M. dichotoma* (Jaquie Maniçoba rubber) and *M. catingea*. These species have non-tuberous roots and are graft susceptible to CMD, but they conferred to their progenies a form of resistance in which plants tended to remain free of mosaic or to produce only mild and frequently transient symptoms.

By the third backcrosses of the hybrids to cassava, tuberous roots of reasonable quality were restored. Progeny derived from the *M. glaziovii* hybrids had the best agronomic performance combined with mosaic resistance. The programme in East Africa was terminated in 1957 and these crosses were never fully exploited there. Seeds from resistant material were distributed to several African countries. The Moor Plantation, Nigeria, introduced seed populations in 1956 and continued selection for resistance. From Jennings' selection 5318/34, Beck selected CMD-resistant hybrid No. 58308, which became the main source of resistance used in the breeding programme started in 1971 at IITA in Nigeria. This hybrid has now maintained very high resistance for almost 50 years under conditions of high inoculum pressure and in a large number of localities; however, it lacks both yield and quality and is highly susceptible to thrips and mites. (See Chapter 1 for additional details on the history of CMD breeding).

The wide range of germplasm assembled at IITA was mostly susceptible to CMD. However, due to prohibitions on importation of clonal material from the Americas, exotic materials could not be evaluated directly. Not until 1990 was a broad germplasm base introduced as seed and adequately evaluated at IITA. Most of this material was, as expected, highly susceptible. A small proportion, however, was resistant or moderately resistant, demonstrating good possibilities for finding valuable new sources of resistance within cultivated cassava (CIAT, 1994). The best crosses were those between CMD-resistant material from IITA and high-yielding Latin American selections, but a low percentage of material of purely Latin American origin also had some resistance. These data place doubt on the long-held hypothesis of absence of CMD resistance in cassava landraces.

Selection for resistance has relied principally on field screening. Hahn *et al*. (1980) recommended that field screening for CMD must be carried out in an environment where inoculum from diseased cassava is present, whitefly populations are high and the average temperature is below 30°C. It will be most effective where annual rainfall is 1 000–1 500 mm, elevation is below 500 m, average temperatures are about 20–25°C, soils have a pH of 4-6 and are rich in N and P and low in Na. Topping seedlings appears to enhance symptom expression.

A variant form of mosaic disease appeared in the Luwero District of Uganda in 1988. Pathologists learned that the new variant was the result of the combination of African CMD and the East African cassava mosaic virus. By the early 2000s, this variant had spread through Africa's cassava belt. Beginning in 1991, several institutions worked together to multiply TMS 6014, TMS 30337 and TMS 30572. By 2000, this massive effort resulted in about 80 000 ha of resistant varieties on farmers' fields (Nweke *et al*., 2002). IITA and Nigerian partners launched an ambitious project in 2003 to pre-empt an outbreak in that country. The project involves producing millions of new disease-resistant cassava plantlets and cuttings and delivering them to Nigerian farmers (Dixon *et al*., 2003).

12.3 CASSAVA BROWN STREAK DISEASE (CBSD)

CBSD, although recognized from coastal East Africa since 1936, only emerged in the 1990s as a major threat to cassava. It is still among the most poorly understood of the crop's major diseases. The virus affects many plant parts, including leaves, fruits, stems and roots. Typical symptoms include various patterns of foliar chlorosis (especially on older leaves) and purple-to-brown lesions on green stems. Under severe infections, stems may die back. Storage roots become brown and corky and are inedible (Calvert and Thresh, 2002). IITA is leading research to: (1) characterize the virus; (2) improve CBSD detection through the development of ELISA and PCR-based diagnostic techniques; (3) carry out comparative epidemiological studies to describe and quantify rates of CBSD spread in different varieties and contrasting agro-ecological environments; (4) determine mechanisms of host plant resistance; and (5) assess the potential of genetically modified cassava to suppress CBSD (Dixon *et al*., 2003).

12.4 CASSAVA COMMON MOSAIC DISEASE (CsCMD)

CsCMD, caused by a potex virus, was first reported in southern Brazil in 1938. Although present in several countries of South America, Asia and Africa, it is generally not economically important. In southern Brazil and Paraguay, it is sometimes necessary to take control measures. It has no known vectors and transmission is apparently exclusively through mechanical means.

12.5 CASSAVA FROGSKIN DISEASE (CFSD)

CFSD is a relatively recently described disease (Pineda *et al*., 1983), although it is unknown how long it may have been present in less accessible areas of the Amazon basin, its supposed centre of origin.

Symptoms vary by genotype and by growing temperature. Affected plants may be symptomless, or may show symptoms only on the roots, only on the leaves or on both roots and leaves. Economically, the root symptoms are clearly the most significant. Roots develop longitudinal fissures. Initially these fissures may involve just a small section of the root, but they can grow to cover the entire root. With time, these fissures tend to become corky, giving the classic rough-skinned appearance from which the disease name is derived. In the most severe cases, roots accumulate almost no starch. Even when symptoms are only moderate, marketability of the roots may be severely compromised.

The causal agent is easily transmitted by grafting. The Colombian variety *Secundina* acts as an indicator plant, showing a mosaic pattern on the leaves when grafted onto infected stock. Research to date suggests a viral cause of the disease. Field studies have shown apparent variation for genetic resistance, but concerted resistance breeding has not been attempted.

12.6 SUPERELONGATION DISEASE (SED)

SED, caused by *Sphaceloma manihoticola,* was first reported in the Tolima Valley of Colombia in 1972. Since then it has been reported throughout wide areas of South and Central America and the Caribbean. Symptoms of SED include necrotic leaf spots, along with hypertrophic leaf vein, petiole and stem cankers, characteristic of scab disease caused by the *Sphaceloma* species. The disease name is derived from marked elongation of the non-lignified internodes of affected plants. *In vitro* the pathogen produces Gibberellin A_4, the direct cause of the stem elongation. Severely affected plants may show dieback of the elongated stems and even plant death.

Programmes with strong breeding efforts, currently or in the past, for SED resistance are INIVIT (formerly CEMSA), Cuba; INIFAP (formerly INIA), Mexico; CNPMF, Brazil and CIAT. Once appropriate sources of resistance are identified, breeding for SED resistance appears to be relatively easy. As the pathogen spreads rapidly by wind and rain in cassava fields, infestations in SED-endemic areas can be managed to create uniform disease pressure. This allows effective selection at field level without any special techniques. Symptoms are distinctive, facilitating selection for resistance.

At CIAT, similar to the example cited for CBB, advanced populations selected at the Carimagua station in the llanos have generally good resistance to SED, whereas the initial germplasm evaluations showed that nearly all clones were highly susceptible (Kawano *et al*., 1983; Iglesias and Hershey, 1991).

12.7 BROWN LEAF SPOT (BrLS)

Brown leaf spot, caused by *Cercosporidium henningsii*, is probably the most widespread of all the cassava foliar diseases. Although it infrequently causes severe damage in cassava plantations, it occurs practically wherever cassava is grown and therefore may be among the diseases causing the highest overall yield loss worldwide (J.C. Lozano, personal communication). Teri *et al*. (1984) evaluated yield loss over a two-year period in Morogoro, the United Republic of Tanzania, in protected–non-protected comparisons. Average losses were 15.5 percent in 1981/82 and 25.0 percent in 1982/83.

Symptoms are characterized by leaf spots on both sides of the leaves. On the upper surface the spots appear uniformly brown with a distinct darker border. On the lower surface the lesions have less distinct margins and in the centre the brown spots assume a greyish cast because of the presence of conidiophores and conidia of the fungus. As the disease progresses, infected leaves turn yellow and dry and eventually drop. Susceptible varieties can be severely defoliated during warm rainy seasons.

Primary infections are initiated in new plantings when wind or rain carry conidia from lesions on old fallen, infected tissues to infection courts on leaf surfaces. Penetration occurs through stomatal cavities and invasion of the tissues is through intercellular spaces. When these lesions mature, conidiophores are produced from the stomata. Secondary disease cycles are repeated throughout the rainy season whenever conidia are carried to new sites of infection by wind or rain. Older leaves are more susceptible than younger leaves.

CIAT began a search for resistance early in its programme development (CIAT, 1973). An artificial inoculation system, by aspersion, was a reliable means of evaluating for resistance. Likewise, Kasirivu *et al*. (1980) found resistance in Africa through field screening. These studies showed that environmental variations affected the efficacy of screening – plants subjected to stress had less damage from *C. henningsii* than those in favourable conditions. Artificial inoculation was more reliable from year to year. Teri *et al*. (1984) found a wide range of yield loss among varieties, but also an apparent high genotype x year interaction. In 1981/82 the clone F279 had the lowest yield loss (1.7 percent) and the next year it had the highest loss in the trial (38.1 percent).

Apparently no programme has undertaken sustained breeding for brown leaf spot resistance as a priority. Currently the levels of yield loss in any given site or year are generally low to moderate and other traits take precedence. Nevertheless, it is a trait that should be kept in mind as one of significant potential due to widespread occurrence of the disease and one that may require greater attention in the future. It is a

disease that could potentially cause devastation in high intensity cultivation, with high plant density and large monoclonal plantations of susceptible varieties.

12.8 WHITE LEAF SPOT (WLS)

WLS, caused by *Phaeoramularia manihotis* commonly occurs in the humid but cooler cassava-growing regions. The disease has been reported in areas of Asia, Africa and Latin America. Lesions are circular to angular, usually 1–7 mm in diameter and white, or sometimes, but rarely yellowish brown. They frequently have a diffuse-coloured border on the lower leaf surface.

Penetration occurs through stomatal cavities and invasion of the tissues through intercellular spaces. When the leaf spots thus produced reach about 5–7 mm a stroma is formed from which the conidiophores are later produced. Secondary disease cycles are repeated throughout the rainy season when the conidia are dispersed by splashing rain. The fungus survives the dry season in old, infected tissues and renews its activity with the coming of the rainy season. Although CIAT has noted apparent genetic differences in levels of resistance based on field evaluations, resistance breeding has apparently not been undertaken in any programme.

12.9 BLIGHT LEAF SPOT (BlLS)

Cercospora vicosae Muller and Chupp is the causal agent of BlLS, a disease of the lowland tropics. Leaf spots are large and brown without definite borders. Each lesion may cover one-fifth or more of the leaf lobe. The upper surface of the lesion is uniformly brown but on the undersurface the centres assume a greyish cast because of the presence of conidia and conidiophores of the fungus. This disease can be locally important, but globally does not have as great a significance as brown leaf spot. No breeding programmes have specifically targeted this disease.

12.10 CASSAVA ANTHRACNOSE DISEASE (CAD)

The name *anthracnose* is actually a generic term, often describing any leaf blight caused by fungal pathogens. At the field level it is often difficult to distinguish among several pathogens causing similar symptoms. Anthracnose in cassava has been reported in many countries, but until recently was considered to be of minor importance. It is now known, however, that it can cause severe yield losses and decrease quality of planting material.

Causal agents reported for CAD include *Glomerella manihotis*, various *Colletotrichum* species, *Gloeosporium manihotis* and *Glomerella singulata*. These pathogens generally rely on tissue wounding for penetration. Breeding for resistance has been pursued mainly in Africa, but pathologists now generally recognize that reducing injury from other pests or from physical causes is sometimes the best approach to controlling anthracnose.

IITA has been the lead institution for work on CAD resistance. During the 1996 to 1999 growing seasons they evaluated 436 African landraces and 497 improved genotypes at Ibadan, a high infection zone (Owolade *et al*., 2005). There were significant differences among genotypes, both in the number of cankers per plant and the size of the cankers. There were several resistant local landraces that had not been previously used as resistance sources in breeding, indicating good potential to make continued progress for this trait.

12.11 CONCENTRIC-RING LEAF SPOT (CRLS)

CRLS is commonly found in the cooler cassava-growing areas of Brazil and Colombia and has also been reported in the Philippines, Africa and India. Several *Phoma* species are reported as inducing the same disease symptom on cassava.

The disease is characterized by the presence of large brown leaf spots, usually with indefinite margins. These lesions are commonly found at the tips or edges of the leaf lobes or along the midrib or leaf veins.

The upper surface of the lesions initially consists of concentric rings formed by brown pycnidia. These rings are frequently absent from old lesions as mature pycnidia are washed off by rain.

During the rainy season, when temperatures fall below 22°C, this disease may cause severe defoliation in susceptible varieties, finally resulting in dieback of stems and in the most severe cases, death of the plant.

Only CIAT has carried out sustained breeding for CRLS resistance, within its gene pool for highland adaptation. At the highland selection sites and surrounding areas of the Cauca Department in Colombia, susceptible clones will not survive an entire growing season in normal years of heavy disease pressure. Resistance sources have been almost exclusively from the narrow genetic base of Andean highland-adapted materials. Over the years, there have been moderate degrees of introgression of middle altitude and lowland germplasm into this gene pool, to give a broader genetic base to the sources of resistance to CRLS. Many advanced hybrids now combine good levels of resistance with high yield potential and good root quality. Although serious in localized areas of the Andes, the disease has an overall low priority in breeding.

12.12 PREHARVEST ROOT ROT

Root rot diseases of cassava are important primarily, but not exclusively, in areas with poorly drained soils with high organic matter content, or during periods of excessive rainfall. *Phytophthora* and *Pythium* species are the most common and important cause of soft rots. *Fusarium*, *Diplodia*, *Seytalidium*, *Armillariella*, *Rigidoporus*, *Rosellinia*, *Verticillium* and *Glomerella spp*. are reported to cause dry rot symptoms. Generally, infection of young plants causes damping-off while infection of older tissues results in partial or complete wilting and a soft or dry-rot of the thickened roots. Frequently, following infection by one or several pathogens, a broad spectrum of weak pathogens and/or saprophytes invade the diseased roots, masking the identity of the initial causal agent and causing many root rots to appear similar.

For many years, breeders were pessimistic about the genetic potential for alleviating root rot problems. Most of these pathogens have a generalized pathogenic activity. They do not have the type of specific pathogen–host tissue interaction that is usually associated with successful resistance breeding. However, after a concerted effort to characterize the pathogens and develop screening methods, CIAT pathologists became more optimistic about resistance. Artificial inoculation of root plugs (*Fusarium* and *Phytophthora* spp.) or stake inoculation in a bath with a fungal suspension (*Diplodia* spp.) could complement field screening as tools to identify resistance. There was a surprising level of resistance in some Brazilian Amazonian materials. After repeated good performance in environments conducive to root rot, state programmes in Brazil released several varieties (Lozano and Fukuda, 1993). Continuing work is showing that strains of *Fusarium solani*, *F. oxysporum* and *Diplodia manihotis* may vary widely in pathogenicity (CIAT, 1995). The implications for breeding are not yet clear, but may indicate the need for broad testing during variety development and intensive local testing before recommending a variety as resistant.

12.13 CASSAVA GREEN MITE (CGM)

Several species comprise the green mite complex, including *Mononychellus tanajoa*, *M. progresivus, M. caribbeanae, M. manihoti, M. Mcgregori* and *M. bondari*. The taxonomy of these species is complex and still a matter of some dispute. Green mites usually concentrate on the growing points of cassava plants, on buds, young leaves and stems; the lower part of the plant is less affected. Affected young leaves are marked with yellow spots, lose their normal green colour, develop a mottled, bronzed mosaic-like appearance and become deformed. Under severe attack, plant growth is stunted, shoots lose their green colour and stems become scarified. Stems and leaves die back progressively from top to bottom.

The *Mononychellus* mites are widespread and can be found almost everywhere where cassava grows. Isozyme studies indicate highest genetic diversity and possible origin, in northwest South America. The spread to new regions has been mainly on infested planting material, while localized dispersal is

primarily by wind. The mites form ballooning threads by which they lower themselves from the leaves. In Africa, *M. tanajoa* was first reported in Uganda in the early 1970s and subsequently spread throughout most cassava-growing areas of the continent.

The CNPMF in Brazil screened the national germplasm collection in high mite pressure sites in the northeast and identified only a small proportion of clones as highly or moderately resistant to *Mononychellus* mites. Breeders intercrossed accessions combining mite resistance and general adaptation to semiarid conditions, in a population improvement scheme, to provide new sources of adaptation/resistance for other dry areas of Latin America and Africa (Fukuda *et al*., 1992).

At CIAT, the Entomology section of the Cassava Programme identified clones with high resistance levels through extensive germplasm screening in field and screenhouse conditions. Breeders extensively used those resistant clones that also combined the best agronomic traits, in crosses with susceptible clones, to combine resistance with even better agronomic traits. The clone MBra 12 has been especially useful as a source of resistance because of the numerous favourable traits it possesses. CIAT has also made use of resistant accessions identified in Brazil, as parental components for population improvement. The first generation of crosses from the mid-1970s produced advanced selections with good resistance and good yield. Many were somewhat inferior in root dry matter content. These resistant hybrids were then used as parents in the second cycle of crosses and the progeny from these are further improved over the original germplasm selections.

Breeders give high priority to providing selection environments that allow the reliable identification of field resistance. High mite populations are now found frequently at the CIAT-Palmira headquarters station and occasionally in the Llanos and the north coast selection sites. Susceptible spreader rows help enhance the severity and uniformity of these infestations. Susceptible checks dispersed throughout trials provide a measure of potential damage levels and resistant checks set a comparative standard for selection of resistant hybrids.

Africa has placed more emphasis on introducing natural enemies from the Americas than on resistance. IITA has had a somewhat difficult time finding sources of resistance, because most African material is susceptible. They have focused on indirect selection for pubescence as a key resistance mechanism. Entomologists have extensively screened wild species for resistance, also with special emphasis on pubescence. *M. tristes* was especially promising (IITA, 1993a).

There is still some uncertainty about the effectiveness of host plant resistance across the various species of *Mononychellus* and some closely controlled experiments in this area need to be carried out.

12.14 RED SPIDER MITE

The red (or two-spotted) spider mite (*Tetranychus urticae*) is a major agricultural pest worldwide, for many crops. On cassava, it appears to be most important in Asia. In India, Pillai and Palaniswami (1982) estimated yield losses of 17-33 percent from a complex of four *Tetranychis* species. This mite affects the older cassava leaves, contributing to severe defoliation when populations are high on susceptible varieties. Initial yellow spotting becomes reddish or rust-coloured as the infestation progresses. Some evaluations for resistance have been carried out in the Philippines and significant differences in resistance reported. It appears that the pest is not yield-limiting and the evaluations have not progressed beyond the level of characterizing varieties and breeding material. In India, the CTCRI made extensive evaluations of their germplasm over a five-year period. From an initial screening of 1 200 accessions in the first year, they narrowed evaluations to 12 consistently resistant accessions in the final year (Pillai and Palaniswami, 1990).

12.15 LACEBUGS

Lacebug (*Vatiga manihotae* and *V. illudens*) damage is reported mainly in Brazil and Colombia but also occurs in several other South American countries. The greyish adults, about 3 mm long, generally feed on the undersurface of the leaves. The whitish nymphs are smaller and usually concentrate on the central

part of the plant. Prolonged dry periods favour high populations. The presence of lacebugs is most easily detected by the small black dots of fecal matter left on the lower surface of leaves. Damage symptoms consist of yellow spots that eventually turn reddish brown. Severe infestations can lead to heavy defoliation. Resistance breeding has been limited to observations of differences in pest damage levels. While these differences appear to be significant, no further work has been carried out to incorporate higher resistance into advanced selections.

12.16 MEALYBUG

Two major species of mealybug attack cassava in Africa and the Americas. *Phenacoccus manihoti* was introduced from the Americas into the Democratic Republic of the Congo (Zaire) during the early 1970s (Hahn and Williams, 1973). It subsequently spread throughout most of the cassava belt of Africa causing severe yield reductions (Herren, 1981). This species is parthenogenic, consisting of only females. *P. herreni*, a very closely related species, causes considerable damage in certain areas of the Americas, especially the northeast of Brazil and the Colombian Llanos (Bellotti *et al*., 1982; Bellotti, 1983). This species reproduces by matings between males and females. Mealybug feeding causes leaf yellowing and curling, defoliation and with high infestations, shoot death. Root yield losses can reach 87 percent (Herren, 1981; CIAT, 1985). As an indirect result of mealybug feeding, sooty moulds may build up on the leaf surface and reduce photosynthesis.

The search for resistance to mealybugs is limited mainly by lack of reliable mass screening techniques. Natural field infestations tend to be uneven and variable across seasons. Due to the importance of plant vigour in resistance, greenhouse or screenhouse evaluations may not reflect field results. Also, the success of biological control, especially in Africa, has reduced the priority for resistance breeding relative to other pests. Resistant material has been identified tentatively in the Democratic Republic of the Congo and at both CIAT and IITA, but genetic and breeding studies have been limited. PRONAM in the Democratic Republic of the Congo reported resistance in three selections of Brazilian origin and showing some wild type characters (Ezumah, 1980). CIAT concluded that high levels of resistance are probably not available in cassava germplasm. The principal mechanism appears to be a tolerance corresponding to good plant vigour. The combination of moderate varietal resistance with biological control should be the ideal management strategy for this regionally devastating pest.

12.17 SUBTERRANEAN SUCKING INSECTS

Nymphs and adults of *Cyrtomenus bergi* (Hemiptera) feed on cassava roots by means of a thin, strong stylet that reaches the parenchyma. These wound sites serve as an entrance for a complex of micro-organisms. The localized symptoms they cause along the path of entry of the stylet are known as smallpox disease. Affected roots may appear normal externally, but are unacceptable for fresh consumption and for some types of processing. First reported only sporadically and distributed in Colombia and Panama, the insect is now reported causing damage in northern Argentina, southern Brazil, Costa Rica, Cuba, Honduras and Venezuela.

Several years of research at CIAT pointed to root cyanogenic potential and especially of the root peel, as a probable resistance mechanism. Severe damage occurs only on clones with low CNP. Nevertheless, many low-CNP clones also had low damage levels, indicating other possible mechanisms. High CNP of the peel did not confer resistance when parenchyma levels were low (CIAT, 1994). Given the conflict of high CNP with user preferences in many regions, other resistance mechanisms or other control methodologies are urgently needed.

12.18 AFRICAN ROOT AND TUBER SCALE (ARTS)

Stictococcus vayssierei is a subterranean insect indigenous to the humid forest zone of Central Africa. It has increasingly become a pest since the 1970s and is reported in the Cameroon, Central African Republic, the Democratic Republic of the Congo, Equatorial Guinea and Gabon. The pest not only causes direct damage but possibly facilitates entry of root rotting pathogens. Yield losses up to 30 percent appear to be common and can exceed 60 percent (Dixon *et al*., 2003). Within a multipronged

pest management approach, IITA envisions the identification and deployment of ARTS-resistant/tolerant germplasm, but this research is still in preliminary stages.

12.19 THRIPS

Several species of thrips are pests of cassava, especially in the Americas (Bellotti and Schoonhoven, 1978). Thrips attacks have also been reported from Africa and India. *Frankliniella williamsi* has received the most attention in resistance breeding. This species damages the terminal bud of the plant and leaflets are deformed, showing irregular chlorotic spots. Leaves develop abnormally or not at all in severe cases. Growing points may die, resulting in growth of lateral buds, which gives a witches' broom appearance to the plant.

Thrips are essentially a problem in areas with an extended dry season. High and uniform populations consistently affect plantings at the CIAT-Palmira headquarters station. Many agronomically good genotypes show high levels of resistance. Breeding is straightforward and usually involves simply including resistance criteria within existing populations rather than any special attention to identifying sources of resistance. The CIAT population developed for adaptation to the high rainfall, acid soil savannas, has the lowest overall levels of thrips resistance. Breeders emphasize exploiting the resistance in parent clones that incorporate some of the high priority disease resistances, such as bacterial blight and superelongation disease. Resistance to thrips may be selected indirectly by rating apical pubescence, but in most areas where the pest is important, damage ratings are more easily accomplished.

Surveys in Africa demonstrated a phenomenon similar to what is found in Latin America, for distribution of varieties with pubescent apical leaves (Nweke *et al*., 2002). In five countries of East and West Africa, only 15 percent of varieties were pubescent. However, in the savanna region, 79 percent were pubescent. It is unclear whether this apparent adaptive selection was a result of insect or mite pressure or whether pubescence may have some other adaptive advantages in drier climates.

12.20 WHITEFLIES

Bemisa whiteflies are important primarily as vectors of cassava mosaic disease, but can also cause direct damage. The CNPMF in Brazil screened for resistance to the most prevalent whitefly species in Brazil, namely, *Aleurothrixus aepim.* CIAT has given some emphasis to breeding for resistance to *Aleurotrachelus sociales*, the most prevalent species of whitefly on cassava in Colombia and believed to be the vector of the virus causing frogskin disease. Five accessions from the germplasm bank were identified as resistant to *A. sociales* and some of these used in crosses. Progeny of the resistant x resistant parents showed a moderate whitefly population but a very low level of damage symptoms (CIAT, 1982). Selection for agronomic traits within these resistant types produced some whitefly-resistant clones with excellent yield and quality (CIAT, 1990). The resistant clone MEcu 72 caused more than double the mortality of whiteflies as compared with the next most resistant clone (CIAT, 1994). Much of this mortality was caused by detachment of the insects from the lower leaf surface. Although leaf pubescence appears to play a role in reducing whitefly damage, it cannot be used reliably as a sole selection criterion to improve resistance (CIAT, 1995).

In 2002 Colombia released the variety Nataima-31, one of the MEcu 72 progeny, believed to be the first whitefly-resistant variety of any crop species. This release was the culmination of a collaborative effort of the Cassava Breeding and Entomology programmes at CIAT and national research and extension agencies over a period of more than 20 years.

13. TRANSGENIC TECHNOLOGIES

13.1 INSECTS

To date the principal transgenic insect resistance technology has been the incorporation of *cry* genes that synthesize the Lepidoptera-specific toxin from *Bacillus thuringensis* (BT). Variants of this gene have a major impact on the commercial production of maize (stem borers) and cotton (bollworm). CIAT

has targeted the cassava hornworm and the stem borer as potential target pests for BT-mediated resistance. Researchers incorporated the *cry* gene into the commercially important varieties CM 3306-4 (ICA-Negrita) and SM 1219-9, in addition to the model African clone 60444. Early testing against the hornworm gave encouraging results, while it appears that stronger transgene expression will be needed for control of the stem borer (Taylor *et al*., 2004a).

13.2 DISEASES

As the most devastating disease of cassava globally, CMD easily warrants the highest level of attention for a transgenic solution. At the same time, because of its prevalence in the poorest cassava-producing countries, there are acute challenges for successfully implementing transgenic technologies at the field level.

Since conventional breeding has been and continues to be, very successful for CMD resistance, do transgenic technologies have a role to play? Breeders have released more than 200 varieties in Africa, many with high levels of CMD resistance (Manyong *et al*., 2000). The contribution of transgenics may not be so much to introduce new forms or sources of resistance, but rather to allow the introduction of resistance genes into numerous locally-adapted and accepted varieties, without changing their best traits. Two groups work with CIAT and IITA to create CMD resistance through transgenics: the Danforth Plant Science Center in the United States (ILTAB) and ETH in Switzerland.

Early studies made use of the model species *Nicotiana benthamiana*, which is highly susceptible to CMD and has been used for years in studies of the causal agent of the disease. Frischmuth and Stanley (1991) demonstrated that a defective interfering sequence derived from a deleted B component of a Kenyan strain of the mosaic virus could impart elevated resistance within transgenic *N. benthamiana* plants. ILTAB followed an alternative route and produced *Nicotiana* transgenic for the AC1 gene. This gene encodes the replication associated protein required to ensure replication of both viral genomic components. Resulting plants had significantly better resistance to CMD.

ILTAB scientists first succeeded in recovering transgenic plants from the West African clone 60444, which is highly susceptible to CMD. These plants showed a small improvement in resistance when first challenged with the virus, but the resistance did not persist (Taylor *et al*., 2003). Verdaguer *et al*. (1996) followed a similar approach and integrated the AC1 gene into 60444 by particle bombardment. Transgenic plants were significantly more resistant and in particular to more than one geminivirus species, than their non-transgenic counterparts. Since plants in farmers' fields may be exposed to various species, this cross protection is an important feature. Screenhouse testing of the transgenic plants began in Kenya in April 2004.

ETH in Switzerland followed an antisense-RNA approach to CMD resistance. Targets for antisense-RNA interference were the mRNAs of the AC1, AC2 and AC3 genes from the A-component of the viral genome. The A-component plays key roles in virus replication and transcriptional regulation. Researchers incorporated these three antisense genes into 24–40 independent transgenic plant lines of the susceptible clone 60444. Assays showed delayed symptom developments and attenuated symptoms in transgenic plants (Taylor *et al*., 2004a).

In a parallel study, scientists at ETH incorporated the barnase and barstar genes from the bacterium *Bacillus amyloliquefaciens* into the cassava genome. When tissue is infected with the mosaic virus, the ratio of barnase–barstar shifts in favour of barnase and this causes local cell death before the virus can spread to adjacent cells, a hypersensitive reaction. Zhang *et al*. (2003b) reported an 88–99 percent reduction of viral replication activity in transgenic leaves.

Progress to date leaves little doubt that transgenic technologies will lead to breakthrough impact on control of CMD in the future. One of the key technological milestones in this process will be the ability to insert resistance genes into a wide range of existing adapted and accepted varieties, such that the technology can have immediate use in affected regions.

14. EFFECTS OF RESISTANCE ON PEST POPULATIONS

Often host plant resistance has the combined effects of reducing damage to the plant and reducing pest populations. This is not always the case, however. For example, the clone MBra 12 shows little damage from cassava green mite, but supports high populations of the pest (CIAT, 1994). The effects of resistance or susceptibility of breeding lines on pest population dynamics can have a strong influence on the efficiency of selection. At the outset of a resistance breeding programme, there is commonly a high proportion of susceptible material. This in turn favours high pest build-up and consequently pest pressure that may be well above what would normally be found in farmers' fields. Low and moderate resistance levels can be masked by this unnaturally high pressure.

To avoid excessively high pest pressure in early stages of selection and thereby risk discarding useful levels of resistance, it may be necessary to reduce pressure by pesticides or other control measures. The use of check varieties with a range of resistance levels is useful for defining an appropriate selection cut-off. If even the moderately resistant checks are highly damaged, some pest control may be advisable. If this is difficult, at least the checks provide a guideline on the level of damage that should be associated with useful resistance for selection of experimental lines.

At the other end of the spectrum of a resistance breeding programme, advanced trials should contain mainly resistant material, if resistance has been a primary breeding goal for several cycles. A generally high proportion of resistance may reduce natural pest populations to the point where even susceptible materials show little damage. If this occurs, clones may be selected from generation to generation under the assumption of having good resistance. As an extreme example, such a clone could be advanced to semi-commercial, on-farm trials before its susceptibility is discovered. To avoid this, the use of known susceptible checks is useful in all selection stages. If these checks are not highly damaged, some form of pest population enhancement, as discussed earlier, will be necessary.

It is impossible to maintain pest populations at strictly optimal levels every year at every stage of selection. However, if some of these precautions are taken, the variations can be reduced to an acceptable range. Over the several years that any resistance programme needs to function, the variations should average out to provide realistic measures of resistance.

The accumulation of molecular markers for resistance traits will provide valuable confidence and efficiency in the selection for host plant resistance. Nonetheless, parallel field confirmation of results will be essential in most cases.

15. LIMITS OF PEST AND DISEASE CONTROL BY GENETIC MEANS

Host plant resistance is rarely, if ever, a means to eradicate a pest completely. The goal instead should be to stabilize populations below economic damage levels. For some pests, even limited advances in resistance may require many years to achieve. For others, it may be possible to develop unnecessarily high levels of resistance, thereby diverting resources from other critical aspects of the breeding programme. Some breeders take the attitude that it is better to achieve excessively high resistance levels, just to be safe. There is of course some justification for this approach, because there are frequent examples in the literature, where pest outbreaks overcome moderate resistance levels through population pressure (apart from the phenomenon of overcoming resistance through genetic changes). However, one always has to balance the various objectives and not concentrate excessively on any single one to the detriment of overall crop improvement.

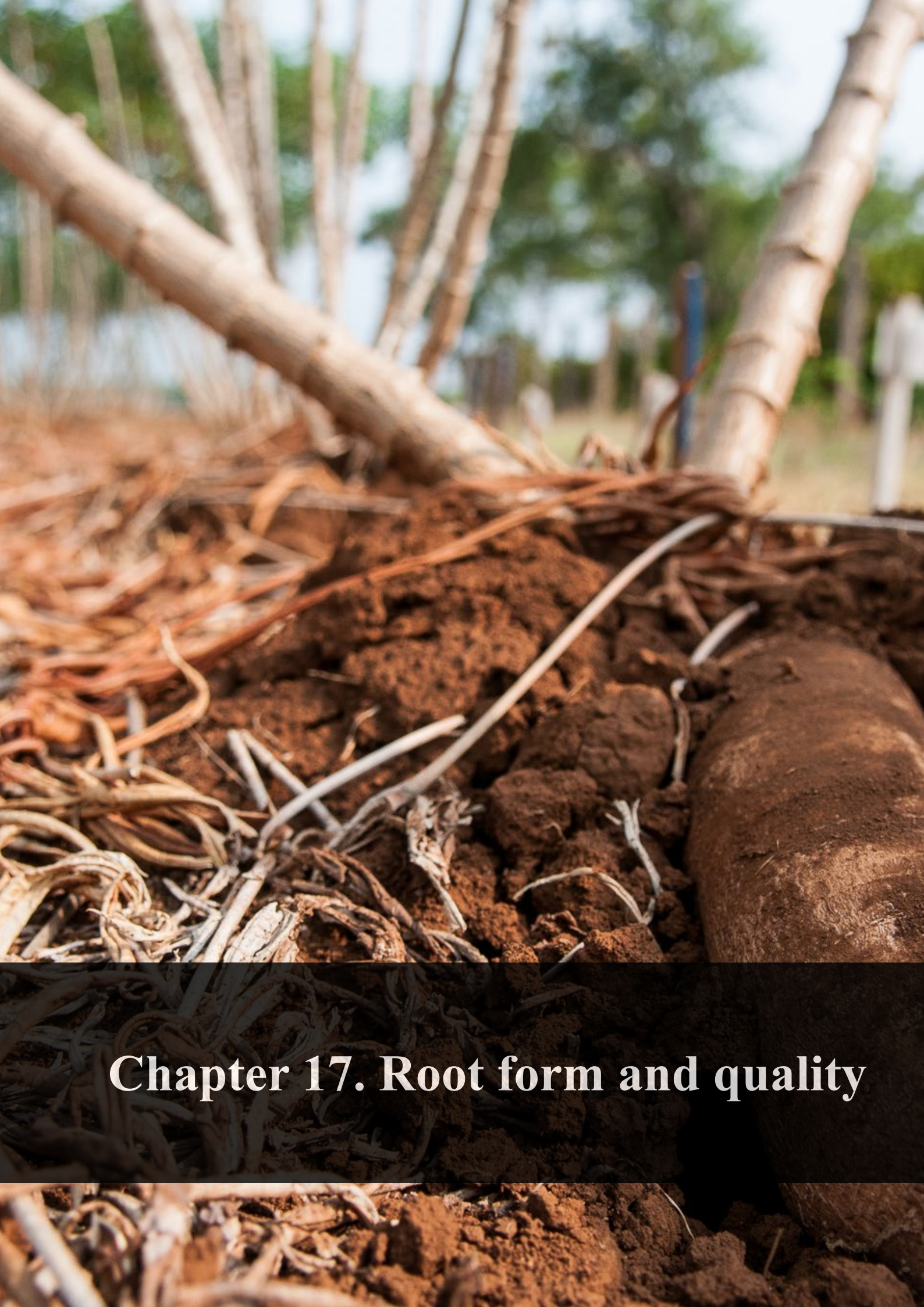
Nelson (1973) described some of the limitations of breeding for resistance to pathogens and these can be extended also to apply to mites and insects. The real or suspected limitations to the use of host plant resistance for pest control include: (1) the absence of genes that can affect pest control; (2) the difficulty in finding resistance to poorly specialized parasites that attack plant parts which do not react actively in

their own defence; (3) the inability to transfer genes for resistance from donor species to agronomically acceptable varieties; (4) close linkage between genes controlling desirable and deleterious traits; (5) the number of genes necessary to confer an acceptable kind of resistance; (6) the rapidity of changes in varieties to meet demands for new crop products; (7) length of the life cycles of plant species; (8) cytoplasmic conditioning of host susceptibility; (9) the production of new races by many plant pathogens; and (10) the number of potential genes for resistance available to plant species. No single means of pest control is universally effective. The goal should be pest management using the various control alternatives in an effective combination.

16. FUTURE PERSPECTIVES

The early impact of molecular approaches to plant improvement has been mainly in the area of pest resistance, due to the level of emphasis in this area and its relative simplicity compared with more complex traits such as yield potential. Areas of research with likely application to cassava in the short to medium term are:

- enhanced selection capability through marker-assisted selection. This will be especially important for identifying resistance in situations where a challenge from the pest or pathogen is not a viable option. For example, as a precaution against potential devastation from introduction of cassava mosaic disease to the Americas, breeding programmes should begin selecting for resistance in adapted genotypes. This might be done by tagging genes for resistance and selecting for the appropriate molecular markers, because the pathogen itself does not exist in the Americas (see Chapter 19);
- understanding mechanisms of resistance, with improved ability to select for stability by combining various mechanisms;
- transformation, using resistance genes extracted from species otherwise inaccessible to the cassava gene pool. In theory, a broad range of genes might be extracted from any number of species for testing in cassava;
- creation of de novo variability for specific traits where natural variability does not exist or is inaccessible. One example is use of anti-sense genes which turn off biochemical pathways to achieve desirable plant responses. Another is the insertion of virus coat protein genes as mentioned earlier;
- understanding pest or pathogen variability through molecular diagnostics, followed by studies of the implications for resistance breeding;
- understanding the interactions between plant genotype and biocontrol agents. IITA has found, for example, that morphological characteristics of the apical leaves affect populations of predatory mites and their effectiveness against the cassava green mite (Dixon *et al*., 2003). There are probably many such examples, but little research has been undertaken in this area.



Chapter 17. Root form and quality

Starchy roots are the principal commercial product from the cassava plant. Their form and quality usually play a decisive role in acceptability of a variety for producers, processors and consumers. The diversity of quality traits required for different end uses and in different regions demands careful study and planning by breeders.

Breeders have often overlooked the importance of the range of root quality traits that consumers consider, believing that as a starch crop, improvement of DM production per unit area per unit time was sufficient. It is now clear, however, that many quality factors and not just total starch production, influence acceptability for nearly all markets. Moreover, desired quality characteristics vary widely from one region to another and within a region varieties of distinct quality characteristics may be required for different markets. In any specific target area, quality-related objectives can be simple and straightforward, but globally the situation is very complex.

There is no comprehensive summary of quality factors important to different regions of the world. The information often is kept only in the minds of the local population. Far more work needs to be carried out in order to better understand local quality requirements, especially where introductions of new germplasm are being made, so that these introductions can include consideration of quality requirements. IITA (1992) reports that in Africa the following characters are important for consumer acceptance of cassava roots for human consumption:

Processed:

- size and shape, including presence of knot-like root constrictions, which affect handling;
- ease of peeling;
- flesh hardness (affects ease of grating);
- fibre content;
- moisture content (affects time required in processing and the yield of the food product);
- starch content and quality (affects swelling capacity and stickiness of gari);
- colour of root flesh;
- sugar content;
- enzyme activity (affects breadmaking quality).

Fresh:

- organoleptic properties of taste, appearance, texture (especially mealiness) after cooking;
- potential to generate cyanide;
- maintaining quality/shelf life.

This chapter covers breeding for root form, DM, starch content and quality, cyanogenic potential, cooking and eating quality, nutritional characteristics and post-harvest deterioration.

1. ROOT SIZE AND FORM

Root size and form gain special importance when cassava roots enter commercial processing and marketing systems. For home use, these traits are less important. This hypothesis is partly supported by the great diversity of sizes and shapes often found in landrace varieties grown in a given region.

Mean size of individual roots is generally not correlated with yield, which should allow the breeder a great deal of flexibility in choosing the root size to aim for, while still having yield improvement as a major objective. Tan (1987) concluded that higher yields would be achieved principally by maintaining a large number of commercial-sized roots.

Root form, although related somewhat to size, is probably even more independent of yield. The IPGRI (IBPGR) descriptor list for cassava describes five basic root forms (Gulick *et al*., 1983): conical, conical-cylindrical, cylindrical, fusiform or irregular. Although no data are available on the heritability of these

root forms, observations suggest it to be relatively high, especially when comparing contrasting forms such as short-conical versus long-fusiform. Progeny of the short, conical-rooted clones are usually of similar root form and progeny of the long-rooted clones are generally long-rooted.

Root form may influence a large number of other components of adaptation and acceptability, though most have not been studied. Based on deduction, rather than experimentation, one could believe that root form may influence drought tolerance, lodging resistance, ease of harvest and ease of processing. Long roots might be advantageous for water or nutrient absorption, or in lodging resistance, though the relevant research has not been carried out. Long roots are clearly negative factors for ease of harvest, ease of processing and generally for urban consumer acceptability. At the other extreme, very short roots are usually not ideal either. Observations at CIAT suggest that very short-rooted plants are quite susceptible to lodging. Also, these roots generally have short necks (*peduncles*), which make detachment of the root from the stem more difficult, increasing the probability of post-harvest deterioration of the roots. Even though correlations between root length and yield are generally not significant, if roots are too short, there clearly is a limit to potential yield. Most programmes will find that selection for roots of intermediate length provides the best integration of potential yield, ease of harvest, suitability for processing and consumer acceptance.

There is almost a universal preference for roots without constrictions and other irregularities. An uneven surface primarily makes peeling more difficult, but also may influence the uniformity of quality of a root. Constricted areas of a root are often tough and fibrous. An uneven-surfaced root has a larger surface area-to-volume ratio, increasing probability of surface damage and water loss. For some industrial purposes, where peeling is unnecessary and quality relatively less important, irregular roots may be acceptable.

Soil conditions certainly have some influence on root form, but the genetic component also seems to be quite strong. It is difficult to produce uniform roots in coarse, rocky soils because they must conform in part to the soil structure. In light, sandy soils, roots confront few physical obstacles and are more uniform.

The breeder should choose a selection environment that permits adequate expression of root form, while at the same time being representative of water and nutrient conditions of the target region. This may mean evaluation, at least at the latter selection stages, in soils that are somewhat coarser than average for the target area, in order to induce expression of negative root form characters. This might insure against the selection of clones that are highly sensitive to soil structural anomalies, in terms of root form.

2. ROOT COLOUR

Colours of three main structural components of cassava roots play a major role in root acceptability in different markets: periderm (surface), cortex (below the corky periderm) and parenchyma (flesh) colours.

2.1 PERIDERM COLOUR

Periderm colour can range from white to very dark brown. Graner (1942) showed that light and dark root colour is controlled by a single gene, with dark being dominant. Although there is some environmental influence that creates a range of shades around each of these two extremes, it is normally fairly easy to classify light versus dark roots. There are also roots of intermediate brown pigmentation, whose genetics are not yet clear.

Although there are no confirmed quality traits associated with root colour, numerous farmers and processors believe light coloured roots are more prone to post-harvest deterioration . If this is the case, it may be due to physical structural differences in the two types. The dark roots have a thicker, corky epidermis and may confer some resistance to wounding. It is also possible, however, that deterioration and discoloration are simply more externally visible on light as compared with dark roots.

In any case, there are strong regional preferences for root surface colour, some apparently based on real advantages and some apparently based only on tradition. Light-skinned varieties are sometimes preferred or required for starch extraction, as peel pigments may discolour the product. However, for many starch processing plants, periderm colour is unimportant.

For fresh consumption, preferences vary from one region to another and seem to be based on assumed association between colour of known clones and good quality. People generally assume that to have acceptable quality any new clone must have roots of the same surface colour as their well-known local varieties. Although this assumption may have no scientific basis, it is usually strongly held and one that the breeder will need to consider. Change in preference is not impossible, but the breeder should be aware of the strength of any such preferences and their basis.

As root surface colour is a monogenically controlled trait (probably with minor modifier genes), its manipulation is quite simple. If white roots are the objective, all white-rooted progeny can easily be produced by selecting all white-rooted parents (all homozygous recessive for root colour). If dark-rooted progeny are desired, at least one of the parents will have to have dark roots and the proportion of white-rooted progeny will depend on whether these parents are homozygous dominant or heterozygous. As this is such an easy trait to manipulate, it is generally recommended not to restrict choice of parents to just one or another category, but rather to base parental selection on other criteria and choose acceptable segregants in the progeny with the desired root colour.

2.2 CORTEX COLOUR

The most common root cortex colours (surface visible when corky periderm is removed) are essentially the result of intensity of purple colouration due to anthocyanin pigmentation, or yellow colour from β-carotene, or a combination. This colour is usually less influential in determining root acceptability than is either root surface or root flesh colour. It is critical in some industrial uses where discoloration of the starch would be unacceptable. In parts of Colombia, purple cortex colour is associated with good eating quality, because some of the traditional, high quality clones have this trait.

Anthocyanin coloration of the cortex can be associated with either light or dark surface colour and with either white or yellow flesh colour. It has not been determined however, whether or not any genetic linkages exist for these characters. Empirical observations and experience with anthocyanin coloration in other species, suggest control by few, rather than many, genes. Intensity of coloration is relatively stable from one environment to another, but may change slightly. This combination of probable oligogenic control and relative environmental stability makes breeding for specific cortex colours straightforward.

2.3 PARENCHYMA COLOUR

Root parenchyma, or flesh, colour may range from nearly pure white to deep yellow or orange, apparently depending almost directly upon concentration of β-carotene (precursor to vitamin A). Some clones also appear to have a purple anthocyanin coloration of the root flesh (Gulick *et al*., 1983), but these are rather rare.

Although higher carotene levels have a nutritional advantage, preferences for white or yellow-fleshed varieties usually seem to be based on other considerations. For fresh consumption, preference for white flesh predominates, but in localized areas, yellow roots are preferred, notably in parts of Amazonia. For dried, processed cassava for human consumption, there is also regional variation in colour preference. Given the importance of vitamin A deficiency as a global nutrition problem, increasing β-carotene content in cassava deserves further attention in affected regions.

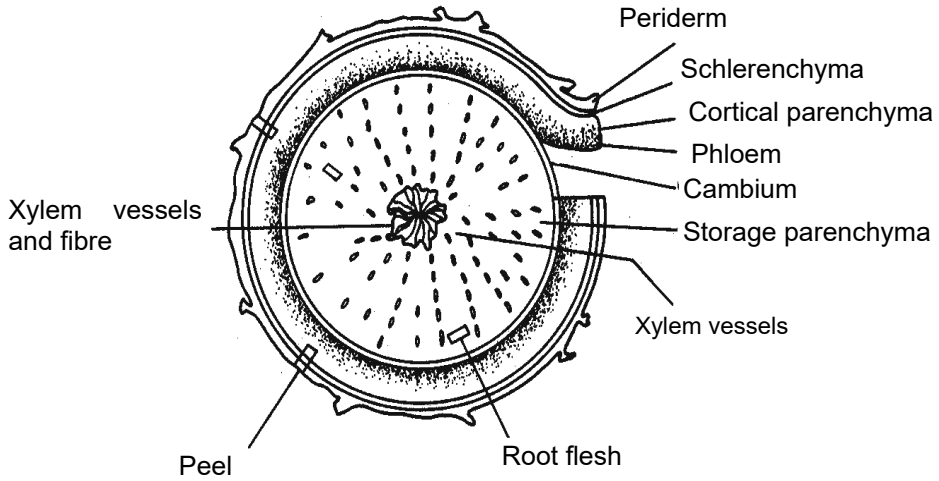
Generally, for starch extraction for industrial purposes, white-fleshed roots are required. For the animal feed industry, at present, no importance is given to root flesh colour, but conceivably, a preference could develop for the nutritional advantage of higher carotene in yellow-fleshed varieties.

Hershey and Ocampo (1989) reported that yellow flesh versus white flesh is controlled by a single gene, with partial dominance (see Table 6.2). Homozygous dominants are a deep yellow and homozygous recessives are white. The heterozygote, in those cases studied, is an intermediate yellow. This ease of identification of the genotype through the phenotype allows definition of a very precise breeding strategy. The approximate segregation from any cross can be predicted based on knowledge of parent genotypes. In further studying this trait, CIAT (1995) found that there may be modifying genes that complicate interpretation of the genotype associated with specific parenchyma colours. The implications of root flesh colour in nutritional value is further discussed in a later section of this chapter, on nutritional characteristics.

3. ROOT PEEL CHARACTERISTICS

The peel is the composite of periderm (or bark), schlerenchyma and cortical parenchyma (or cortex) and usually is removed as a unit from around the parenchyma, or root flesh, when roots are peeled (Figure 17.1). Few programmes have considered peel characteristics among selection objectives. The main characteristics that seem to be of importance in the market place are ease of peeling and peel thickness.

Figure 17.1 Cassava storage root components in cross-section



Source: CIAT (1981)

3.1 EASE OF PEELING

Many uses of cassava require peeling as part of the processing before use. Peeling may be carried out by hand or by a variety of mechanical peelers. There are large differences among varieties in the ease with which the peel can be removed. This appears to have at least two broad components: firstly, the toughness and thickness of the peel and resistance to breaking as it is removed from the parenchyma; and secondly, the intensity of attachment of the cell layers at the interface between the peel and parenchyma. No research has taken place in order to quantify these components of ease of peeling separately, and even where any overall rating has been utilized, it is usually highly subjective. Ease of peeling is a very important component of acceptability for some end uses and research should be carried out to define more precisely the factors involved to develop a rapid, effective methodology for evaluation and to study gene control and heritability. Until such information is available, the breeder working for a target area where ease of peeling is important, should routinely screen material in the intermediate to late selection stages, based on a simple subjective evaluation. This may be carried out either manually or mechanically, as long as the results correlate well with the methods that are used commercially.

3.2 PEEL THICKNESS

The full range of implications for different thicknesses of root peel is not well understood. Hypothetically, peel thickness could be involved in resistance or tolerance to root-feeding pests and root pathogens and resistance to post-harvest handling damage (and subsequently, post-harvest deterioration). Not only are these basic functions of the peel poorly understood, it is also unknown how the environment influences peel thickness, or the nature of genetic control of this character. At the present time, most breeders will have little basis for including peel thickness as a selection criterion, but it could, with further study, turn out to be an important trait for selection.

One relationship that can be surmised is that a thicker peel will probably mean a higher proportion of DM partitioned to a non-usable product, or at least a less valuable product. Even where the peel is used, it must be recognized that its starch content is well below that of the parenchyma and cyanogens are usually much higher than in the parenchyma. In terms of efficiency of carbohydrate distribution, one would like to see a thin peel, with a high proportion of DM in the parenchyma. However, until more is understood about the functions of the peel, this cannot be unequivocally recommended as a selection objective.

4. DRY MATTER (DM), STARCH CONTENT AND STARCH QUALITY

Cassava root starch, which constitutes on average 85–90 percent of the root DM, is by far the most important component of cassava yield. Therefore, the quantity and sometimes the quality, of this starch are of basic importance in most breeding programmes. A great deal is known about breeding for starch content of roots and very little about breeding for starch quality. The latter has received some attention indirectly where breeding programmes make product evaluations a part of the selection process. For example, in Nigeria, IITA evaluates cassava for its garification rate, which apparently is related to some biochemical differences in starch. Likewise, for breadmaking, where cassava flour partially substitutes for wheat flour, dramatic differences in bread quality can result from different cassava varieties, or even the same variety grown under different conditions. Starch quality probably plays a major role in these differences.

Apart from the many potential and poorly understood, implications of starch quality, starch quantity *per se* has a key influence on root characteristics. With some exceptions and up to certain limits, high root starch content is preferred over low starch content. This applies to preferences for fresh consumption; processing and drying for human consumption; chipping and drying for animal feed; and starch extraction for feed, food or industrial purposes. Limited exceptions include use of special low-starch varieties in parts of the Amazon basin for making a cassava-based drink. Consumers in some regions

prefer a mealy texture typical of very high starch content and others prefer the wetter consistency of intermediate starch levels.

4.1 STARCH AND DRY MATTER CONTENTS

As 85–90 percent of cassava's DM is starch, the measure of DM is often used to estimate starch content. Various methods are available to the breeder to measure or estimate these two closely related quality components.

4.1.1 Starch extraction

Starch extraction methods have been well developed for many years. They rely on rupturing cells in a liquid medium to release starch granules, followed by sedimentation. These procedures are routine but they are time-consuming and require laboratory facilities that may not be available to many breeding programmes (Table 17.1). Starch extractions are rarely justified as a means of discriminating among large numbers of genotypes in breeding programmes, but may be necessary in later stages for some specific markets. For the breeder's purposes, starch content can be adequately estimated from the more easily measured parameter of DM content.

4.1.2 Fresh and dry weight measurements

A simple, straightforward procedure for measuring DM content, not only of cassava roots but of practically any plant tissue, is to measure fresh weight of a sample, dry and weigh the sample and divide dry weight by fresh weight to obtain percent DM. Drying should be carried out in a forced air oven, because under ambient conditions, sample moisture level normally will not fall below about 14 or 15 percent. Continued respiration of the root during the relatively long natural drying process could lead to additional measurement error.

Roots should be sampled randomly from several plants within the plot, with a minimum of five to eight roots per plot. Measurements may be taken on peeled or unpeeled roots, depending upon which form is utilized in the region. DM content of the peel is lower than that of the parenchyma, so measures will differ for whole versus peeled roots. For animal feed uses, it is probably more appropriate to measure whole roots, while for human consumption, roots should be peeled. It is also possible, for convenience, to measure unpeeled roots and use a conversion factor to estimate DM content of peeled roots.

Roots are cut into pieces not more than about 1 cm thick and then weighed. If samples are weighed before they are chopped up, some DM loss can occur, which will cause underestimation of DM content. Sample size may vary, but probably should be in the order of about 0.5–1 kg of fresh roots per plot. Smaller samples may result in too high an error, but very large samples add little additional precision to the measurements.

Table 17.1 Comparisons among methods of measuring starch content

Method	Accuracy	Relative cost	Speed
Birander	High	High	Low
Whole root dry weight/wet weight	Medium	Intermediate	Intermediate
Peeled root dry weight/wet weight	Medium-high	Intermediate	Intermediate
Specific gravity	Medium	Low	High

Drying should be for 48 to 72 hours in a forced air oven at minimum 60°C and maximum 70°C. Minimum drying time can be determined by periodic sampling to see when weights stabilize. Samples should be weighed immediately after removal from the oven, before they begin to absorb moisture. As starch is highly hygroscopic, this absorption of water occurs rather quickly.

4.1.3 Specific gravity

As with many roots and tubers, there is a close relationship between root specific gravity and either DM or starch content of cassava. This relationship is the basis for the principal method used commercially

by buyers of cassava for the starch and animal feed industries. Many breeders may find that it is also the fastest and most efficient way of selecting for DM or starch content in a breeding programme.

The relationships between DM, starch and specific gravity of cassava roots have been established by several researchers, as well as some commercial enterprises. CIAT (1976) reported on one simple and effective procedure. Root samples of 3 to 5 kg, from a broad range of genotypes, were weighed in air and in water (to obtain specific gravity), chopped, dried and weighed again (to obtain DM content). Samples were also taken for laboratory analysis of starch content. The procedure was repeated for both peeled and unpeeled roots. Results showed a close linear relationship between specific gravity and either starch or DM content. DM and starch were plotted as a function of specific gravity and the regression equation calculated. To estimate DM content of peeled roots from the specific gravity of unpeeled roots, the equation is: **Percent DM = (158.3x - 142)**, where x = root specific gravity. Root specific gravity can be measured or estimated by several possible methods. Two general procedures are considered here.

Weight in air and weight in water. A sample of roots, normally 3–5 kg, is weighed in air, on any suitable balance, with a precision of not less than ±25 g. Although it is not necessary that roots be washed and scrubbed clean of all debris, they should be generally free of soil.

Two alternatives are commonly used. One is to adjust the sample size to a given weight (for example, 3 kg) for all samples. Then, when samples are weighed in water, the weights can be converted directly to DM or starch estimates, based on previously prepared tables. Some balances are calibrated specifically to give a direct reading of root starch content, when a given weight of roots is weighed in water (e.g. the Reiman scale, used widely in Asia).

The second method is to use variable-sized samples (no adjustment of sample size by cutting roots). Then, specific gravity must be calculated for each sample and subsequently converted to starch or DM based on the appropriate formula.

Practically any kind of container that is conveniently handled can be used to weigh roots. The most efficient method is to use the same container for weighing both in air and water, because transferring roots from one container to another can actually be one of the most time-consuming steps of the procedure. A type of sturdy wire basket works well, because it allows soil and debris to fall through and also allows easy weighing in water.

Salt solutions. CIAT developed a variation of the specific gravity method for DM estimation, which employs salt solutions of progressively higher specific gravity (Hershey, 1983). Small root samples are passed through the solutions, beginning with the solution of lowest specific gravity. Solutions are prepared so that the specific gravities at the lowest and highest levels bracket the anticipated range in the trial. A section can be sampled from the centre of the root with a cork borer or a knife. With a small wire or plastic basket, the sample is passed progressively from lowest to highest density solution. At the point at which a sample floats, it is known that its specific gravity is between that solution and the previous one.

The precision of the estimates can be increased by decreasing the density increments between solutions. However, because this method would normally be used in the early stages of a breeding programme as a method for rapid elimination of low DM clones, a range of about five different solutions should give adequate precision. One of the major conveniences of the method is that relatively small sample sizes are used and this may be important in some situations. Time involved in sampling of roots may be a limitation unless some type of quick sampling device is available.

4.2 STARCH QUALITY

The environment can strongly influence starch quality in cassava. Many types of stress decrease *quantity*, but their effects on quality have been elusive. For virtually all uses for which starch quality is important, the quality is still measured indirectly in the final product, or some intermediate product.

More direct physical or chemical measurements are apparently not routinely taken by any breeding programme at present. One of the factors proposed as a potential influence on starch quality is the proportion of amylose relative to amylopectin. IITA has suggested that this may be a critical factor in determining quality of processed roots for human food. CIAT is further investigating this relationship to see in what other ways it may influence root quality. The analysis may be too tedious for routine use in a breeding programme, but, if found to be useful, might be employed for parental selection and in the latter stages of selection when fewer genotypes are being evaluated.

4.3 GENETIC VARIATION AND HERITABILITY

4.3.1 Range in cassava germplasm

Evaluations of CIAT's large cassava germplasm collection for root DM content have clearly shown a wide genetic variation in DM content. At CIAT-Palmira headquarters, a range of <17 percent (the minimum that can be measured by the specific gravity method) to about 45 percent was observed. Reports from other parts of the world confirm this wide variability to be found in cassava germplasm. Quantitative evaluation of CIAT's core collection in the early 1990s confirmed the range of DM content found earlier by semi-quantitative methods (Table 17.2).

4.3.2 Regional variations

Evolutionary pressures created regional variations in the DM content of local varieties. Mapping of the origin of cassava collections in Colombia and their respective DM contents, illustrates the point (see Figure 4.1). For example, in the north coast region, local varieties tend to have very high DM content (perhaps amongst the highest in the world), while in the Amazon region, DM content tends to be low. It is not clear to what extent these trends are the result of environmental influence on evolution, or human selection for different forms of utilization. Until recently, most cassava in the north coast region was used for fresh consumption (high DM required), while in the Amazon region, most is still processed and dried (DM content less important). It seems logical to assume that regional variations were strongly influenced by selection for certain end uses.

4.3.3 Heritability

Heritability for DM content is high, reported in the range of 80–92 percent for h_b^2 (IITA, 1981; Tan, 1981; 1984) and 62 percent for h_n^2 (Kawano, 1978). These data, as well as extensive experience by various breeding programmes, indicate that solid progress in breeding for root DM content is not difficult.

4.3.4 Mutation for starch traits

Breeders have used chemicals or irradiation to induce mutations and generate genetic variability, especially in the 1950s and 1960s, with mixed success. However, for cassava, the recessive nature of most mutations makes it difficult to identify them through phenotypes. With the advent of molecular biology tools, there has been a renewed interest in mutation breeding. A system known as DNA tilling (targeted induced local lesions in genomes) has been successfully used in different plant species to tag mutated DNA sequences (McCallum *et al*., 2000; Till *et al*., 2003).

Table 17.2 Evaluation for root quality parameters in CIAT core collection

Variable	N	Mean	SD	Max.	Min.	Range
Parenchyma						
DM (%)	566	34.2	6.2	48.9	13.0	35.9
Total cyanogens (mg/kg, dry weight)	566	315	417	4 126	17	4 109
Total cyanogens (mg/kg, fresh weight)	566	102	124	1 041	7	1 034
Amylose (% of total starch)	503	22.3	2.1	28.8	15.3	13.4
Starch (% of DM)	559	84	4	93	71	22
Peel						
DM (%)	566	27.0	4.5	46.1	15.4	30.7
Total cyanogens (mg/kg, dry weight)	566	1 871	1 103	8 415	204	8 211
Total cyanogens (mg/kg, fresh weight)	566	498	287	1 983	55	1 928
Ratio peel: parenchyma						
DM	566	0.8	0.1	1.4	0.5	0.1
Total cyanogens (dry weight)	566	11.0	7.4	77.6	0.5	77.2
Total cyanogens (fresh weight)	566	8.4	5.0	42.7	0.5	42.1
Source: CIAT Annual Reports (1992 and 1994), Utilization Section						

The end-product of the tilling process is a plant (and its offspring) that has been identified with a change in a specific gene of interest. That plant line is then used to determine the overall effect/role of that gene on properties of the plant. Tilling is a high-throughput method to identify specific gene knockouts in mutant populations, useful as a tool of reverse genetics. Tilling utilizes PCR-based screening of plants generated through chemical mutagenesis (generally via ethyl methane sulfonate [EMS] treatment), often resulting in the isolation of missense and nonsense mutant alleles of the targeted gene(s). Tilling permits the high-throughput identification of mutations in target genes without production of genetically modified organisms and it can be an efficient way to identify mutants in a specific gene that might not confer a strong phenotype by itself.

4.4 ENVIRONMENTAL INFLUENCES ON DRY MATTER CONTENT

4.4.1 Temperature

There appears to be a fairly clear and marked relationship between temperature (within the normal range for cassava growth) and root DM content, higher temperatures result in lower DM, other adaptation factors being equal. Studies by Irikura *et al*. (1979) demonstrated increasing DM as temperature decreased with a rise in altitude above sea level. This generalized response does not hold when a clone is highly temperature sensitive and has a very narrow range of temperature adaptation. In that case, moving either up or down in temperature, away from the optimum, can result in decreased DM content.

4.4.2 Soil water

Soil water availability strongly influences root DM content, probably indirectly through foliage growth and photosynthate partitioning. When a cassava crop endures an extended drought, it is normal for the plant to begin to draw on the carbohydrate reserves in the roots for continued survival. When the rains arrive after a long dry season, root DM decreases even more dramatically, as the starch reserves are converted to sugars and translocated to the tops for new foliage production. There are marked differences among varieties in their ability to maintain root starch content during drought and at the beginning of the rains. Some of the local varieties in the north coast region of Colombia have the ability to tolerate three to five months of drought and still maintain 35 percent or more of DM, while others fall to below 20 percent. This apparently has to do in part with building up very high starch levels during more

favourable growing conditions prior to water stress. There may also be other mechanisms for reduced transformation of starch to sugars during the dry season and early rainy season. Plants that retain a good leaf area during drought might be expected to draw less heavily on starch reserves for canopy regrowth, but data have been inconsistent.

4.5 BREEDING METHODOLOGY

All the components for successfully breeding for improved DM seem to exist: high genetic variability, high broadsense and narrowsense heritability and simple, effective screening techniques. These, in addition to the market importance, make it logical that DM improvement should be a goal of many cassava breeding programmes. As broadsense heritability is high, selection beginning in the early generations is justified. Effective selection can begin as early as the segregating F_1 populations, where evaluations are made on individual plants. However, the evaluation of this large number of samples may not be justified for most programmes. Selection for root DM content beginning in the single row trial can be generally recommended.

CIAT has identified several clones with high or low levels of amylose in their starch. One group of 29 clones averaged only 11.2 percent amylase, whereas a high amylose group of 35 clones averaged 22.7 percent. A divergent recurrent selection scheme will look at the potential to move towards both higher and lower amylose levels.

One of the breakthroughs in cassava quality modification came out of a selfing project at CIAT begun in 2004 (Ceballos *et al*., 2007a). More than 20 000 seeds were obtained from the selfing of 74 different parent clones. Starch samples were subjected to a range of analyses, including colorimetric and differential scanning colorimetry amylase determination. AM206-5 was first noticed when it showed a unique and distinctive staining pattern when treated with an iodine solution. Roots and stems of this plant stained reddish-brown, while other genotypes showed the typical dark blue staining. This led to clonally multiplying the plant to perform a range of other tests. All analyses converged to support the hypothesis that this genotype has amylase-free (waxy) starch. This is the first report of such a discovery after thousands of evaluations made in different landraces and improved cassava germplasm. Crosses of AM206-5 are underway to transfer the mutation to germplasm adapted to the most important cassava growing environments.

The efficacy of mutation by ionizing radiation, followed by selfing, was demonstrated in follow-up studies of other putative mutations, including a small granule type, and a genotype with hollow starch granules (Ceballos et al., 2007b).

4.6 TRANSGENIC APPROACHES

Cassava is increasingly channelled to markets that rely on specific starch traits. Transgenic modification of biosynthetic pathways offers potential opportunities to modify starch naturally, for a range of speciality markets. Modified gene regulation could change the length and distribution of the amylose side chains to create a broad range of functional characteristics. The use of antisense genes could partially block starch synthesis and result in accumulation of the precursor sugars, for example, as a raw product for the industrial alcohol market. Since much of the work on starch transformation is sponsored by the private sector, the processes are often proprietary and details are not available.

ADP-glucose pyrophosphorylase (AGPase) converts glucose-1-phosphate to ADP-glucose, the first step in starch formation, while granule-bound starch synthase (GBSS) converts ADP-glucose to amylase. Munyikwa *et al*. (1998) reported the cloning of cassava c-DNAs encoding AGPase, GBSS and branching enzyme (BEI and BEII), involved in the formation of branched molecules in amylopectin.

The *glgC* gene encodes AGPase, which is the rate-limiting step in cassava starch biosynthesis. Researchers at Ohio State University incorporated a modified version of the *glgC* gene from *E. coli* into cassava and succeeded in increasing AGPase activity by over 65 percent compared with controls (Ihemere *et al*., 2003). In greenhouse trials, the seven transgenic clones significantly increased shoot

and root biomass and accumulated almost twice the dry weight starch content compared with the mother plants.

Raemakers *et al*. (2003) at Wageningen cloned the *GBSS* gene from cassava in the antisense orientation and were able to recover 50 transgenic plants with the *GBSS* antisense gene. Subsequently two of these plants were able to produce amylose-free starch (known as waxy starch) in their storage roots.

CIAT is working on producing waxy cassava through an antisense and sense construct of a full-length *GBSSI* gene. The construct has been successfully incorporated into the model clone 60444 and tests are continuing (Taylor *et al*., 2005a).

4.7 ADVANCES AND FUTURE PERSPECTIVES

It is difficult to quantify advances in breeding for root DM content, because in many cases the objective of breeding programmes has been to maintain certain highly desirable quality characteristics found in local clones, rather than to improve upon them. Thus, it is common to find that even where breeding programmes have emphasized DM content or starch quality as selection criteria, the result has been to maintain the quality of already existing clones, while making improvements in other areas. As mentioned previously, it appears that in many cases farmers have given rather high emphasis over centuries of selection to root quality, so it cannot be expected that large gains over local germplasm will be made in this trait. An exception will be where new markets are developed and changes in root quality are required.

Thailand is a clear example of broad success in improving starch content compared with the local variety. This was possible, firstly because the market paid a premium for starch and secondly because massive introduction of sources of high DM from Latin America gave a good opportunity for selection. This success in DM improvement is spreading throughout Asia.

5. CYANOGENIC POTENTIAL

All cassava clones so far studied contain the glycosides linamarin (95 percent) and in smaller amounts, lotaustralin (5 percent). The glycosides accumulate in the vacuoles, while the enzymes for their degradation, mainly linamarase, are located in the cell wall. When tissues are damaged, such as in many forms of processing, the compounds are brought together to generate cyanohydrin and glucose. At pH greater than five, or at temperatures above 35°C, acetone cyanohydrin breaks down spontaneously to produce acetone and hydrogen cyanide. Alternatively, the reaction is aided by the enzyme hydroxynitrile lyase.

Cassava clones that have a high cyanogenic potential (CNP), which are normally bitter to the taste, can cause acute poisoning if the roots are eaten without processing. This type of poisoning is rare, however, due to the traditions of processing cassava through pounding, grating, drying, fermenting or other means that liberate most of the HCN. However, the long-term ingestion of low levels of cyanide from cassava has been associated with goitre, cretinism, tropical ataxic neuropathy and tropical diabetes (Cock, 1985).

Cyanide is detoxified by the formation of thiocyanate from thiosulfate, which is formed from sulphur-containing amino acids. Thiocyanate inhibits thyroid uptake and iodine transport and is thus associated with goitre and cretinism. Tropical ataxic neuropathy is associated with protein malnutrition and extremely low levels of sulphur amino acids in the blood. When sulphur-containing amino acids are limiting, due either to deficient protein intake or to protein that lacks balance of the essential amino acids, thiocyanate in the blood increases.

Dufour (1988) studied health and nutrition among the Tukanoan Indians in northwest Amazonia. Over 70 percent of their food energy comes from very high CNP varieties, with no apparent toxic effects, because of a well-defined traditional processing.

Surveys in sub-Saharan Africa showed that about 70 percent of total cassava production is processed. Nweke *et al*. (1994) suggested that with a continuing trend toward greater commercialization, the importance of low-CNP varieties will likely decline even further.

Populations in northeast Brazil consume large amounts of farinha, made mostly from bitter varieties, but there is no evidence of chronic cyanide toxicity. During farinha production most of the cyanide is eliminated when the cassava mash is squeezed and the water, containing much of the cyanide, is discarded. More cyanide is eliminated when the resulting mash is roasted.

Apparently there are many areas where bitter cassava is preferred for processing. Some consumers claim that bitter cassava results in a superior product, either in flavour or in starch quality. For many years there was no evidence of any biochemical or physical variations that could account for this. However, these beliefs were so widely held by farmers and processors, that they could not be dismissed. In 1994 CIAT reported significant differences between low- and high-cyanogen clones, for several physical starch properties: viscosity, cooking time, gel instability and gelification index (Table 17.3). Except for cooking time, none of these is easily identified with the qualities that consumers perceive. It is certainly an area that needs to be further investigated by programmes aiming to reduce root cyanogenic potential in new varieties. Nweke *et al*. (1994) also support this hypothesis with their findings that farmers in sub-Saharan Africa believe bitter types contain more starch, give a better quality of the finished product and enhance storability of some products, such as the dried cossettes of East Africa.

Table 17.3 Evidence of relationships between cyanogen levels and cassava starch quality

Trait	Low cyanogen group	High cyanogen group	Significant difference (P=)
Total cyanogens (mg/kg fresh basis)	13	461	10^{-3}
Amylose (% of starch)	23	22	n.s.
Gelatinization temp. (°C)	64	65	n.s.
Maximum viscosity (BU)	509	359	10^{-4}
Viscosity at 90°	391	230	10^{-4}
Ease of cooking (min.)	10	6	10^{-4}
Gel instability (BU)	277	222	10^{-2}
Gelification index (BU)	158	65	10^{-4}
Source: Adapted from CIAT Cassava Programme Annual Report (1994)			

It is not known what may be the biochemical function of the glycosides in the cassava plant, if any. In most crops where cyanogenic glycosides exist, they apparently play no critical role in primary plant functions. In several species (e.g. white clover and sorghum bicolour), cyanide has been eliminated with no detrimental effects. It is more probable that cyanogenic glycosides are secondary chemicals, evolved as defence or adaptation mechanisms, for certain environments. In the case of cassava, it has been proposed many times that the cyanide probably evolved as a defence against insects or larger animals. HCN as a defence against cassava insect pests is as yet unsubstantiated, with the possible exception of the subterranean sucking insect, *Cyrtomenus bergi*. Controlled studies and empirical evidence indicate that neither root nor leaf cyanogen levels are related to damage by leaf-feeding insects or mites, when such studies have been conducted with a broad germplasm base. One theory holds that pests having a long association with cassava may have evolved efficient detoxification pathways, while cyanogens continue to be effective against potential new pests. Perhaps ultimately, the best approach to answering these questions will be to create acyanogenic experimental clones along with their isogenic wild type counterparts and then to observe their reaction to a range of environmental variables.

The ability of high CNP types to tolerate drought better has been suggested, but no clear relationship has been shown. Root cyanogens generally increase under drought conditions, creating the impression that high CNP types are better-adapted. Nevertheless, there has not been sufficient research in this area.

Mkumbira (2002) reports on the first molecular evidence that low and high CNP differ genetically in molecular markers. He used eight SSR markers and principal component analysis to show distinct grouping of high and low CNP types.

5.1 METHODOLOGY FOR MEASUREMENT

Two general methods are used for estimating cassava tissue CNP: quantitative and semi-quantitative. An enzymatic assay, developed by Cooke (1978), is the standard quantitative analysis. The assay is based on hydrolyzation of cyanogenic glucosides by incubation with purified linamarase and colorimetric measure of released cyanide. Sensitivity of the assay may be to the level of <0.1 mg HCN/100 g fresh tissue. As such, it could be used to screen effectively for zero-CNP clones. IITA has automated the method and reports carrying out up to 300 samples per day (Rao and Hahn, 1984). The Essers assay involves the same procedure for sample extraction and also uses spectrophotometry as the analytical basis, just as the Cooke method. The major difference is that the Cooke method employs use of the noxious solvent pyridine. The Essers method uses a mixture of isonicotinic acid/barbituate, a much safer solvent (CIAT, 1994).

A rapid, semi-quantitative procedure, the Guignard test (Table 17.4), is commonly used for screening large numbers of samples in the early selection stages. The test is based on intensity of the colour reaction of alkaline picrate-soaked filter paper to HCN released from a tissue sample. The method has a rather broad range of error when compared with quantitative methods. Advantages of the test are its simplicity and the large number of samples that can be handled. Three people working together at cutting root samples, weighing and adding picrate solution to the sample, can process in the order of 50 to 80 samples per hour. This kind of sample volume makes it practical to begin to estimate cyanogen levels even in the earlier stages of selection, such as the single row trials.

5.2 SAMPLING METHODS

Information on the distribution of cyanogens among roots within a plant and within individual plants, is critical to developing a sampling methodology that best estimates the mean or the range of cyanogen levels in a variety. A strong radial gradient exists, moving from higher concentration next to the peel and lower towards the centre of the root. There is usually a small longitudinal gradient, with higher concentrations at the proximal as compared with the distal ends. Cooke (1978) suggested that the content of a central disk is usually within 15–20 percent of the root mean content.

Variation among roots in the same plant, among plants in the same plot and across environments can be very high. For example, IITA (1993a) reported up to three-fold differences among roots within a plant. To make valid comparisons among clones, large numbers of samples are required. This may be appropriate when looking at advanced stages of selection, but in the preliminary selection stages, such precision is usually impractical. Simple and rapid procedures can be used to eliminate the highest CNP genotypes (if that is a goal), with the expectation that some will be missed. These should be picked up in repeat evaluations at later stages of selection. The very minimum number of samples, a single sample per plot, taken from a single average-sized root, for each replication, may be adequate if root CNP is not a very stringent selection criterion and if selected materials will continue evaluation across several years and locations. If low CNP is a major selection objective, more precision is required. IITA (1993a) suggested sampling a minimum of four plants per plot, three roots per plant and four replications, in the case of replicated yield trials. At 48 samples per clone, most programmes will find this level of precision can only be applied at advanced evaluation stages, where relatively few clones are being evaluated.

Cyanogen levels in the peel are generally well above those in the parenchyma. Virtually all clones with high levels in the parenchyma are also high in the peel, but the reverse is not necessarily true. For markets where unpeeled roots are used, CNP of the whole root rather than of just the parenchyma should be sampled.

As most cyanogens are synthesized in the leaves and transported to roots, several authors have suggested leaf analysis could be an effective rapid screening method. Correlations between 0.36 and 0.59 are

reported between leaf and root parenchyma CNP (Mahungu *et al*., 1994; Moh *et al*., 1976; and Cooke *et al*., 1978). These levels indicate a moderate potential for reducing root CNP by leaf screening. This methodology is somewhat called into question by the more recent discovery that cyanogens also appear to be synthesized in the roots themselves (McMahon *et al*., 1995). In parts of the world where people eat cassava leaves, the leaf screening test could have direct value in selection, apart from any relationship to levels in roots.

5.3 GENETIC VARIATION AND HERITABILITY

No acyanogenic cassava landrace varieties nor hybrids have been confirmed, although it is conceivable that they might exist. IITA, which gives high priority to breeding low CNP clones, reported finding clones with a minimum of 30 mg HCN/kg fresh root. The minimum level CIAT found in the core collection was 7 mg/kg (Table 17.2). Such levels are certainly acceptable for most uses, including fresh human consumption, even at very high levels of cassava in the diet.

There has been less interest in searching for clones at the upper end of the scale for cyanogen content. The highest levels reported in CIAT's germplasm collection are in the order of 1 000 mg HCN/kg fresh weight of the parenchyma.

There are wide variations among regions for cyanogen levels of local clones. These variations generally correspond to the requirements of traditional cassava markets. What is not clear is to what extent certain end uses of cassava were developed on the basis of whether high or low cyanogen clones were available and to what extent high and low cyanogen types were selected for suitability to certain desired uses in the region. Non-random distribution patterns for sweet and bitter types have already been in existence for several centuries and possibly several millennia. The high-cyanogen types were dispersed along the major rivers, while the low types were found in drier areas (savannas). In modern times, however, both low and high cyanogen varieties have spread throughout South America.

Mapping the origins of CIAT's Colombian collection and comparing regions for CNP, shows clear regional differences (Figure 4.1). Most cassava here is consumed fresh, so it can be expected that most clones will have low CNP. However, there are concentrations of high-CNP clones in the Amazon region and also the eastern plains, where cassava is generally processed. Similar patterns can be expected for other countries having diverse end uses.

Table 17.4 The picrate (Guinard) test for rapid semi-quantitative evaluation of cyanogenic potential

Reagents

Picric acid ($C_6H_3N_3O_7$) (5 g per preparation)
Sodium carbonate (Na_2CO_3) (25 g per preparation)
Toluene (at least 10 ml)

Materials

1. 15 ml test tubes (125 x 16 mm) with stoppers
2. Whatman #1 qualitative filter paper
3. Balance, preferably with accuracy to +/- 0.05 g
4. Knife or scalpel
5. Non-absorbent pad for cutting samples, such as synthetic kitchen cutting board.
6. Wire or plastic test tube racks
7. Permanent ink marker for glass
8. Non-corrosive tray to hold solutions (minimum of approximately 10 x 10 cm x 2 cm deep)

9. Volumetric flasks, beakers, dark bottles for storing solutions, pipettes or eye droppers, magnetic agitator
10. Scissors
11. Tweezers
12. Paper towels and tissues
13. Protective equipment (goggles, latex gloves, laboratory-coat)

Procedures

A. Prepare the alkaline picrate solution as follows: (note: alkaline picrate solution and filter paper strips may be prepared up to several weeks prior to use):
   1. Dissolve 25 g sodium carbonate in 0.5 litres distilled water
   2. Disolve 5 g picric acid in 0.5 litres distilled water. Filter
   3. Mix both solutions (from steps 1 and 2) to obtain the *alkaline picrate* solution (2.5% Na_2CO_3 and 0.5 percent $C_6H_3N_3O_7$)
   4. Cut filter paper into 1 x 6 cm strips (at least as many strips as expected number of samples)
   5. Half-fill a tray with alkaline picrate solution. Submerge filter paper strips in the solution until completely saturated. Pull strips from the solution one at a time with tweezers and lay, separated from each other, on an absorbent paper towel to remove excess solution. Paper strips can be soaked and extracted from solution in lots of 20–40 at a time, so that they do not dry out while waiting to process samples
   6. Cut a 1 g root sample. The easiest procedure is normally to cut slightly larger than a 1 cm cube and then shave small sections to get down to exact weight. With some experience, one can estimate very closely to 1 g
   7. Place root sample in a test tube using tweezers
   8. Add 5 drops of toluene, taking care to place the drops directly on the sample and not on the sides of the test tube
   9. Immediately, using tweezers, place a paper strip, saturated with alkaline picrate, in the test tube. Holding the paper strip just at the tube opening, insert a stopper such that it maintains the strip in place above the sample. The entire strip should be inside the test tube so that it cannot dry out due to a wicking effect by exposure to outside air.
   10. Write the sample identification on the test tube
   11. Leave samples at room temperature for 24 hours
   12. Rate on a 1-9 scale on the basis of intensity of red colour of the saturated filter paper (see semi-quantitative scale below)

***NOTE**: It is important to clean instruments that have been in contact with the sample, between each sample, with paper tissues, to minimize contamination and false readings*

Approximate potential HCN liberation from the roots:

No.	Description		HCN content (ppm)
1	Bright yellow (no change of colour)	=	<10
2		=	10-15
3		=	15-25
4		=	25-40
5		=	40-60
6		=	60-85
7		=	85-115
8		=	115-150
9	Intense, deep red	=	>150

Although there are large effects of the environment on root cyanogen levels, interaction effects (G–E) appear not to be so pronounced. Both broadsense and narrowsense heritability values are high (0.87 – 1.07) (Kawano, 1987). Breeding for low CNP contents should be fairly straightforward through selection of low CNP parents, followed by progeny screening from the early testing stages.

5.4 ENVIRONMENTAL INFLUENCES

In spite of the general knowledge that environmental factors can strongly influence cyanogen levels in roots, practically no systematic studies have been carried out to quantify cause and effect relationships in this area. Most of the available information is empirical and based on observations of limited genetic and environmental variation.

5.4.1 Temperature

Apparently the only studies on temperature effects have been carried out under natural conditions. As these comparisons involve evaluation at different sites, there is little assurance that factors other than temperature are not also influencing cyanogen levels. Observations in Colombia at altitudes ranging from near sea level to 1 800 masl suggest, but do not confirm, that lower temperatures reduce cyanogenic potential (Irikura *et al*., 1979).

5.4.2 Soil water availability

Both scientists and producers have long believed there is an association between drought and high cyanogens. The interpretations, however, have varied widely. Water deficits raise cyanogen levels in some varieties, but not others. Pre-drought conditions and type of drought experienced can also have an influence (CIAT, 1990; Nwosu and Onofeghora, 1994). In very dry areas, such as the interior of Brazil's northeast, where high cyanogen varieties predominate, there is no substantive proof that cyanide potential has any adaptive value in drought. This remains an open question that needs further research.

5.4.3 Soil nutrients

The most comprehensive data on environmental effects on cyanogen levels have been derived from fertilizer studies. This is logical because it is one environmental component that the farmer can reasonably hope to modify, as compared with temperature and soil water levels. It is also a factor that can be readily studied at different levels in a given environment, keeping other factors constant. Farmers frequently comment that fertilizer reduces root quality and sometimes causes bitterness.

De Bruijn (1971, 1973) reviewed genetic and environmental factors affecting CNP and noted several tendencies among the varied results in the literature. Nitrogen fertilization generally increases cyanogens. Several authors reported that potassium deficiency has similar effects, but results are less consistent than for N. Root quality evaluations should be made across the range of conditions to which a new variety will be exposed. In the case of soil nutrient status this means testing the CNP level across the expected range of fertility gradients in the target region. Clones that show good stability of cyanogen level across these gradients would usually be preferred.

5.5 BREEDING OBJECTIVES AND METHODOLOGY

As nearly all programmes that include cyanogenic potential as a selection criterion are aiming at low rather than high levels, this discussion will be limited to that objective. Some of the principal prerequisites to success are already in place. Good, rapid evaluation procedures are available and heritability is high. The principal variables influencing potential progress towards low CNP for most programmes will be the germplasm base available and selection intensity. Obviously it is going to be very difficult and time-consuming to attempt to breed low CNP clones when beginning with a gene pool where all clones have high levels. If most of the locally adapted varieties are high in cyanogens (not an uncommon scenario), introduction of outside sources may be the best option.

Given the moderately high heritability, lowering of CNP through breeding can be achieved relatively easily. The main components of breeding are selection of low CNP parents and progeny screening from

the early to intermediate stages of evaluation. Accumulation of multiple genes controlling low levels of cyanogenesis, through recurrent selection, has had proven success. The breeder should, however, be prepared for the fluctuations in CNP that will probably occur across years, locations and agronomic practices. Evaluation must be sufficiently extensive to document a range of variation to be expected and to identify conditions that may raise or lower CNP for specific varieties.

Whether or not to begin screening in the F_1 will depend upon resources available for evaluating such a large number of samples, as well as the relative priority given to breeding for cyanogen levels. To date, only IITA has made a concentrated effort to select for low cyanogen levels in the F_1. Even if the breeder opts for F_1 evaluation, it is unlikely that it would be necessary to screen every F_1 plant. This normally would be made only after some proportion of the population has been discarded on the basis of other, more evident criteria, such as plant type, root form and yield or pest resistance.

5.6 TRANSGENIC APPROACHES

Through the early 2000s, most of the molecular work to reduce cyanide potential was carried out at Ohio State University, USA and at KVL University, Denmark. The first procedure involved blocking the synthesis of linamarin and lotaustralin with antisense insertions. Siritunga and Sayre (2003) targeted the genes *CYP79D1* and *CYP79D2* encoding the cytochrome P450s, which in turn catalyse the first dedicated step in linamarin and lotaustralin synthesis. Using an antisense strategy, they introduced the 5' ends (650 bp) of the *CY79D1* and *CYP79D2* genes into cassava in reverse orientation, via *Agrobacterium*-mediated, Ti-plasmid transformation. Five transformants with altered *CY79D1* and *CYP79D2* had up to a 94 percent reduction in leaf linamarin content. Authors noted that transformants that had 60-94 percent reductions in their leaf linamarin content all had root linamarin contents that were less than one percent of wild type levels.

These results appear to confirm previous indications that cyanogens are mainly synthesized in the leaves and transported to the roots. Siritunga and Sayre (2003) also suggest that there may be a threshold leaf linamarin content required for its transport to roots, since plants having 40 percent of the wild-type leaf linamarin content had less than one percent of wild type levels, just as for plants with up to a 94 percent reduction in linamarin content. If this result showing a threshold is consistent, it should in fact facilitate the production of acyanogenic types.

This laboratory (Ohio) was able to successfully transform the Colombian clone MCol 2215 (Venezolana), which is among the lowest of accessions in CIAT's germplasm collection for cyanogenic potential. It remains to be seen if the procedure is equally effective at reducing root linamarin contents to almost zero for clones with very high levels, such as MVen 25.

The second approach was based on the finding by White *et al*. (1998) that levels of hydroxynitrile lyase (HNL) (which catalyses the release of HCN) in cassava storage roots were only six percent of that detected in leaves of the same plant. They hypothesized that programming root cells to overexpress HNL would accelerate the detoxification process and ultimately decrease the risk to consumers.

To achieve this, cDNA from a cassava hydroxynitrile lyase gene was cloned and integrated into the accession MCol 2215 (the same clone in which the antisense genes were inserted, described above) (Siritunga *et al*., 2004). The strategy has been very successful through the preliminary evaluation stages. Transgenic plants in the greenhouse showed between 800 and 1 300 percent increase of HNL in the storage root tissues compared with non-transgenic plants. Overall levels of linamarin and linamarase remained unchanged, but the increased HNL reduced residual acetone cyanohydrin in homogenized roots by as much as a factor of three. This system has the advantage that cyanogen levels remain unchanged in the plant during growth and development, which may have protective or physiological functions. When processed prior to human consumption, endogenous detoxification is much more efficient than in non-transformed types, in greenhouse trials (Taylor *et al*., 2004a).

5.7 ADVANCES AND FUTURE PERSPECTIVES

IITA in Nigeria has had the longest concerted effort of any programme to reduce cyanogen levels of cassava roots through breeding. They consider this to be an important objective for many of the end uses of cassava on the African continent. Progress has been relatively slow because many of the parental sources, including the mosaic-resistant lines derived from *M. glaziovii* crosses, are high in cyanogenic potential. Eventually, however, breeders produced clones with levels well below those of local check varieties, while maintaining superior yields. CIAT placed little emphasis on cyanogenic potential for many years, following instead a strategy of creating a wide range of variability from which national programmes could select levels appropriate for their own needs. With increasing concerns about the human health aspects of cyanogenesis in new cassava areas that do not have a tradition of processing, CIAT began to develop specific low-cyanogen populations (CIAT, 1989).

The prospects for continued improvement for low CNP are good. However, breeding for acyanogenic clones does not offer much promise through conventional breeding methods. One approach with potential, which has not been attempted to date, is large-scale selfing of a wide germplasm base with the objective of identifying recessive genes for acyanogenesis. In other crops with a similar pathway for glycoside biosynthesis (e.g. sorghum and white clover) single gene control of acyanogenesis has been found and utilized in breeding. Mutagenesis could be used to increase the possibility of creating recessive mutants. Selfing would still need to be carried out in order to create the homozygous recessive condition required for expression of the phenotype. Alternatively, expression of recessives could be achieved by creating and screening haploids or dihaploid plants, although this is not yet a routine technology for cassava.

If a protocol is developed that allows transformation of any existing clone and insertion of genes to block cyanogen synthesis, it will be possible to make virtually any existing clone acyanogenic while retaining all its other attributes. Also, the only positive way to learn about the physiological or pest resistance implications of acyanogenesis is to develop and test such materials. Transgenic approaches have already been successful and will be able to answer some of the critical questions on the role of cyanogens in the plant. Even if ultimately found to be unsuitable for agronomic or consumer-related reasons, acyanogenic cassava will provide a basis for learning much more about the role of cyanogens in the plant and better develop breeding and utilization strategies.

For a transgenic approach to be most useful, it is critical that a protocol be developed that allows transformation of a wide range of existing clones, already locally adapted and utilized by farmers.

6. COOKING AND EATING QUALITY

About one-fourth of cassava is utilized for fresh human consumption worldwide. For fresh consumption, the main traits of interest are cooking characteristics and eating quality. As cassava is primarily starch, some breeders have operated on the assumption that quality for the fresh market is strictly a function of starch quantity. In fact, consumers are quite discriminating about cassava quality and look for very specific features. However, the features of importance vary considerably from one region to another.

Surveys from the north coast region of Colombia indicate that consumers consider cassava to be a desirable food product, preferred at about the same level as rice or potatoes. In some countries, however, cassava is less preferred than other major staples and is consumed because of availability or cost advantage. The inherent qualities of cassava itself are probably not a deterrent to its expanded use as a food product in most regions. Experience also shows, however, that varieties may have any number of desirable traits, including high yield, but if they are not comparable to local varieties in eating quality, they will not be accepted for the fresh market.

This section considers several traits associated with cooking characteristics and organoleptic qualities. Eating quality is a complex of many different traits and food specialists are only beginning to understand the individual components. The components considered here are: cooking time, texture, bitterness and

flavour. Each of these, in turn, is almost certainly the function of several to many subcomponents. It quickly becomes clear that trying to maintain or improve cooking and eating quality through breeding is usually a complex objective.

6.1 COOKING TIME

Cassava is commonly cooked in plain or salted boiling water, or directly in various types of soups or stews. Average cooking time required to soften roots is usually 15–20 minutes, but some varieties may take over half an hour. Generally, longer cooking times are associated with poorer quality. As cooking time increases, fuel use also increases and this may be a major consideration in acceptability of a variety. There is no knowledge about the physical or biochemical factors that influence time of cooking. Experienced persons can predict cooking time from various textural aspects of the raw root, but these have not been quantified. The only reliable method to rate a root for cooking time is to perform cooking tests, periodically testing the roots with a fork for softness.

6.2 TEXTURE AND FLAVOUR

Many terms are used to describe texture; several of them are interrelated and few have a well-defined means for quantification. The preferred or accepted texture varies broadly across regions. A few commonly used terms are listed here, though there are many others.

6.2.1 Hardness

Roots will cook to varying degrees of softness or hardness, even when fully cooked. Generally, roots should not be so soft that they completely fall apart when cooked, nor should they be hard. Penetrometer readings from raw roots are generally positively correlated with hardness of the root after cooking, though only a small portion of the variation in post-cooking hardness can be explained by pre-cooking hardness (CIAT, 1985). Densely packed starch contributes to hardness of raw roots, but high-starch varieties are often quite soft when cooked. The correlation may arise from non-starch components such as cell wall structure. The method has not been used in breeding programmes.

6.2.2 Glassiness

The characteristic of glassiness is one that is apparently not universally known. A vitreous texture of the root appears to be caused most commonly when roots have lost starch due to transformation of starch to sugar, for translocation out of the root for leaf and stem growth. This occurs at the beginning of the rainy season, when tops are pruned and new growth has begun, or after regrowth from other types of damage such as insect feeding. This trait, though not easily detected prior to cooking, is loosely related to hardness of the raw root. Glassy roots are generally undesirable for consumption. Screening may be carried out by a penetrometer test to discard the obviously undesirable clones, followed by a cooking test for the remainder. As there is a large environmental component to glassiness, the breeder needs to try also to identify changes in management to overcome the problem.

6.2.3 Poundability/mealiness

In Africa, the terms poundability and mealiness are used to define important components of texture. Mealy or poundable roots are soft and dry when cooked. The descriptions may be nearly the opposite of glassy and hard, terms used in Latin America, though there have been no attempts at cross cultural comparisons in organoleptic tests. The physical/chemical influences are not understood, but IITA has suggested some association with amylose content (IITA, 1993a). Genotype and season, especially rainfall, strongly influence mealiness and poundability.

6.2.4 Bitterness

Bitterness is typically associated with levels of cyanogens in the roots. Indeed, roots with high cyanogenic potential are nearly always bitter. However, low CNP roots can also be bitter, possibly owing to tannins or other secondary compounds. For this reason, correlations between a bitter taste scale and cyanogenic potential are variable and sometimes insignificant. If roots are high in cyanogens, there should be no need for taste-testing. In fact, it would not be recommendable to do so because of danger

of toxicity problems. Roots low to intermediate in cyanogens should be tasted after cooking to assess degree of bitterness.

6.2.5 Fibre content

Cassava roots contain about 2–4 percent fibre (dry basis). This varies with variety, environment and age of the plant. Fibre analysis is a routine procedure and can be made easily in appropriately equipped laboratories. However, laboratory fibre analysis is not always well correlated with consumer perceptions of fibrousness of cooked roots. Thus, again, the best standard procedure is qualitative evaluation after cooking, in combination with laboratory tests.

The wild species are typically very high in fibre and this may be a serious constraint for their use in breeding (CIAT, 1995). Levels may reach 40–50 percent of total DM.

6.2.6 Sweetness

Many consumers consider sweetness in cassava to be the absence of bitterness factors, but it can also be related to sugar content. The degree of sweetness desired by consumers appears to be a regional characteristic, so this needs to be known before any breeding objectives can be set. Chavez *et al*. (2005) found very large differences in a subset of CIAT's germplasm collection, for total and reducing sugars. Total sugars ranged from 0.2 to 12.9 percent (dry weight basis). This variation has important implications both for human consumption and industrial uses. No programmes have implemented routine quantitative analysis of sugars to screen for sweetness of roots in breeding nurseries, which probably indicates that this is not yet generally a priority objective. It may be included as one of several characteristics in taste tests.

6.2.7 Cassava flavour

Flavour is the composite of many factors. Without further extensive biochemical analysis, there is little possibility of laboratory tests to evaluate variations in factors that influence the characteristic cassava flavour. Currently there are simply no alternatives to taste tests, with the exception that high CNP clones generally are perceived not to taste good. These could be eliminated *a priori* from taste evaluations.

To summarize: taste tests are an essential part of varietal evaluation for situations where cassava is destined for the fresh market. Due to highly regionalized preferences, evaluation criteria are not generally standardized throughout breeding programmes. Comparison of information gathered by different programmes on varietal evaluations is difficult. Cassava consumers have quite definitive tastes, but cassava scientists have not given sufficient attention to analysing the importance of various criteria, or standardizing evaluations.

6.3 GENETIC VARIATION AND HERITABILITY

Wide variation certainly exists for all the cooking and eating-quality traits discussed above, although only a narrow germplasm base has been evaluated for most of these. Except for cyanogens, the only information that exists on heritability of traits directly associated with eating quality is in the minds of breeders who have made casual observations of behaviour of materials in different environments and perhaps have informally compared progeny with parents. Parents known for good eating quality tend to produce good progeny and parents with poor eating quality produce poor progeny. These observations, although logical and probably correct, have yet to be experimentally confirmed.

6.4 ENVIRONMENTAL INFLUENCE

Eating quality may change dramatically with changes in environment, a fact that causes considerable frustration to producers, consumers and breeders alike. As so many different components make up eating quality, it is not surprising that the environment would have large effects; each of the components may be affected individually and in different ways. This is why it is often difficult to exceed, or even match, the quality traits of local varieties long-selected for stability of quality. It may also indicate that wide adaptability of clones for fresh consumption will be very difficult and often impractical to achieve.

One of the most common environmental influences on quality is soil moisture. As DM content decreases after a protracted drought, quality declines as well. If cassava is left in the ground until after the rains begin, an even more dramatic decline in starch and in fresh eating quality occurs.

Farmers in the north coast region of Colombia report that roots become vitreous (or glassy) if no weed cover is left during the dry season. This may well be the result of high soil temperatures, higher root respiration rates and the resulting decline of starch content. Environmental factors affecting cyanogens would directly influence eating quality –, generally those factors causing higher cyanogens also result in lower eating quality.

Little is known about environmental influence on cooking time, root hardness, or fibre content. Plant age, independently of environmental changes, affects eating quality. Fibre content tends to increase with age, while for most other factors there can be more of a genotype–age interaction (i.e. less predictability of change due to effects of plant ageing on eating quality).

6.5 BREEDING METHODOLOGY

As overall eating quality appears to have low to intermediate heritability, this suggests that an effective approach is to select for some of the components of quality that have highest heritability and/or are the easiest to measure, in the early stages of selection. Evaluation and selection of the integrated measure of eating quality (the taste tests) can be left until the later stages, where the breeder utilizes multilocation testing, large plots and replication.

Root DM and CNP are certainly two of the most critical criteria for eating quality and can be tested in the early stages. The aim should be to eliminate those clones with unacceptably high CNP or unacceptably low DM content. Concentrating only on these two factors in the first two or three cycles of selection will greatly increase the possibility of finding clones of good eating quality in intermediate to advanced yield trials, when the number of clones for testing is reduced.

For taste tests, until standard evaluation procedures are broadly agreed upon, each programme will have to develop its own guidelines based on local needs and preferences. A minimum of two replications should be sampled for tasting at each site and a minimum of two sites. Environments for testing should be chosen across a reasonable range of variability for the target region. Testing should be carried out for at least three years before any recommendation is made on the quality of any new clone.

6.6 ADVANCES AND FUTURE PERSPECTIVES

Local traditional varieties used for fresh consumption, when they exist, are usually appropriate standards by which to measure the acceptability of the quality of new varieties. Very often, because these varieties are already well accepted on the market, it would be impractical and counterproductive to introduce varieties with eating quality which is substantially inferior. Rather, the goal generally will be to achieve similar quality in new clones having, in addition, other improved traits such as pest resistance or higher yield. Breeders who combine superior yield or resistance, with an eating quality clearly superior to the better local clones, will have made exceptional progress.

7. NUTRITIONAL CHARACTERISTICS

7.1 β-CAROTENE

In nutritional terms, cassava is primarily an energy source. There is probably little justification for including a broad range of nutritional characters among breeding objectives. Increased productivity and starch content are the most obvious ways to improve cassava's contributions to human nutritional status. Nonetheless, other nutritional components can be locally important, such as vitamins A and C and minerals such as zinc and iron. Leaves, used both in animal feeding and for human consumption, have high levels of protein, vitamin C and β-carotene, a precursor of vitamin A.

Visual characterization of yellow pigment intensity is highly correlated with β-carotene content. Breeding for increased β-carotene can make an important contribution to preventing nutritional-induced blindness. Daily human requirements are about 3 mg. This could be supplied by just 150 g of fresh roots having 2 mg β-carotene/100 g fresh roots, assuming it was 100 percent available.

Hershey and Ocampo (1989) suggested single-gene control of yellow pigmentation. After further genetic studies, CIAT (1995) proposed a two-gene system controlling root colour: Y_1, with complete dominance, allowing for transport of β-carotene at high levels to the roots; and Y_2 with partial dominance allowing for its accumulation in the roots. The quantitative variability within root colour classes suggests that a number of genes with smaller effects is also involved in the accumulation process (Iglesias *et al*., 1997).

Breeding for this trait seems to be relatively easy, but acceptance of yellow roots by people accustomed to white roots is usually problematic. In the Democratic Republic of the Congo for example, Dongala *et al*. (1994), tested five yellow-fleshed varieties with up to 31.5 mg β-carotene/100g, but none of these was generally accepted on the market.

Processing reduces β-carotene significantly, although there may be genetic variation for the degree of this reduction (Iglesias *et al*., 1997). As compared with fresh roots, the following levels of reduction occurred for different products: oven-dried cassava flour, 44 percent; sun-dried cassava flour, 73 percent; and boiled roots, 34 percent. Although the correlation among different processing methods across genotypes was significant, the relative magnitude of the effects indicated that the genotypes with the highest carotene concentration in the fresh roots may not be the same as those that are highest after processing. Thus, after routine screening of fresh roots, post-processing evaluation also needs to be carried out to test stability (Iglesias *et al*., 1997).

7.2 PROTEIN

Given the cassava root's basic physiology as a starch storage organ, many scientists believe significant improvement of root protein content is an unrealistic goal. Nonetheless, breeders have periodically given some attention to protein improvement, but never as a long-term concerted effort. The earliest work on breeding for higher protein was that of Bolhuis in Java, begun in 1932 (Bolhuis, 1953). He describes a lack of success through either inter- or intraspecific breeding. Some clones from Indo-China had protein contents >1 percent, but these were very poor agronomically. All the materials and much of the data from this programme were lost during World War II. Bolhuis concluded, "Little success may be expected from the search for cassava varieties with a higher than normal protein content in the roots." In India, breeders reported that colchicine-induced tetraploids contained higher root protein than their respective diploids, but these levels were apparently not maintained over various cycles of propagation (Hrishi, 1978; Bai, 1987).

Preliminary studies by CIAT (Chavez *et al*., 2005) showed several clones with about three times the normal protein contents. MCol 2436 had about 9 percent crude protein (DM basis). The high protein content seems to be most frequent in accessions from Central America and southern Mexico. The authors speculate that this may be the result of introgression from *Manihot* species that grow only in that region. Several elite hybrids are also among the high-protein types. Since none of these hybrids was evaluated for protein content during the selection process, it appears that protein does not have a negative influence on the expression of other traits of commercial importance.

In Africa's cassava belt, the crop contributes, on average, more than 50 percent of calorie intake. Due to high dependency on cassava, minor increases in protein content could make a significant contribution to the dietary protein intake of consumers. In the early 1990s IITA initiated a programme to hybridize cassava with *Manihot tristis*, a wild species reported to have high protein in the roots. The studies are ongoing and potential for success remains to be evaluated.

Soon after formation of the cassava biotechnology network Jaynes (1988) suggested protein could be enhanced via insertion of synthesized genes into the cassava genome. Researchers at ETH in Switzerland transformed the clone 60444 with an artificial storage protein ASP1 gene, especially designed to be rich in essential amino acids (Kim *et al*., 1992). Regenerated tissues expressed the transgene at both the RNA and protein levels. Total leaf protein of these tissues did not change, but the amino acid profile did. However, transgenic plants did not grow normally and root sampling was not possible. More recently ETH has succeeded in recovering phenotypically normal plants in the greenhouse, whose leaves strongly express the ASP1 protein (unpublished results of ETH, cited by Taylor *et al*., 2004b).

While these results represent an important hurdle in cassava nutritional improvement, the expression of improved protein levels is the first of many steps that will be needed. The protein should accumulate preferentially in the roots, it must be non-alergenic and it should be stable (not washed out or degraded) during processing. Since one of the special values of cassava starch for industry is its low protein content, there would clearly be a segregated market for high and low protein types. Furthermore, the plants with modified protein should not adversely affect yield, pest resistance, cooking, palatability or storage qualities. The highly complex nature of enhanced protein for cassava means that research is at the very first stages of many years of product development and promotion. Breeders and the organizations that fund them must have a realistic long-term vision if protein improvement is a goal.

If cassava nutritional value is to be significantly improved, the combination of conventional and transgenic breeding methods will be most productive. In general, breeders should not be overly concerned about monitoring nutritional changes in the roots, possibly with the exception of β-carotene in areas where cassava is the main source for the population. For most populations where nutritional status is critical, cassava is consumed in a processed form, which can facilitate fortification through food additives to compensate for critical dietary components. Generally, a multipronged approach to alleviation of nutritional deficiencies is most appropriate.

7.3 MINERALS

The Consultative Group on International Agriculture (CGIAR) identified iron and zinc as the two principal minerals for which cassava breeding has good potential to contribute to improved human nutrition. Both CIAT and IITA screened their germplasm collections as an initial step in determining the feasibility of breeding for improved mineral content (Maziya-Dixon *et al*., 2000; Chavez *et al*, 2000; Chavez *et al*., 2005). In a large scale screening of 600 accessions CIAT found a range of 6-230 mg/kg and a mean of 17.1 mg/kg (dry weight basis) for Fe. IITA found a range from 3.5-48.8 mg/kg in 162 clones. For zinc, in the same sets of clones, the CIAT clones ranged from 2.6-37.5 mg/kg and at IITA, 4.3-18 mg/kg. These wide ranges indicate a good potential to exploit genetic variation to improve nutritional quality, but actual progress has not been reported to date. There is continuing debate over the most appropriate approaches to confronting micronutrient deficiencies, including possibilities for food additives and crop diversification. Nonetheless, breeders have already demonstrated that a genetic approach is worthy of continued exploration.

8. POST-HARVEST DETERIORATION

As cassava changes from primarily a subsistence to a commercial crop, its rapid post-harvest root deterioration (PHD) is becoming a significant constraint. Harvest of larger lots, on-farm storage, off-farm transport and storage during marketing or pre-processing, all contribute to the need for longer shelf-life. At present, medium- to large-scale commercial processing relies on very carefully coordinating all the steps from harvest to processing, with little margin for error or flexibility for contingencies. FAO estimates a fairly modest 8 percent post-harvest waste for cassava. Continued commercialization will require greater control of post-harvest quality traits in cassava. There are both genetic and management alternatives to solving some of the problems.

8.1 METHODOLOGY FOR EVALUATION

Wheatley (1985) suggested a standard evaluation procedure for assessing resistance to physiological post-harvest deterioration. The method involves cutting 15 cm thick sections from the middle of undamaged roots, covering the distal end with PVC film (in order to maintain the moisture content of the exposed root tissues and hence inhibiting the onset of deterioration from this end of the section), storing roots for three days and finally, evaluating transverse sections for degree of deterioration, based on a semi-quantitative scale.

CIAT used simple field evaluations in the past to assess combined resistance to physiological and microbial deterioration. This involved leaving a sample of roots from harvested plots lying in the field in a shaded or partially shaded area of the plot. Evaluations were made by cutting roots into transverse sections, with half the roots evaluated after one week and half after two weeks. This method may be useful as a means of obtaining an integrated measure of the various processes that decrease cassava post-harvest acceptability.

Elucidating the biochemistry of deterioration will lead both to better evaluation methods (biochemical indicators) and to strategies for increasing shelf life (suppressing PHD). Based on hypothesized pathways implicated in PHD, CIAT proposed intensified research into two enzymes (phenylalanine ammonia lyase [PAL] and chalcone synthase [CHS] and one metabolite [scopoletin]). In field trials, scopoletin was positively correlated with PHD (r=0.61–0.82) (Wheatley, 1985; CIAT, 1994). Root DM was inconsistent in its correlation with PHD. As intra- and interroot variation for scopoletin concentration is quite high, the assay should involve sampling of the whole root parenchyma, several roots in a plot and at least two replicates.

8.2 GENETIC VARIATION AND HERITABILITY

Cassava has no distinct period of physiological maturity; roots may be harvested (at the extremes) soon after they begin to accumulate starch, up to several years after planting. Average time to harvest is about one year to 15 months. Probably there was almost no selection during evolution for longer post-harvest conservation. Early cultivators of low-CNP varieties simply harvested roots as they needed them for family use. For high CNP types, processing to eliminate the toxin was necessary and this also served the purpose of transforming a perishable root into an easily stored dry product. Roots are not used for propagation and thus there would be no natural selection for post-harvest conservation for this purpose. Rogers and Appan (1973) reported on one wild species, *M. walkerai*, native to northern Mexico and southwestern United States, having roots with adventitious buds, which can be used for propagation. This needs to be confirmed and its post-harvest physiology investigated.

In spite of the limitations of studies to date, it seems clear that there is considerable genetic variation for resistance to post-harvest deterioration, at least within the period from 0 to 14 days. For example, Wheatley (1985) evaluated a small group of clones and found a range from 2.1 to 90.1 percent deterioration at CIAT, Palmira. CIAT revisited the question of variability for PHD in the early 1990s and evaluated a broader germplasm base to reassess potential for progress by conventional breeding methods (Tables 17.5 and 17.6). Again, the variation is substantial. It should be noted, however, that when considered in a larger perspective, where several weeks, or even months of storability would be desirable, virtually no clone can meet the criteria.

Most recently, a genotype highly tolerant of PHD was reported in an irradiated S_1 population at CIAT. This clone did not show post-harvest physiological deterioration even after three weeks in conditions where normal roots would begin to show deterioration symptoms after three days. These preliminary results are being retested after multiplying materials clonally (Ceballos *et al*., 2007b).

Table 17.5 Comparison across two sites in Colombia for reaction of a selected set of clones to physiological post-harvest root deterioration (PHD)

	Villavicencio, Meta		CIAT-Palmira, Valle	
Clone	**PHD (%)**	**DM (%)**	**PHD (%)**	**DM (%)**
SM 627-5	2	34	5	35
SM 979-20	7	39	16	37
CM 7033-3	11	39	13	40
CM 7251-1	56	39	90	36
SM 985-9	64	34	91	33
CM 6986-10	76	37	79	33
Overall mean (86 clones)	38	35	34	35
Source: Adapted from CIAT Cassava Programme Annual Report (1993)				

Table 17.6 Comparison across harvest times for post-harvest root deterioration in a highland ecosystem of Colombia

	12-month harvest		18-month harvest		Yield increment at late harvest (%)	Type of clone
Clone	**Yield (tonnes/ha)**	**DM (%)**	**Yield (tonnes/ha)**	**DM (%)**		
SG 700-3	30	33	39	36	+31	Versatile
CG 402-11	33	32	38	31	+14	Versatile
SG 427-87	18	34	31	37	+76	Late
CG 501-2	19	34	26	38	+38	Late
SG 427-64	31	34	29	36	-5	Early
SM 526-3	30	35	26	35	-12	Early
Mean (37 genotypes)	20	33	21	34	+3	
Source: Adapted from CIAT Cassava Programme Annual Report (1993)						

In spite of many evaluations for genetic variability, no sustained effort has been directed at genetically improving cassava for post-harvest storability. The available data suggest that progress could be made within fairly restricted limits, but achieving conservation of several weeks or more through conventional breeding seems unlikely. Even these limited objectives, however, could probably solve many of the problems related to deterioration in current processing and marketing systems. Longer-term conservation through genetic engineering would open up vast new possibilities for cassava as both an industrial and food crop. At a minimum, breeders should monitor post-harvest deterioration of new materials to make certain that new experimental varieties are not extremely susceptible.

During several years of evaluations of post-harvest deterioration in breeding and utilization trials in Colombia, there was consistently a positive correlation between DM content and level of deterioration. Clones with high DM (a positive trait) tend to have rapid deterioration (a negative trait). No physiological basis for this relationship has been confirmed. It is somewhat problematic for breeding, but the correlation is not so high and it should be possible to select clones having both high DM and good shelf life.

Kawano and Rojanaridpiched (1983) estimated narrowsense heritability of deterioration at 0.44–0.62, when measured as the combination of physiological and microbial effects. These values seem

surprisingly high for a complex trait so influenced by the environment and so subjective in its evaluation. Some proportion of the heritability is indirectly due to the high heritability of the correlated trait, DM content. The authors concluded that reducing post-harvest deterioration by breeding is feasible if one can accept lower root DM content. Later results from CIAT (1994) suggest that with rigorous selection of genotypes, root DM may not need to be sacrificed to extend shelf life.

8.3 ENVIRONMENTAL INFLUENCE

Evaluations of physiological deterioration of various cassava clones, each harvested in five sites in Colombia with different edaphoclimatic characteristics, demonstrated substantial year and location effects on physiological deterioration. The studies indicated that clones most affected by stress factors (e.g. insect and disease attack, or drought) suffered more defoliation and were more resistant to physiological deterioration than less stressed clones. Experiments with plants defoliated manually reacted similarly. A partial explanation may be that stress tends to reduce DM content, which in turn (for reasons yet unknown), extends shelf life. In any case, preharvest stress can confound the evaluation of genetic differences among clones, especially when there is wide differential reaction to the stress itself.

8.4 FUTURE PERSPECTIVES

Farmers, processors and consumers have learned to manage post-harvest deterioration in cassava and do not usually consider it a priority feature for improvement. However, post-harvest deterioration will increasingly become a constraint for cassava to enter new processing systems and markets. The problem will best be solved by a combination of management and genetic approaches. A workshop sponsored by FAO in 1991 explored the possibilities for the application of molecular biological techniques to extend the shelf life of cassava substantially. A better understanding of the biochemistry of deterioration is a necessary first step towards any genetic modification through transformation.

Beeching *et al*. (1994) suggested that the most promising approach would be to enhance the root wound response. The sealing and wound healing aspects necessary to the successful completion of the wound response, now poorly expressed and/or localized in harvested roots, might be improved. Possibly wound-induced signals and responses could be suppressed after early localized suberization and lignification, rather than extending through the whole root. Huang *et al*. (2001) looked at differentially expressed genes during the early deterioration process to gain molecular insight and identify important metabolic pathways. The goal of such research is to be able to eventually apply reverse genetic approaches to delay or even prevent physiological post-harvest deterioration.

Solving cassava's post-harvest deterioration problem will be a long-term endeavour, but if successful, it could revolutionize the way cassava is managed, both pre- and post-harvest.

A common need for many of the strategies aimed at improving root quality is the availability of a high capacity root quality analysis laboratory to screen large numbers of samples (>15 000) in search of those with novel pasting properties or enhanced nutritional value. CIAT has developed jointly with the National University of Colombia, a laboratory that can to generate thousands of amylograms per year using a battery of rapid viscoanalyser, Brabender, DSC and other standard equipment and protocols.

Chapter 18. Balanced improvement

Only rarely does a cassava breeder limit objectives to a single trait. Usually improvement is sought for a few or several traits. In addition, one must also nearly always consciously select in order to maintain expression of many others, at levels found in local varieties. If it is assumed that most traits of interest to the cassava breeder are multigenically controlled, then breeding for the appropriate balance of multiple traits can very quickly become quite complicated. How many breeding objectives can be reasonably managed? What are the trade-offs between the strict focus on one or two objectives, versus including a broad range of target traits to improve?

Most of the literature on crop breeding is based on experience from extensively researched crops in developed countries. In these situations, the breeder is often working with a crop that is already greatly improved through intense selection. Usually improvement can be focused on one or only a few traits within any given breeding programme. Genes for these traits are generally sought in existing local germplasm collections (except where genetic transformation is anticipated), which are often narrow in relation to the global variability for the species. When the trait is found, it is commonly backcrossed into the adapted genetic background and a new variety is created. The process may then be repeated for other traits.

Balanced improvement is more than the sum of strategies for meeting individual objectives of a breeding programme. It needs to take into account not only the relative priorities for different traits, but the often complex interactions among them. This subject area has already been discussed in some detail in the section on subdivision of breeding objectives through defining distinct agro-ecosystems (Chapter 7). In the following sections the implications of a strategy for integrating numerous traits in a breeding programme are looked at.

Successful integration of breeding objectives depends on four areas of programme management: setting objectives, selection of the initial germplasm base, choice of breeding methodology and management of the selection environment(s). Previous chapters frequently referred to all of these, with respect to individual selection criteria.

1. INTEGRATING OBJECTIVES

Achieving overall varietal acceptability involves integrating a wide range of specific objectives into an overall performance evaluation within a selection environment representative of the target production area. Quantification of performance is easiest when an overriding trait, such as yield, is used by producers as the measure of acceptability. This would essentially allow the breeder to ignore most individual traits and focus on yield selection. However, as has already been seen, neither yield nor any other single measure is usually adequate to describe varietal acceptability. In most cases it is necessary to subdivide objectives into nearly-independent groups of components.

Root yield can serve to integrate a multitude of elements related to general physiological adaptation (temperature, photoperiod, rainfall patterns, soil structure and fertility), pest resistance, plant architecture and photosynthetic efficiency. Market acceptability may include the additional components of root form and size, external and internal colours, starch content and quality, cyanogenic potential, post-harvest deterioration and a multitude of traits relating to acceptance for specific end uses. Cropping system compatibility includes early vigour, plant architecture, fibrous root system architecture, water use efficiency and others.

A first step could then be to balance objectives among these three principal integrating criteria. Existing common varieties can be an initial basis for defining traits needing improvement. For simplification, priorities can be classified as low, intermediate, or high. Often, only intermediate or high level priorities can justifiably be addressed.

A second step could be to list the known components requiring improvement, within each group. As a hypothetical example for yield-related factors, this might be: sprouting ability under dry conditions,

yield potential and green mite resistance; for acceptability: low levels of cyanogens and yellow-fleshed roots; and for cropping system compatibility: later branching.

As a third step, the breeder may wish to list traits that already exist in local varieties and are of high priority to retain at current levels. Following the same hypothetical example, these could be, for yield-related traits: mid-season drought tolerance and thrips resistance; for market-related traits: starch content and mealy texture when cooked; and for cropping system compatibility: suited to intercropping with maize. In this simple example, the breeder is already faced with 11 traits to consider in selection. If all are quantitative traits (multigenically controlled) and each is given the same level of priority, one can imagine a very slow rate of progress. Strict prioritizing and focusing more strongly on one or just a few traits is essential to demonstrating a level of genetic gain that will satisfy client needs.

Achieving balanced improvement does not mean all traits of interest need to be considered simultaneously in each selection cycle; in fact, it will rarely be the most efficient procedure. Firstly, no single selection environment is likely to provide the appropriate balance of selection pressure consistently year after year; and secondly, the breeder needs to apply selection criteria to different stages of selection based on heritability of those traits considered. In this way, multiple-character, integrated selection is carried out in a step-wise process where groups of traits of manageable size are evaluated at any given stage.

2. ESTABLISHING THE GERMPLASM BASE

A broad genetic base is an essential, but not sufficient, prerequisite for multiple objective breeding. Even with a broad genetic base, it is possible that certain traits being sought will have low genetic variability within a given gene pool. Where possible, a genetic base for breeding should be chosen on the basis of variability to the specific characteristics being sought and not on some artificial criteria of variability unrelated to objectives. Perhaps the most logical procedure for most cassava breeding programmes is to obtain a combination of landrace varieties from ecosystems matching that of the target production area (matching in terms of edaphoclimatic and pest characteristics) along with improved breeding lines with similar adaptation traits and improved agronomic value.

Although this is in theory a practical approach to establishing a germplasm base, in reality there is little ecosystem data associated with cassava germplasm collections. Therefore, the breeder must often rely on secondary data – those obtained from evaluation of the germplasm by gene bank curators or breeders. If this evaluation has been carried out well and in an appropriate set of environments, the information may be just as valuable, or more so, than information directly from the ecosystem of origin. Evaluation of germplasm accessions, simultaneously under uniform conditions, may be the best means of comparing suitability for a particular breeder's needs. This type of comprehensive evaluation across diverse ecosystems is available only from a few of the larger germplasm collections, such as the one at CIAT.

3. MANAGING THE SELECTION ENVIRONMENT

Management of the selection environment is a key part of balanced selection. Choice of an appropriate environment has already been discussed at length. Enhancing selection pressures and opportunities may be necessary even if the selection site is considered to be highly representative of the target environment and especially so if it is not highly representative. Management may be for the purpose of increasing or reducing the levels of certain factors, or of increasing their uniformity. The essence of balanced selection is that the various components of the environment are kept uniform and at appropriate levels.

Some controversy surrounds the question of whether greater progress can be made when selection is practised under stress or non-stress conditions, even when the final objective is yield under stress conditions. Unfortunately, there are few data from breeding programmes actually making these types of comparisons. The common theory expounded by many is that rate of genetic advance under stress

conditions is low because of high environmental variability. Several examples have shown that selection under favourable conditions resulted in greater advance in both favourable and stress conditions. Most studies, however, have examined a single stress factor, under highly managed conditions and with an inadequate germplasm base for expression of genetic variability under stress.

There is no innate reason why high stress needs to be associated with high environmental variability. The choice of as uniform an environment as possible, combined with management techniques which further enhance that uniformity, can probably often reduce variability to acceptable levels. Furthermore, when stress is the result of seriously yield-limiting pests and diseases, it is untenable to suppose that higher yield could be achieved by selecting under pest-free conditions compared with stress conditions.

4. HOLISTIC VERSUS REDUCTIONIST BREEDING METHODOLOGIES

Breeders must strive for an appropriate balance among research thrusts in multiple-objective breeding programmes. In very broad terms, two distinctive approaches are possible: reductionist versus holistic. The reductionist approach is an attempt to define, for example, individual mechanisms of stress tolerance and to select independently for each. The theory is that selection can be most efficient when direct selection for physiological processes is possible. The holistic approach says that the whole is more than simply the sum of its individual parts. For example, the interaction of different mechanisms of stress tolerance is so complex, that it is virtually impossible to effectively design a selection scheme based on the proper balance of tolerances in an environment where multiple physical and biological stresses interact. Buddenhagen (1983) developed strong arguments for an holistic approach with reference to stress tolerance.

The same can also be applied more broadly to other components of varietal acceptability. Extending the arguments to market acceptability, the reductionist approach would attempt to understand and select for each biochemical pathway that influence quality, or each root morphological trait relates to market preferences. The holistic approach would concentrate on identifying genotypes that have good market acceptability by looking at the same criteria as consumers.

Probably strict adherence to either the reductionist or the holistic approach is unrealistic. Where mechanisms or components are well understood, easily selected for and their relation to other components clear, it is logical to utilize them individually as selection criteria. On the other hand, a breeder should move ahead to establish a breeding programme even without having a very clear understanding of all the components of varietal performance.

As more detailed information becomes available on all facets of cassava breeding objectives, breeders often find themselves pushed towards increasing reductionism. Sometimes this is appropriate and sometimes it is not. It is certain that few breeding programmes are well enough endowed to afford the luxury of delving in great detail into any single focus area. Networking among programmes is one of the most effective ways to bring together and integrate specialized and reductionist lines of research.

5. SELECTION PROCEDURES FOR INTEGRATING OBJECTIVES

As appropriate selection is the core of successful plant breeding, developing an accompanying philosophy and strategy warrants a considerable investment of a breeder's time. He or she should become familiar with a range of methods, experiment with different ones and compare results from other crops and programmes. Every trial can be a means of re-evaluating methods used and exploring possible improvements.

In a strategy of integrated improvement, the breeder normally limits final selection to only a few principal criteria, such as plant type, yield, pest resistance and quality. The hypothesis is that optimum balance among component factors for these traits will automatically be achieved by selection for overall performance. This type of selection does not mean that evaluation for many individual components is

not useful. These individual data can provide an additional basis for selection, an understanding of contributions of individual components to overall performance and a continuing readjustment of objectives. Always the question to be asked is: how best to achieve balanced improvement, rather than how best to demonstrate improvement of any single measurable trait. Three general strategies for balanced improvement are reviewed here: stepwise selection; independent culling; and index selection. Most programmes will not strictly follow any single strategy, but rather combine and modify to suit individual situations.

5.1 STEPWISE SELECTION

The essence of stepwise selection is to improve one or a few characters at a time. When each individual character reaches the desired level of expression, the breeder begins to concentrate on another. This is probably the most common of all strategies throughout crops. The breeder is able to focus on few traits and usually to see visible progress for each in a reasonable time frame. With this strategy, the breeder will often have something new to offer farmers much more quickly than when several traits are considered simultaneously. The key to its successful employment is the correct prioritization of traits, a process further discussed in the next chapter. This approach is most successful when one or a few major constraints can clearly be identified, such as a major pest problem or quality characteristic. It is less appropriate when a range of traits of nearly equal importance need to be improved for a variety to be successful.

Stepwise selection towards balanced improvement does not imply a sharp division between the progressive steps. The breeder will continually review client demands and the breeding populations to decide whether to revisit traits already considered. For example, after having increased levels of resistance to CBB and commenced concentration on root dry matter content, a breeder may see the need to further increase resistance levels if cultural practices change the pathogen dynamics.

5.2 INDEPENDENT CULLING

Independent culling is the procedure by which the breeder sets minimum or maximum acceptable levels of expression of each trait and includes in the selected group only those genotypes having all characters of interest within the defined range. The advantage of this method is that it is very simple and straightforward to apply, after the cut-off points for each trait have been defined. Defining the cut-off points, on the other hand, is likely to be complex.

Independent culling is probably most appropriate when the purpose of a particular trial or set of trials is to identify clones for recommendation to farmers. It is less appropriate when the purpose of the trial is to identify clones to enter the next cycle of breeding as parents. In the former case, clones will need to have certain standards across a range of traits in order to be accepted by farmers. On the other hand, independent culling, when strictly applied, will reject a genotype that may be excellent in all but one trait that falls below the established cut-off level. While such a genotype could make an overall positive contribution to future breeding cycles, it will not have the opportunity to do so. Usually the breeder will need to make some compromise between ideal levels of expression and what is practical in terms of retaining adequate genetic diversity in the breeding population for long-term genetic improvement.

Table 18.1 illustrates how the application of independent culling would narrow the identification of clones adapted in three agro-ecologies of Colombia. The success of independent culling resides with the breeder's ability to set appropriate cut-off levels for each trait, such that: (1) selection is not so severe that insufficient variability remains at the end of the process; (2) selection is not so lenient that too many clones remain at the end of the process; and finally, (3) that the cut-off point for each variable provides an appropriately balanced level of expression of all selected traits in the final selected clones.

Table 18.1 Examples of independent culling for selection among 3028 accessions in CIAT's germplasm bank

Target area/Test site	Basic breeding objectives[a]	Target level of expression	Accessions meeting criteria
Subhumid tropics/Colombia North Coast			
Root yield (kg/plant)		>3.5	1 007
plus: Harvest index		>0.5	432
plus: Root dry matter content (%)		>35	313
plus: Cassava green mite damage[b]		< 2.0	57
plus: Thrips damage[b]		<2.0	42
Acid soil savanna/Carimagua, Meta Department			
Bacterial blight damage[b]		<3.0	55
plus: Superelongation disease damage[b]		<3.0	21
plus: Cassava green mite damage[b]		<3.0	6
plus: Lacewing damage[b]		<3.0	4
Highlands/Popayan, Cauca Department			
Phoma leaf blight damage[b]		<2.0	17
plus: *Oligonychus* mite damage[b]		<2.0	10

[a] Data from CIAT-Palmira: root yield, harvest index, root dry matter, mite and insect ratings; data from Carimagua: bacterial blight and superelongation ratings; data from Popayan: Phoma ratings
[b] Ratings on a 1 to 5 scale, where 1 = very low damage; 5 = very high damage

Source: Hershey (1984)

5.3 SELECTION INDICES

Plant breeding theory amply demonstrates that optimum multiple-trait selection is best achieved through use of selection indices, a means of weighting individual traits and combining their values into a single index for ranking tested materials. Formal selection indices have been little-used in cassava breeding. The classical indices which require economic weights and genetic values for each trait are often impractical, because of lack of reliable genetic information on complex traits. CIAT (1994) analysed data from ten years of trials in Colombia to compare alternative approaches to the definition of a selection index. The objective was to find a linear combination of phenotypic values that would maximize the expected genetic gain. Four estimation procedures were considered: (1) factor analysis without rotation of factors; (2) with rotation of factors; (3) principal component analysis; and (4) modified base index. The primary variable (objective) was dry root yield and secondary variables used in building different functions were plant height, number of stakes per plant, branching index, canopy depth (length of stems with leaves) at harvest time, harvest index, numbers of commercial roots and cyanogenic potential. The different alternatives were compared with the modified base index, considered to give the closest estimate to the true genotypic value of an individual. The study showed that greater progress could be made by subjecting data from each trial to factor analysis, determining the importance and relative weight to be assigned to each trait, followed by selection based on the scores. Other factors could then be considered in order to adjust the final group of selected genotypes, such as pest resistance or root dry matter content.

Selection indices can also be developed through a combination of field experience, rudimentary genetic information and intuition. CIAT (2003) reported using a selection index based on an informal weighting of four key factors, where root yield is the principal selection objective:

$$SI = (FRY*10) + (DMC*8) – (Plant\ type*3) + (HI*5)$$

where: *SI* = selection index; *FRY* = fresh root yield; *DMC* = dry matter content of roots (percent); *plant type* is on a 1–5 scale, where 1 is best and 5 is worst; and HI = harvest index. In order to remove the inherent weighting effects of the different variables, data are normalized (mean = 0 and standard deviation = 1) prior to applying the selection index. Appendices VIa to VId illustrate the application of several alternative indices, where different characters are selected and differentially weighted to create the selection index.

Mahungu *et al*. (1994) compared efficiency of selection for root yield by selecting for yield itself; and by applying a selection index that incorporated root yield, number of storage roots, storage root size and stem girth. In general, the selection index identified nearly the same set of genotypes for selection as did direct yield selection.

In spite of the relatively few examples where index selection has been tested, the reality is that most cassava breeders will begin to find selection indices practical to develop and apply, with the power of personal computing now available to virtually every scientist. These indices will need to be of a less formal type well into the future, because the genetic information required for classical indices will be slow to accumulate.

Selection indices can be viewed in a continuum from very informal, to those based strictly on the classical definitions that apply heritabilities and economic weights to each character. The most informal level is the purely visual selection a breeder may make in the field – a mental (subjective) summing up of any genotype's positive and negative traits to make a select or reject decision. This has often been referred to as the breeder's eye. The effectiveness of this method relies almost totally on the skill and experience of the breeder. For a skilled breeder, it may be one of the most efficient and effective methods. He or she will understand the economic importance of many individual traits, their heritability, relationships among traits, influences of different types of environmental variations and G–E interactions.

One method breeders use to combine subjective and objective evaluations into a sort of subjective, or mental, index is to ask the question, "Taking into account all traits of importance, how does this clone rate?" The answer to the question can be tabulated as a rating scale, for example, from one to five. CIAT has used a system that takes this method one step further by asking the question, first about the above-ground plant parts (referred to hereafter as the foliage) and secondly, about the roots. Both are scored at harvest. Each is rated on a scale where one is excellent and five is very poor. The components included in this evaluation may vary according to the goals for the breeding population and the environment. Foliage evaluation usually includes factors such as plant type, reaction to pests and diseases, lodging and leaf retention. Root evaluation includes factors such as yield (subjective evaluation), root form and root colour (if that is a part of the selection criteria). These evaluations are used in conjunction with a wide range of other subjective and objective criteria. They provide a guideline against which the breeder can compare these other criteria. If, for example, the sum of evaluations indicates good performance of a clone, but the subjective foliage evaluation is poor, the breeder may want to take a second look to see why there is an apparent discrepancy. Another way to use these evaluations is to consider them as one more criterion among others in a selection index (see Appendix VI).

Most cassava breeders do not strictly adhere to any one defined methodology for multiple trait selection. They combine personal experience and genetic theory in some locally appropriate scheme. Good breeders recognize that personal experience, flexibility and common sense contribute as much to success as does theory and they will not hesitate to make creative use of many resources.

Chapter 19. Marker-assisted selection[5]

5 Contributed by Martin Fregene and Chikelu Mba

1. INTRODUCTION

Marker-assisted (or aided) selection (MAS) refers to the use of molecular markers to follow the inheritance of genes in a breeding programme with or without phenotypic selection (Bernardo, 2003). Usually these are the genes that are difficult to evaluate in a population due to low heritability, the phenotype is expressed only at maturity, or environmental conditions that allow expression of the trait are sporadic (for example absence of a disease or pest, or the confounding effect of the environment). In essence, the breeder takes advantage of the linkage between the allelic variants of a molecular marker and the agronomic trait of interest in making selections. The selection of progenies based on genetic values derived from molecular marker data can substantially increase the rate of genetic gain, especially if the generation intervals can be reduced (Meuwissen *et al.*, 2001).

MAS is made even more appealing by the fact that DNA from any tissue on the plant could be used in such assays. For some crops, the procedure has been so well developed that no destructive steps are involved. At the SCRI, Dundee, Scotland, for example, tissue is drilled out of the endosperm of rice seeds for DNA extraction. Such seeds could still be planted if they carry the desired alleles. As a means of expediting the generation of data, Ikeda *et al.* (2001) also reported a very simple way for extracting rice DNA for use in MAS.

The integration of MAS into breeding programmes as predictors for trait genotypes is, however, not always straightforward. In most cases, especially for quantitative traits, there is a need to validate the trait–marker association through large scale field experiments and statistical methods in order to make valid estimates of target genome segments (so-called quantitative trait loci [QTL]), contributing to the genetic variance of a trait. Once valid assumptions are met, the breeder selects genotypes that are superior at target loci. The environment, or even gene interactions, do not affect the markers. It is for these reasons that selecting for favourable effects due to QTL on the basis of marker data has become accepted as having great promise even for the improvement of polygenic traits. For qualitative traits with clear-cut delineation between phenotypes, the situation is different and far simpler, as the mapping of the marker is synonymous with mapping the trait and vice versa (assuming no crossing over between the marker and the gene of interest).

A MAS programme normally involves three basic steps. The first is genome (linkage) mapping where markers are placed on a molecular genetic framework map on the basis of their segregation in a mapping population. For ease of use of these markers in assays, they are usually Polymerase Chain Reaction (PCR)-based markers such as AFLP, RAPD, microsatellites, ISSRs, SNPs, etc. In the second step, genome linkage mapping is followed by QTL mapping. In this step the genome location of markers that co-segregate with the traits of interest are located on the linkage map. Hospital and Charcosset (1997) as well as Spelman and Bovenhius (1997) used simulation studies to investigate the optimal location of a marker relative to the QTL in order to be efficiently used in MAS. The smaller the flanking QTL bracket, the easier it is to trace the QTL transmission from one generation to the other on account of the linkage disequilibrium. The third stage involves the selection of molecular markers at such QTL during the evaluation and selection processes.

Once marker–trait association has been validated, the transmission of trait genes from parent to offspring is monitored through closely linked markers (Stam, 2003).

Deliberate crosses and selection from progeny facilitate the accumulation of desirable genes.

2. SUCCESSES AND LIMITATIONS OF PHENOTYPIC SELECTION

The shy and asynchronous flowering of cassava is a major constraint for breeders. Some landraces and improved varieties flower very sparsely, which severely limits the quantity of recombinations that can be made. In addition, it can be difficult to synchronize flowering between two clones selected as cross parents. Virtually all cassava genotypes are heterozygous and little inbreeding has been practised. This

confounds the selection of parents, as the good phenotypic attributes of a parent might be due to dominance effects, which cannot be passed to the progeny.

Another bottleneck in cassava breeding is the need to exercise low selection pressure at the seedling trial stage because of low heritabilities found with selections based on a single plant. Although this is in some ways the equivalent to an F_1 in seed-propagated crops, selection in cassava is confounded by the fact that some traits are expressed differently when derived from seed as compared with subsequent clonal generations. Other constraints include: the low multiplication ratios, meaning replicated trials are conducted only during the third cycle; the long growth cycle, making cassava breeding programmes lengthy; and the limited resources available worldwide for breeding the crop. On account of these problems, varietal development is typically very slow, normally requiring eight to ten years, and another five to ten for economic impact.

Cassava genetic improvement can be made more efficient through the use of easily assayable molecular genetic markers that enable the rapid identification of the genotype without the confounding effect of the environment or developmental stages. These DNA sequences represent a limitless source of reliable markers for tagging traits in crop improvement programmes. Molecular genetics has special potential in three areas, namely:

- development of molecular tags that can inexpensively and rapidly identify desirable genotypes early in the breeding cycle, thereby eliminating the need to evaluate large numbers of plants, and obviating the confounding effects of the environment;
- facilitation of the accumulation of genes influencing agronomic traits of importance from different sources. For example, parents carrying different traits or different sources of genes can be recombined without resort to time-consuming field trials, reducing the breeding cycle to a year and a half, the time required to produce seeds. Nonetheless, in most situations, breeders will also be evaluating nurseries for traits controlled by genes that have not been tagged. These traits will normally need to be evaluated in field trials.

MAS can complement the description and analysis of the structure of genetic diversity, with the goal of exploiting new diversity. Thousands of local varieties held by small farmers represent a critical resource for the future productivity and stability of production of the crop. How to evaluate and use the vast amount of variability in a systematic manner is still a challenge to most cassava breeding programmes, in spite of considerable investment in collecting and conserving cassava germplasm resources.

As the name implies, molecular marker-assisted selection, MAS, is complementary to field-based selection methods and does not replace them. Development and use of markers does not make economic sense for all traits. Some will remain easier to select phenotypically compared with the use of markers, for example, traits that have a high heritability and appear early in the crop cycle, such as plant architecture. Furthermore, many traits of agronomic interest in cassava are quantitatively inherited, controlled by many genes that often interact with each other and are affected by the environment. Unravelling the genetics and the development of markers for such traits is still many years down the road and field-based selection methods will remain the principal means of making genetic gains for these traits.

3. MOLECULAR MARKERS: A BACKGROUND

One common issue in MAS introgression of traits is the reduction or elimination of undesirable donor genome content, transferred to the progeny for multiple generations through linkage drag. The simulation of Stam and Zeven (1981) indicates that this could be substantial and would significantly impede the success of foreground (recurrent parent genome) selection. Tanksley *et el.* (1989) concluded that with marker-assisted backcrossing, a significant proportion of the recurrent parent genome is recovered as compared with a selection scheme without molecular markers. Hospital *et al. (*1992) corroborated this in achieving a reduction of two backcross generations with the use of molecular marker selection. Frisch *et al. (*1999), through a simulation study, found that use of molecular markers for the

introgression of a single target allele saved two to four backcross generations. This situation applies even with small population sizes and marker data points. It was inferred that MAS had the potential to reach the same level of expression as the recurrent parent genome in generation BC_3 as reached in BC_7 without molecular markers. The same authors also advocated concentrating the selection of recombinants on the carrier chromosome for the desired allele in early generations of selection, underscoring the need to use mapped markers as also suggested by Meuwissen *et al. (*2001). One of the conclusions from the Meuwissen *et al.* (2001) simulated study is that with a dense marker map covering all the chromosomes, it is possible to accurately estimate breeding values even in the absence of phenotypic data and progeny. Stam (2003) recommended the inclusion of a background marker that distinguishes between the recurrent and donor parents' genomes, in order to attain greater levels of efficiency. This considerably reduces the number of generations of recurrent selection required for recovering the recurrent parent genome. The same author also stresses that genome size is an important factor to be considered in planning the total size and duration of a successful introgression programme. Larger genome sizes generally require larger populations as well as the scoring of more markers. Van Berloo *et al.* (2001) determined the optimum sizes for the populations and markers.

The utility of MAS can be assessed using the following equation attributed to Lande and Thompson (1990):

$$RE_{MAS:PS} = \text{square root: } [\ (V_M/V_A)/h^2 + (1-V_M/V_A)^2/(1-h^2(V_M/V_A))]$$

Where $RE_{MAS:PS}$ is the relative efficiency of MAS compared with phenotypic selection and V_M/V_A = the ratio of variance explained by the marker compared with total additive genetic variance, and h^2 is the narrow sense heritability. If V_M/V_A is high (a marker for a major gene or markers for QTLs that control a large proportion of additive genetic variance) and a trait has very low narrow sense heritability (h^2), then $RE_{MAS:PS}$ will be very high.

There are many traits of importance in cassava breeding with low h^2 (such as the F_1 seed generation). Some examples are:

- most traits, when evaluation is based upon a single plant, particularly for quantitative traits and resistance to several pests and diseases;
- disease resistance traits where the pathogen pressure is absent or low, such as cassava mosaic disease resistance in the Neotropics or CGM during the wet season;
- very variable experimental fields due to natural variability and/or poor management; and
- traits that are often affected by stage of plant growth, e.g. DM.

Markers may permit the efficient elimination of undesirable genotypes at the seedling stage. For example, the number of genotypes at the seedling stage can be reduced by 50 percent if a trait is controlled by a single gene, or by 87.5 percent if controlled by three genes. This is one of the most crucial selection stages, since it contains the highest level of genetic diversity for the breeder to find the trait combinations of interest. Often, up to 90 percent of genotypes are discarded in the seedling stage.

The economic benefits of MAS relative to phenotypic selection are a critical consideration in breeding programmes. Moreau *et al.* (2000) stated that economic returns from adopting MAS decrease with increased cost for genotyping, therefore restricting the utility to traits with low heritability. The benefit is greatest if the investment is high enough to evaluate large population sizes necessary for using molecular markers to explain genetic variations. Moreau *et al.* (2000) cautioned that their study was based on one cycle of selection and for just one trait and that this may explain the relatively lower economic value for MAS obtained when compared with earlier studies such as that of Xie and Xu (1998).

MAS offers great potential for an accelerated improvement of quantitative traits in crop plants. Based on theoretical studies, the following seem to hold true and should guide decisions for the adoption of MAS as a breeding strategy:

- best results are achieved in making selections when MAS is combined with phenotypic data as compared with either approach in isolation (Hospital *et al*., 1997; Moreau *et al*., 1997). Gimelfarb and Lande (1994) stated that phenotypic data would reduce the cost of genotyping, especially if phenotypic evaluation is conducted in early generations of evaluations. This not only reduces the cost of MAS but also increases its efficiency;
- the relative efficiency of MAS as an alternative to phenotypic selection is improved in situations of lowered heritability estimates. Yousef and Juvik (2001) inferred that for the introgression of quantitative traits in sweet corn, MAS is more efficient than phenotypic selection when traits are difficult and costly to measure and that the higher gain from MAS could compensate for its higher cost. This is echoed in the simulation study of Moreau *et al.* (2000);
- MAS based on uncertain QTL estimates, or where QTL are in repulsion phase linkage with markers, would be of limited value, underscoring the need to validate QTL before using them in MAS. The ideal solution would be to develop universally applicable QTL-specific, or even to develop QTL allele-specific, direct markers derived from the DNA sequences of the target genes of interest;
- the greatest use for MAS may be found in the introgression of exotic germplasm into breeding programmes, and in the improvement of materials derived from mapping populations;
- substantial investments into the development of molecular marker maps, and research to detect associations between phenotypes and markers, have led to the availability of molecular marker maps for a wide range of crop species. Initially some assumed that once all of these tools became available, MAS could become a panacea in crop improvement. Such enthusiasm is, however, now being tempered by some of the aforementioned issues. The judicious approach is to identify the situations in which MAS is best suited. Indeed, there is not much information in the literature on the successful use of MAS in introgressing genes in breeding programmes. This, however, may be attributed to the fact that MAS has been most widely used by private breeding companies whose work is not always in the public domain. Also, it is becoming more evident that the use of MAS may hold greater promise for well-known and characterized genes than for unknown genes.

4. TYPES OF MOLECULAR MARKERS

Molecular markers should not be confused with genes; unlike genes, markers do not necessarily have any biological functions or effects. Rather, markers are unchanging landmarks in the journey through the usually complex genome. Markers are DNA sequences that can be identified, and whose genome locations are precisely known, usually by way of linkage mapping. They are inherited from the parent by offspring in the classical genetic models. The presence or absence of molecular genetic markers, in contrast to phenotypic (morphological) markers, is determined through DNA assays. Such assays could be hybridization- or PCR-based techniques. Morphological traits, usually quantitative, on the other hand are tangible and therefore can be measured. Another variant of markers is the biochemical markers, which are based on proteins produced by genes (e.g. isozymes and seed coat proteins).

The most commonly used molecular markers are RFLPs, RAPDs, AFLPs, microsatellites and SNPs. These differ in a variety of ways: the underlying principle, type and origin of polymorphism, abundance of marker in the genome, level of polymorphism, whether marker system is co-dominant or dominant, amenability to multiplexing, DNA quantity required per assay, whether or not sequence information is required, development costs, operational costs, technical demands and amenability to automation. It follows logically also that the type of information that can be obtained from a marker system would vary accordingly and the choice of a system should be guided by the type of information needed as well as a consideration of the differences listed above. The FAO Biotechnology Forum (www.fao.org/biotech/forum.asp) dedicated a conference to the theme, “Molecular marker assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries," and the following apt review of marker systems was provided as part of the background information for participants:

4.1 RFLPs

Restriction Fragment Length Polymorphisms (RFLPs) are markers detected by treating DNA with restriction enzymes (enzymes that cut DNA at a specific sequence). For example, the EcoR1 restriction enzyme cuts DNA whenever the base sequence GAATTC is found. Differences in the lengths of DNA fragments will then be seen if, for example, the DNA of one individual contains that sequence at a specific part of the genome (e.g. tip of chromosome 3) whereas another individual has the sequence GAATTT, which is not cut by EcoR1. RFLPs were the first molecular markers to be widely used. Their use however, is time-consuming and expensive and simpler marker systems have subsequently been developed.

4.2 RAPDs

Random amplified polymorphic DNA (RAPD) markers were first described in 1990. They are detected using the polymerase chain reaction (PCR), a procedure allowing the production of multiple copies (amplification) of specific DNA sequences. The analysis for RAPD markers is rapid and simple, although results are sensitive to laboratory conditions.

4.3 AFLPs

In the mid-1990s, another PCR-based method of generating molecular markers was described, giving rise to amplified fragment length polymorphism (AFLP) markers. With this technique, DNA treated with restriction enzymes is amplified with PCR. It allows selective amplification of restriction fragments giving rise to large numbers of useful markers which can be located on the genome relatively quickly and reliably. Unlike other methods described here, the technique is patented.

4.4 SSRs

Simple sequence repeats (SSR), or microsatellites, are simple DNA sequences (e.g. AC), usually two or three bases long, repeated a variable number of times in tandem. They are easy to detect with PCR and a typical (SSR) marker has more variants than those from other marker systems. Initial identification of SSR markers is time-consuming.

4.5 SNPs

In recent years, single nucleotide polymorphisms (SNPs), i.e. single base changes in DNA sequence, have become an increasingly important class of molecular marker. The potential number of SNP markers is very high, meaning that it should be possible to find them in all parts of the genome, and micro-array procedures have been developed for automatically scoring hundreds of SNP loci simultaneously at a low cost per sample.

Molecular marker technologies have found applications in the determination of the genetic basis of phenotypic expression and the manipulation of phenotypic variation in plants. These have been mostly through the use of markers in understanding heterosis; prediction of hybrid performance; identification and mapping of QTL; and in MAS. Markers have also been used to improve breeding success through the expression of heterosis in crosses; marker-facilitated introgression (backcrossing); and in the exploitation of near-isogenic lines in breeding (Stuber *et al.,* 1999).

5. ESTABLISHING AND OPERATING A MAS PROGRAMME

To assist national and international programmes around the world, a brief description of how to set up a MAS programme for cassava and to establish priorities is provided. The principal facility required is a well-ventilated modest-sized room (for housing molecular marker equipment for DNA isolation procedures, polymerase chain reaction, gel electrophoresis and staining) and a refrigerator (for storage of laboratory consumables and samples). Table 19.1 summarizes a list of equipment, laboratory

consumables and costs for a modest sized MAS programme that generates 10 000 data points in a single year. The basic equipment is also shown in Figure 19.1. Human resources to run such a laboratory will be a research assistant with a minimum qualification of a first degree in the physical sciences, preferably biochemistry, chemistry or biology. As in any breeding programme, proper data management is very important in a MAS programme and molecular and field data should be collected and stored using a single medium. At CIAT, spreadsheets of the Excel© programme (Microsoft) have been found to be useful for storing both molecular and field data (Figure 19.2).

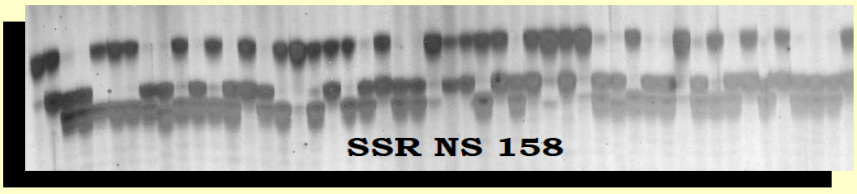
Table 19.1 Cost estimates for running a MAS facility for cassava

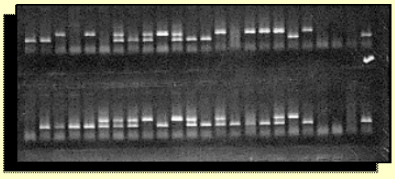
Item	Cost (US$)
Capital costs	
PCR machine	10 000
PAGE and agarose gel rigs (3)	4 500
Silver stain tanks (4)	960
Gel electrophoresis accessories	250
Gel rig power supply (2)	4 100
DNA isolation accessories	600
Microwave oven	110
-20ºC freezer	600
Computer and related software	2 500
Total	**23 620**
Operating costs (per 10 000 data points)	
One laboratory assistant	7 500
PCR consumables	1 200
PAGE gel and silver stain consumables	1 500
DNA isolation	500
Other lab consumables	350
Total	**10 550**
Grand total	**34 170**

Figure 19.1 Basic equipment for a MAS laboratory facility, clockwise from top left: a drill to grind plant tissue, a PCR machine, gel staining equipment and gel rigs

Figure 19.2 Microsoft Excel© spreadsheet for recording and storing pedigree, molecular and field evaluation data on MAS for cassava mosaic disease resistance breeding at CIAT

FORMATO EVALUACIÓN PARA MAS.				EVALUACIÓN:	CMD	PLACA No9			
ITEM	CODIGO	MADRE	PADRE	NÚMERO DE FRASCOS CC	EVALUACIÓN SSRY 158	EVALUACIÓN SCAR RME 1	POSO No.	PARA CAMPO/ENVIO	CÓDIGO TEJIDO
1	CR52A-32	C-243	SM1219-9		-	S	1	NO	998
2	CR52A-37	C-243	SM1219-9		R	R	5	YES	1003
3	CR52A-38	C-243	SM1219-9		R	S	6	NO	1004
4	cr52a-39	C-243	SM1219-9		R	R	7	YES	1005
5	CR52A-40	C-243	SM1219-9		R	R	8	YES	1006
6	CR52A-41	C-243	SM1219-9		R	R	9	YES	1007
7	CR52A-43	C-243	SM1219-9		R	R	11	YES	1009
8	CR-52B-1	SM1219-9	C-243		-	S	12	NO	1010
9	CR53-2	C-243	MCOL 2206		R	S	13	NO	1012
10	CR53-3	C-243	MCOL 2206		R	R	14	YES	1013
11	CR53-4	C-243	MCOL 2206		S	R	15	NO	1014





SCAR RME1

6. MOLECULAR TOOLS FOR CASSAVA BREEDING

Use of neutral molecular genetic markers for cassava improvement commenced with the construction of the framework molecular genetic map of cassava, essentially with RFLP markers (Fregene *et al*., 1997). Due to the difficulties inherent in the use of RFLP markers, especially for resource-challenged NARS, which constitute a majority of cassava researchers, there was a need to develop cheaper and easier-to-use markers. SSR markers became the next generation markers for cassava. By 2004 there were over 600 SSR markers available in the public domain (Chavarriaga *et al*., 1998; Mba *et al*., 2001; CIAT research team, personal communication). Training of network partners in the use of these markers is an important ongoing process.

Other molecular genetic resources for analysing the cassava genome and cloning genes include BAC libraries jointly developed by CIAT and the Clemson University Genome Institute, several EST libraries for resistance to CMD (CIAT; Genebank; Fregene *et al.,* 2004); CBB and cassava starch biosynthesis (CIAT; Université de Perpignan, France [Anderson *et al*., 2004]). Several cassava genes have also been cloned. Munyikwa *et al*. (1997) cloned and characterized the genes coding for the main enzymes involved in cassava starch biosynthesis. They code for the ADP glucose pyrophosphorylase B and S subunits, Branching Enzyme, Granule Bound Starch Synthase (GBSS) and their isoforms. Genes involved in the biosynthesis and degradation of cyanogenic glucoside genes have also been cloned (Anderson *et al*., 2001; Siritunga *et al*., 2002).

7. APPLICATION OF MAS IN CASSAVA BREEDING

The most powerful use of MAS in cassava breeding will probably be in the development of improved progenitors that carry favourable alleles for resistance to pests and diseases, high dry matter yields, and preferred root quality, in a genetic background that is adapted to different agro-ecologies. The success of conventional cassava breeding is inversely proportional to the number of traits it attempts to improve. Combining many genes controlling quantitative traits from diverse sources into a single variety is a long-term, high-risk venture that requires every available tool for success. MAS can be used to achieve these goals more efficiently. For example, CMD-resistant donor parents can be crossed to other parents with excellent resistance to CGM, and markers used to select recombinants that combine resistance to CMD and CGM in a single generation, without the need for field trials. Resulting selections can then be crossed to other genotypes that carry, for example, high β-carotene content to produce multitrait hybrids, again without need for field evaluations. The best of these selections are then crossed to elite progenitors of the appropriate gene pool, to capture genes for yield and adaptation, and the resulting hybrids are selected with markers to eliminate those progenies that do not have resistance to CMD or CGM, and have low β-carotene content, leaving a smaller number of progeny to be thoroughly evaluated in the regular breeding scheme. Selected progenies could then be distributed to partners for use as progenitors or as potential varieties. Multiple generations of crossing are possible, to combine multiple traits, but the scheme will normally end with the cross to a good general combining ability elite parent. This strategy is currently being tested at CIAT to combine resistance to CMD, CGM, CBB, high protein and β-carotene content, and delayed post-harvest deterioration. For any character to be incorporated into a MAS programme, a key prerequisite is the development of genetic markers for each trait of interest.

7.1 RESISTANCE TO CASSAVA MOSAIC DISEASE

An obvious target for the implementation of MAS in cassava breeding is to prepare for the possible introduction of CMD to the Americas, where it does not currently exist, by breeding for resistance in adapted clones (see Chapters 1 and 16 for additional details on CMD). In the early 1990s a new biotype of *B. tabaci*, biotype B (also referred to as *B. argentifolia*), appeared in the Americas, with a wide host range including cassava (Polston and Anderson, 1997). This would appear to increase the possibility that CMD, Eastern African cassava mosaic virus, South African cassava mosaic virus, Indian cassava mosaic virus or a native American gemini virus will become established on cassava in the Neotropics. This is a frightening prospect for cassava production in Latin America, considering that most of Latin American cassava germplasm is very susceptible to CMD (Okogbenin *et al*., 1998). This susceptibility

complicates the utilization of cassava germplasm from its centre of diversity in the Neotropics, for these key cassava production regions. Breeding for resistance to CMD in Latin America, where the disease does not exist and cannot be introduced due to very strict quarantine controls, requires the powerful tools of MAS.

IITA in Nigeria and CIAT in Colombia have been collaborating, with support from the Rockefeller Foundation, to develop molecular markers to this major cassava production constraint. Although evaluation for CMD resistance in sub-Saharan Africa is relatively easy and most areas have sufficient disease pressure to permit proper evaluation of resistance in the field, overlapping outbreaks of CGM, CBB and CMD are common (Legg *et al*., 1998; Opio, personal communication). Consequently, breeding for CMD resistance has to go hand-in-hand with breeding for other biotic stresses, as well as agronomic and quality traits. Combining these multiple traits can benefit from MAS.

Breeding efforts at IITA have uncovered an excellent source of resistance to CMD in some Nigerian landraces (Dixon 1989, unpublished data). This resistance is effective against all known strains of the virus, including the virulent Ugandan variant (UgV) (CIAT, 2001; Akano *et al*., 2002). Molecular and classical genetic analysis revealed a single dominant gene, designated as *CMD2* (Akano *et al*., 2002). Three markers have been identified that are tightly associated with *CMD2,* the closest being RME1 and NS158 at a distance of less than 2cM and 5cM, respectively (CIAT, 2001; CIAT, 2003; Akano *et al*., 2002). The dominant nature of *CMD2* and its effectiveness against a wide spectrum of viral strains makes its deployment very appealing in protecting cassava against the actual or potential ravages of CMD in both Africa and Latin America. More recently Lokko *et al*. (2005) identified three additional DNA markers associated with resistance to CMD (SSR markers SSRY28-180 and SSRY106-207, and AFLP marker E-ACC/M-CTC-225).

CIAT and IITA undertook a project to verify the utility of MAS for CMD resistance and also to work out the details for applying MAS on a routine basis in cassava breeding. Plant materials were full-sib families obtained from crossing resistant Nigerian landraces and CMD susceptible or tolerant varieties. Crosses and their reciprocals were made in 2000 between TME3 and TME9 (two landraces from Nigeria that carry *CMD2*), and TME117, TMS91934 and TMS30572 (a susceptible Nigerian landrace, a susceptible improved variety and a tolerant elite variety, respectively). A total of six families were obtained with progeny sizes ranging from 36 to 840. These progeny were evaluated for CMD resistance over two seasons, the first at Mokwa, a low CMD endemic region and the second at Ibadan, a high-CMD pressure area (both in Nigeria).

Molecular analysis was with the SSR marker NS158, PCR amplification and polyacrylamide gel electrophoresis, and silver staining were carried out as described by Mba *et al*. (2000).

IITA evaluated a total of 2 490 genotypes in unreplicated field trials for resistance to CMD in Nigeria in 2001. For DNA isolation, 60-70 samples were processed per person per day using dried leaf tissue and a mini-prep protocol (Dellaporta, 1983). Yield of total DNA was 10–20 µg per 200 mg of leaves, which provides enough DNA for more than 200 PCR reactions. This DNA also stores very well and can be used again at a later time to confirm results. Since this DNA isolation method is not ideal for routine MAS with a very large number of samples, a shorter protocol was tested in subsequent marker evaluations. PCR and polyacrylamide gel analysis were performed on 192 samples per person per day with the marker NS158. Results of the marker analysis and phenotypic evaluation of CMD resistance in the field revealed that the marker NS158 SSR is an excellent prediction tool for CMD resistance in some crosses (a prediction accuracy of 95–96 percent), but not in others. Crosses with TME117 and TMS91934 produced an unusually large number of recombinants (20 percent) that had the marker allele associated with *CMD2,* but which were susceptible in field evaluations. Scrutiny of the marker alleles for the parents revealed an allele from the susceptible parent that had the same size as the allele associated with *CMD2* in the resistant parent. The allele of marker NS158 that is associated with resistance in TME3 and TME9 is 180 bp in size and an allele of similar size also exists in the CMD-susceptible parents TME117 and TM91924. Two other markers tightly linked to *CMD2*, an SSR marker SSRY28 and SCAR marker RME1, were evaluated in the parents, and RME1 was found to have very

low frequency (one out of six) in the susceptible parents. This highlights the need to develop many markers around a gene of interest in a MAS programme and then to use those markers to evaluate the parents and identify the best markers for the different cross combinations.

Through MAS CIAT and IITA preselected 156 genotypes to evaluate in Nigeria between 2004 and 2006 (Okogbenin *et al*., 2007). Of the original group, 14 combined CMD resistance with high yield. This is the first time that a methodology has proven highly effective for introgressing Latin American germplasm to CMD-endemic areas of Africa.. Although large quantities of germplasm have previously been introduced, the susceptibility to CMD has limited breeders' ability to recover useful genes.

The project attempted to analyse costs of introducing MAS into a breeding programme for CMD resistance. Field and laboratory costs were calculated based on an advanced yield trial evaluation at CIAT headquarters in Palmira, Colombia. The costs of molecular marker analysis were based upon current estimates of US$0.30 per data point (CIAT, 2002, unpublished data) for SSR analysis at CIAT's cassava genetics laboratory. For SSR analysis of multiple traits, a multiplex of the markers in the PCR is considered, with an additional cost of US$0.05 for every data point.

Standard breeding costs of establishment, maintenance and evaluation of one hectare of cassava, about US$1 330, at the cassava breeding unit of CIAT were used in calculations (Table 19.2). In assuming a modest sized breeding programme with a seedling trial of 10 000 genotypes per year, MAS will provide savings of US$1 160-US$3 280 per year, depending upon whether one or more traits are targeted (Table 19.1). The cost savings were obtained by calculating the cost of field evaluation with or without MAS, i.e. costs of field evaluation of 2 000 genotypes x 6 plants, minus the cost of molecular analysis of 10 000 genotypes, and field evaluation of a much reduced single row trial (first clonal generation). Using MAS will realize a reduction in field trial size of 50 percent for CMD alone; 75 percent for CMD and CGM together; and 87.5 percent for CMD, CGM and CBB, all combined. Under the conditions operating at CIAT, MAS will provide estimated savings of US$750-US$837 per year, depending on a number of traits evaluated.

7.1.1 Resistance to CMD in Latin American cassava germplasm

Eighteen progenies from TME3, carrying the *CMD2* marker, were established from embryo axes and imported to CIAT from IITA[6]. They were crossed extensively to elite parents of four cassava gene pools defined by adaptation to distinct agro-ecologies: the subhumid lowland tropics, the acid-soil savannahs, mid-altitude valleys and tropical highlands. The CMD-resistant progenies were also crossed to high carotene and high protein content genotypes. Seeds harvested from the crosses were germinated *in vitro* from embryo axes according to standard protocols for cassava (Fregene *et al*., 1998; CIAT, 2002) to permit sharing of the CMD resistant genotypes with collaborators in Africa and India. Each plantlet was multiplied after three to four weeks of growth to obtain three to five plants. After another four weeks, leaves of all plants were removed for molecular analysis and the plants multiplied again to obtain 10-20 plantlets. DNA isolation was by a rapid mini prep method developed for rice (Nobuyuki *et al*., 2000). DNA obtained is sufficient for 100 reactions and can be held in the Costar© plates for two months at -20°C without any degradation.

PCR amplification, PAGE or agarose gel analysis of SSR marker NS158 and RME1 were as described by Mba *et al*. (2001). Gel image from the marker analysis was entered directly into a spreadsheet that contains information on the parents, tissue culture and greenhouse records, and subsequent phenotypic evaluation of the progenies. After molecular analysis, genotypes that carry the marker allele associated with *CMD2* were further multiplied to obtain at least 30 plants. Ten plants were sent to the greenhouse for hardening and later transferred to the breeding programme for routine evaluation. Five plants were kept *in vitro*, while 15 plants were shipped to partners in India and Africa. A flow chart of the different steps described above is shown in Figure 19.3.

[6] Phytosanitary conditions for the exchange of cassava germplasm between Africa and Asia are very stringent, but appropriately-indexed *in vitro* cultures of embryo axes are permitted for experimental purposes.

One person requires approximately eight hours to pick leaves from *in vitro* plantlets, extract DNA and completely fill a 96-well plate. To set up two 96-well PCR reactions and complete the temperature cycling requires four hours for the same person. Running the amplification product on a 6 percent acrylamide gel and silver stain requires another four hours, or alternatively, two hours for agarose gel analysis. In total it takes 23-25 hours, or three working days, for a single person to complete DNA isolation and marker analysis for 192 genotypes. As an example, two persons working on MAS for CMD could process 640 genotypes per week, or over 32 000 samples in a year. At US$0.30 for a single SSR marker data point analysis, processing 32 000 samples in a year requires a budget of US$9 600. This number of samples is far in excess of the capacity of most cassava breeding programmes, in terms of number of genotypes that can be evaluated at the field level.

Table 19.2 Cost estimates of MAS compared with conventional breeding for improving resistance to cassava mosaic disease and other biotic stresses

No. MAS traits	Initial pop.	80% selection	MAS selection pressure	Total Plants to SRT[a]	Area of land required (ha)	Cost SRT[b]	Cost of MAS (US$)	Total Cost (US$)	Savings over conventional (US$)
0	10 000	8 000	1.0	64 000	6.4	8 320	0	8 320	0
1	10 000	8 000	0.5	32 000	3.2	4 160	3 000	7 160	1 160
2	10 000	8 000	0.25	16 000	1.6	2 080	3 500	5 580	2 740
3	10 000	8 000	0.125	8 000	0.8	1 040	4 000	5 040	3 280

[a]*Single row trial*
[b]*Area x US$1 300*

The biggest bottleneck in the process is the isolation of DNA. While methodology improvements continue, currently only 192 samples can be analysed by PCR and polyacrylamide gel electrophoresis in a day. A high throughput method for DNA isolation is clearly needed. Several simple two-step DNA isolation methods have been tested but the most promising is the use of Whatman FTA cards (Whatman Bioscience, United Kingdom) for DNA isolation. It consists of making leaf squashes onto the FTA cards and punching out 1 mm discs followed by washes and direct use in PCR.

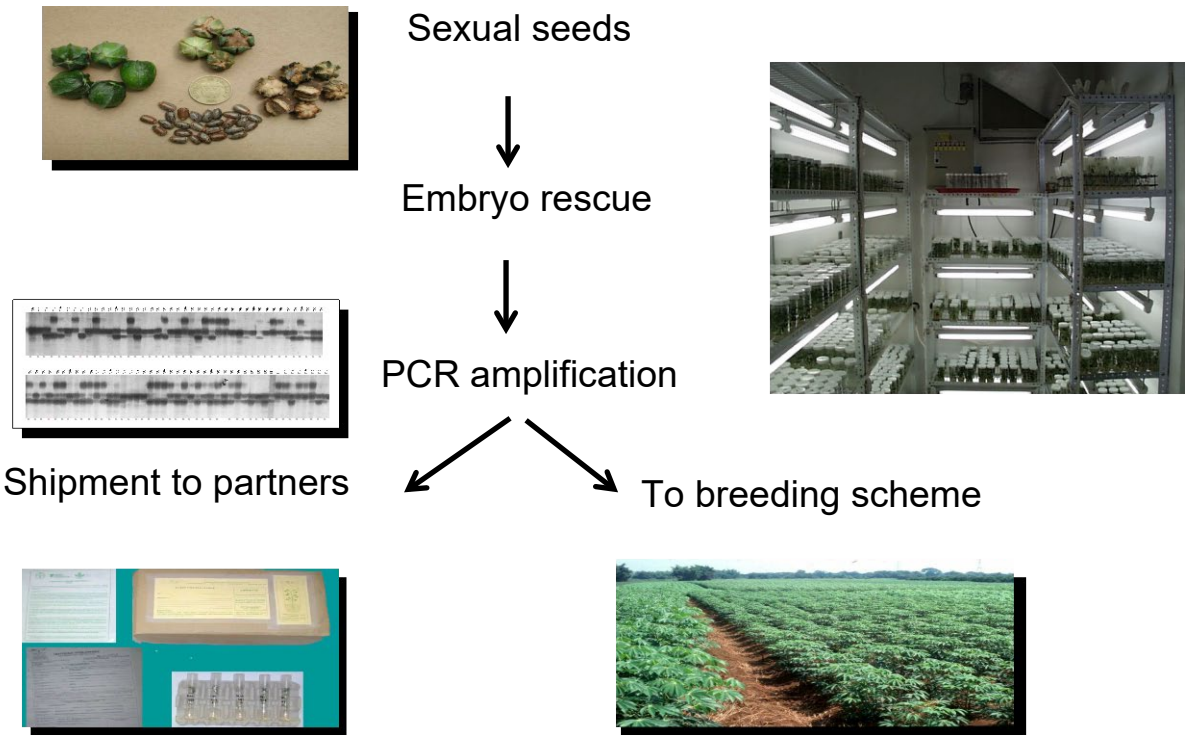
7.1.2 Resistance to CMD and CGM in the United Republic of Tanzania

The United Republic of Tanzania is the fourth largest producer of cassava in Africa with average yields of about 8 tonnes/ha (FAOSTAT). This is somewhat below the continent's average of 10 tonnes/ha, and well below the average yield of 14 tonnes/ha of Africa's (and the world's) largest producer, Nigeria. The low yield is due to many factors, including susceptibility of commonly grown varieties to major diseases and pests such as CMD and the CBSD. Cassava varieties grown by small farmers in the United Republic of Tanzania are, however, very diverse and could be the basis of a successful breeding project. A farmer participatory, molecular marker-assisted, decentralized breeding scheme was proposed as a means to speed up the process of improving local cassava germplasm for resistance to pests and diseases in the United Republic of Tanzania. The project proposes to take farmer-preferred germplasm, stratified by agro-ecology, and cross it to improved introductions that are resistant to CMD and to CGM. The project will also seek molecular markers associated with the resistance genes.

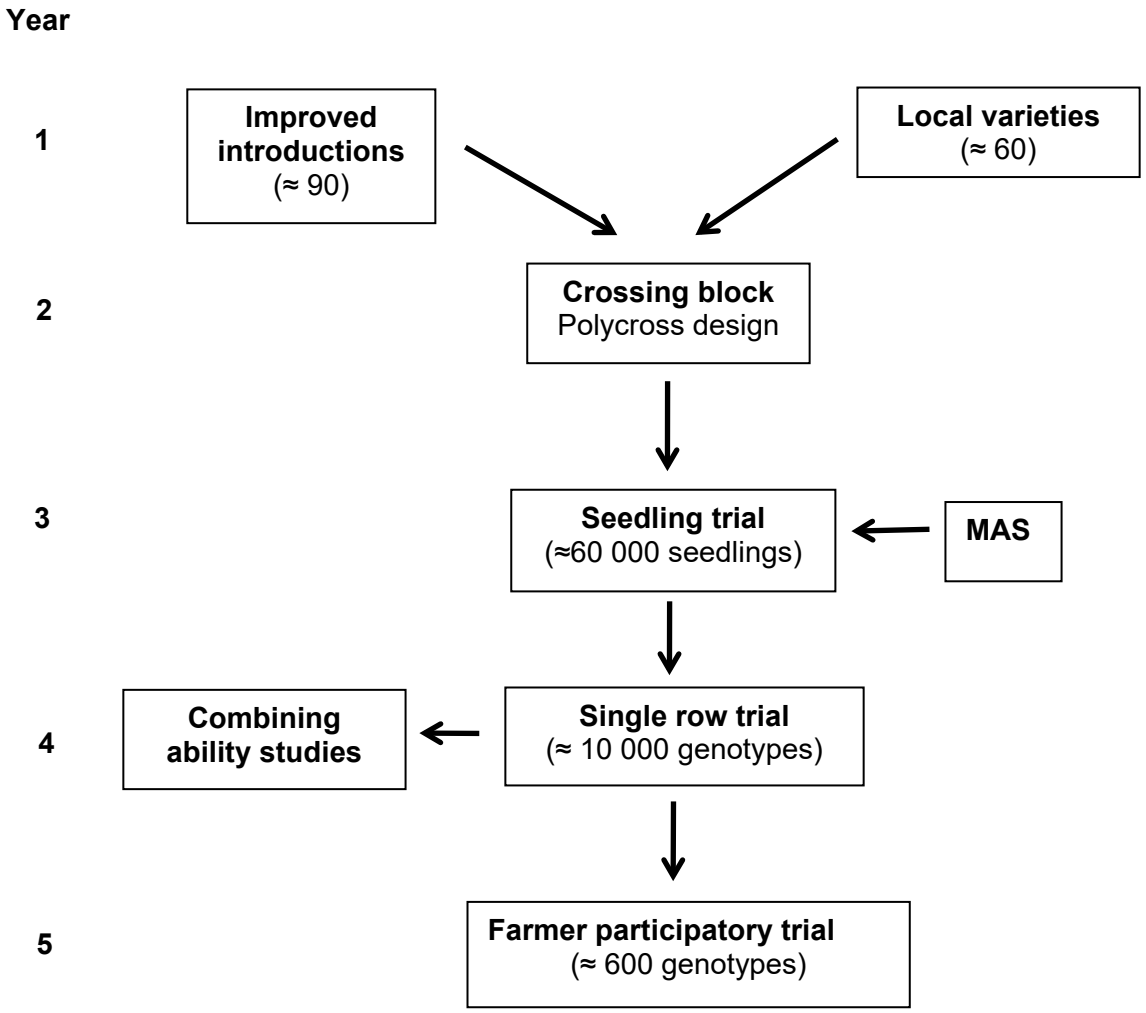
CIAT scientists developed elite parents that combine high levels of resistance to CMD and to CGM using the *CMD 2* genes. Four interspecific hybrid families showed a very high level of resistance to CGM. Bulk segregant analysis, using 500 SSR markers, was used to identify several putative SSR markers associated with resistance. At the same time, selected interspecific hybrids having high CGM

resistance and good storage root formation were crossed to elite parents of CIAT gene pools. The backcross progenies were evaluated for resistance to CGM and also analysed with the SSR markers associated with resistance. Genotypes that showed resistance were crossed extensively to CMD donor parents at CIAT to produce thousands of progenies. All progenies were established from embryo axes as *in vitro* plants to aid shipment to Africa. Molecular markers associated with resistance to CMD and CGM were used to screen and select progenies that combine resistance to CMD and CGM. Resistant plants, a total of 300 genotypes and ten plants per genotype, were shipped to the United Republic of Tanzania as *in vitro* plantlets for use as improved parents. The introduced germplasm was hardened in the greenhouse and then transferred to the field after molecular diagnostics to ensure that they were free of frogskin disease. Thirty of the best genotypes will be crossed to selections from 60 local varieties from three regions where cassava is an important crop. Given the fairly large number of parents to be used, the molecular markers associated with CMD and CGM resistance will be employed to reduce the number of progeny to a manageable number prior to field evaluation. The progeny selected by MAS will be evaluated in a single season in the corresponding agro-ecology and then evaluated over two cycles in collaboration with end users (rural communities and cassava processors). Figure 9.4 illustrates this scheme.

Figure 19.3 Steps employed in utilizing MAS for breeding for resistance to the cassava mosaic disease in Latin America cassava gene pools[a]



[a]*The entire process from sexual seeds to tissue plants for shipment or transfer to the screenhouse takes approximately three months*



7.2 ROOT QUALITY FOR ADDED VALUE

7.2.1 Post-harvest physiological deterioration (PHD)

With the successful development of markers for resistance to CMD and CGM, efforts have turned to development of markers for another major source of loss to cassava production, post-harvest physiological deterioration (PHD). Dramatically delayed PHD was found in *Manihot walkerae*, a wild relative of cassava found in Mexico and the United States (state of Texas). An accession of *M. walkerae* (MWal 001) was crossed extensively to elite cassava varieties. A single successful genotype was found with delayed PHD. The storage roots of the hybrid remained intact a month after harvest. Backcrosses of this hybrid to elite progenitors of the CIAT cassava gene pools and selfed (S_1) populations were made for genetic mapping of the delayed PHD traits. Genetic mapping of the delayed PHD genes is progressing and following identification of genes involved in the regulation of PHD, MAS will be used to combine these genes with progenitors that already have combined CMD and CGM genes. These progenitors, along with low cost marker technologies will be distributed extensively to national programmes in Africa to produce improved varieties that have reduced losses to post-harvest physiological deterioration, CGM and CMD.

7.2.2 Root β-carotene content

CIAT and a number of partners have initiated a project to genetically fortify cassava with the inherent ability to produce higher levels of β-carotene. This is one way of combating the deficiency of this key micronutrient in areas where cassava is a major staple. The experimental approach to increasing cassava

β-carotene content includes conventional breeding and genetic transformation. The discovery of a wide segregation pattern of root colour in two S_1 families from the Colombian landrace MCol 72 (cross code AM 273) and the Thai variety MTAI 8 (AM 320), was the basis for molecular genetic analysis of β-carotene content in cassava. Three markers – NS251, NS980 and SSRY240 – were found to be associated with β-carotene content. NS251 explained 30 percent of phenotypic variation for β-carotene content in the population used for this study. The homozygous state of certain alleles of these markers translates into higher β-carotene content, suggesting that breeding can benefit from molecular markers to assist in combining these favourable alleles in breeding populations. The work is continuing with the search for additional favourable alleles in high β-carotene germplasm to give the best possible phenotypic expression of the trait.

7.2.3 Cyanogenic potential

A collaborative project between the Swedish Agricultural University (SLU), Uppsala, the Medical Biotechnology Laboratories (MBL), Kampala, and CIAT, is aimed at the genetic mapping of cyanogenic potential (CNP) in cassava. An S_1 family, AM 320, derived from the bitter variety MTAI 8, is the basis for the study. This family has been evaluated for cyanogenic glucoside content and has been genotyped with more than 200 diversity array technology (DarT) markers at CAMBIA, Australia, and 100 SSR markers at CIAT. The discovery of molecular markers for CNP will provide a tool to efficiently select for low cyanogenic potential in cassava.

Also ongoing is the genetic mapping of the two cytochrome P450 genes, CYP79D1 and D2, that catalyse the rate-limiting step of the biosynthesis of the cyanogenic glucosides, linamarin in the S_1 family AM 320. The group is also looking for an association with QTLs for CNP. It is expected that markers associated with CNP will be identified at the end of the study.

7.2.4 Root dry matter content (DM)

Few key traits in cassava hold greater potential for increasing cost-effectiveness via MAS, than root DM. This trait is usually measured at the end of the growth cycle. A number of genetic and environmental effects influence DM. It is usually highest before the onset of the rains, but drops after the rains begin as the plant mobilizes starch from the roots for re-growth of leaves (Byrne, 1984). Defoliation from pest and disease attacks can lower DM. Breeding programmes have been quite successful in improving DM, especially for industrial markets.

The entry point for developing markers associated with DM was three diallel experiments carried out from 2000 to 2002. Diallels, in this case made up of 90 families, are an ideal method to identify genes controlling DM that are useful in many genetic backgrounds. Estimates of general and specific combining ability (SCA and GCA, respectively) for many traits of agronomic interest were calculated, with emphasis on DM. Based on GCA estimates, parents were selected to generate larger sized progenies for DM mapping. Sizes of families in the original diallel experiment were about 30 progenies, a rather small size for genetic mapping. Parallel to the development of mapping populations was the search for markers associated with DM using two F_1 families, GM 312 and GM 313, selected from the diallel experiment having parents with high GCA for DM.

Initial marker analysis using BSA led to the discovery of two molecular genetic markers (SSRY160 and SSRY150) which explain about 30 and 18 percent, respectively, of phenotypic variance for DM. These markers are being analysed on approximately 700 genotypes derived from 23 crosses with parents having high GCA for DM in order to confirm their utility across genetic backgrounds. Parallel to this, larger families are being developed from selected parents for QTL mapping of DM.

8. FUTURE DIRECTIONS

As seen from the equation of Lande and Thompson (1990) (commenced earlier) the utility of MAS is highest when molecular markers associated with traits of agronomic interest explain a significant proportion (usually greater than 30 percent) of additive genetic variance and the traits in question have low narrow sense heritabilities. Genetic studies in cassava have revealed that certain traits are controlled

predominantly by additive gene action, for which MAS would lead to rapid genetic gain. These traits include DM, resistance to most pests and diseases, harvest index and foliage weight, while others may be controlled mostly by dominance and epistasis, for example, yield (CIAT 2003). Narrow sense heritability is also low to moderate for the above-mentioned traits in selections based on a single plant, which is the case in the first step of evaluation in cassava – the seedling trial. Development of markers for root quality, resistance to pests and diseases, harvest index and foliage weight should therefore be the most important priorities for MAS.

Given that this list of traits is still rather extensive, further prioritization needs to be made; development of markers is a costly venture if they do not already exist. Top priorities should be given to MAS for the most important pests and diseases prevalent in the region for which durable sources of resistance genes exist. Consideration should also be given to DM, as this is another trait that is significantly affected by non-genetic factors. Although DM normally has a high narrow sense heritability during most of the period near maturity, the onset of the rains creates high phenotypic variability and reduces h^2_n. Selecting for higher and more stable DM after the onset of rains broadens marketing options for growers and may reduce needs for storage of planting material.

There are several initiatives to assist national programmes acquire new molecular tools to increase the cost-effectiveness of breeding. Prominent among these is the African Molecular Marker Network (AMMANET) www.africancrops.net/AMMASSNET.htm funded by the Rockefeller Foundation, and a recent attempt by FAO to assist several African countries acquire know-how in MAS for cassava improvement (FAO project TCP/RAF #). The Generation Challenge Programme of the CGIAR (www.generationcp.org) and the CIAT cassava project (www.ciat.cgiar.org) also have training programmes on molecular breeding that are open to national programme scientists. Research programmes interested in utilizing MAS in cassava breeding can obtain information from these sources.

9. CONCLUSIONS

The role of molecular markers in identifying genes and their locations in the genomes will expand to include the more practical functions of efficiently introgressing these genes into desired backgrounds. As automation of the molecular assays continues to develop, costs will progressively cease to be a critical factor for large-scale applications. In cassava, MAS is already being used to breed for CMD resistance at CIAT in the absence of the disease. MAS is also being used to quickly convert local susceptible genotypes in the United Republic of Tanzanian to CMD- and CGM-resistant ones. The greatest impact of MAS should come from combining useful genes from both cultivated and wild germplasm, in conjunction with field-based selection to produce well-adapted elite varieties that will contribute to the well-being of producers and consumers. Some important considerations for setting up a MAS facility are also discussed.

Chapter 20. Client participation in variety development

1. GENERAL CONCEPTS

Client (technology user) support for plant breeding activities is absolutely necessary for long-term continuity and eventual success. Whether breeding is part of the public or the private sector, growers and consumers ultimately pay for the research. Private, for-profit companies rely on farmers' willingness to pay for superior varieties and public research usually depends on citizens' perception of receiving fair value for their taxes. Farmers are usually the first group of clients who need to evaluate and accept a new variety in order for it to succeed. However, without the acceptance as well of processors and consumers, acceptability in farmers' fields is of little benefit.

Ashby and Lilja (2004) give an excellent overview of the philosophy behind participatory research (PR) in general and specifically participatory plant breeding (PPB). The following introduction draws heavily on that paper. They define participatory research as "approaches that involve clients who are usually not trained researchers, in actively understanding and making decisions on how to conduct research and use its results, together with scientists." In advanced systems of plant breeding, there are generally good lines of communication between producers and scientists. Feedback from markets effectively reaches both breeders and farmers and the characteristics of the target area allow broad adaptation of new varieties.

PPB has evolved to address some of the discrepancies between a breeder's ability to work in advanced systems as compared with those of poor farmers in developing countries. In most cassava production systems, the formalized feedback from farmers to breeders is weak. PPB is most often applied when breeders believe that their most effective strategy will be to aim for relatively specific adaptation. In these situations, farmers are more likely to depend on their own knowledge of local soils, plants, insects and microclimates to choose best varieties.

Participatory research may be divided into what are commonly known as functional and empowering approaches to participation (Ashby and Lilja, 2004). These are in reality different ends of a continuum. The functional approach addresses the objective of improving the efficiency of research processes by involving prospective users of the results. Researchers tend to retain the decision-making power. The empowering approach changes the balance of power in decision-making in the research process – both the end product and how the research is carried out.

Ashby and Lilja (2004) describe five types or modes of participation (not mutually exclusive), depending upon who makes decisions at different stages of the research process. In these definitions, there are two groups of decision-makers*:* scientists (which can include research programmes and extension agencies); and farmers (a category which has actually expanded to include also other intended users of varieties, e.g. consumers, traders and processors).

Conventional: No farmer participation; scientists make the decisions alone without organized communication with farmers.

Consultative: Scientists make decisions alone, but after organized communication with farmers.

Collaborative: Decision-making authority is shared between farmers and scientists based on organized communication and from this, plant breeding decisions are made jointly.

Collegial: Farmers make plant breeding decisions collectively and are in organized communication with scientists.

Farmer experimentation: No scientist participation. Farmers make decisions on how to experiment with and introduce genetic material without organized communication with scientists.

PPB can intervene at any of the many steps of plant breeding: from programme planning, to identifying and collecting basic genetic diversity, to selection in segregating populations, to testing in regional trials, to varietal release and impact assessment. In broad terms, these steps are (Ashby and Lilja, 2004):

- design: setting breeding goals and generating variability;
- testing: narrowing down the variability;
- diffusion: varietal release, demonstration under farmer management and seed production and distribution systems.

When carried out effectively, PPB improves research efficiency and leads to more acceptable varieties. More acceptable varieties in turn accelerate adoption, which should lead to improved livelihoods for farmers.

Every breeder, with a greater or lesser degree of detail, has a concept of the combination of traits necessary for a variety to be accepted. Rarely, however, do breeders fully appreciate all of the details that can influence the farmers' decisions. Systematic feedback from farmers at various stages of breeding goes a long way towards assuring that recommended varieties will be accepted and successful.

Much of the push for participatory breeding across crops, is based on statements such as the following: "The formal crop improvement system has concentrated generally on the increase of yield potential in favourable environments with the use of irrigation and agrochemical inputs. The importance of adaptation to variable and risky and low-input rainfed conditions, secondary crop uses and cultural preferences, have received little or no attention" (Almekinders and Elings, 2001). This will often not hold true for cassava, however. Most programmes, at least in the last 20 years, have concentrated selection under conditions that attempt to approach those of farmers' fields in terms of physical and biological stresses. Nonetheless, farmers were usually not specifically brought into the process of evaluating new materials until after formal release.

Of course, this does not mean that breeders will automatically be cognizant of all the details of farmers' needs, but it does probably take them a good distance toward that goal. On the other hand, the application of participatory research *per se* does not assure that researchers will adequately learn farmers' interests in variety improvement. Successful programmes result from a careful choice of research goals, targeting of environments and selection of user communities (Ashby and Lilja, 2004).

Precisely determining farmers' and consumers' needs is especially difficult for a crop grown over a broad diversity of agro-ecological variations and with different types of markets. Under high input conditions, physical and biological variations over large areas are reduced and one or a few varieties may uniformly suit the needs of farmers. Some markets can accept greater variation than traditional food markets while others may be very stringent. In low-input situations, or where there is a diversity of end uses, any individual cassava variety is likely to be acceptable over a limited area.

It is common for farmers to associate acceptability with certain characters typical of their familiar traditional varieties. Plant type, leaf shape and colour of leaves, stems and roots often seem to play this role. If these traits, which many times have nothing to do with variety performance, are markedly different from what a farmer is accustomed to, he or she may be reluctant to accept a new variety. Given this complexity, a sensible approach to selection seems to be to involve farmers in evaluation relatively early in the variety development process. Farmer participation can greatly reduce the risk of rejection of a proposed new variety for a defect that passed undetected during several years of development.

Rapid rural appraisals can be an excellent starting point to guide decisions on an appropriate research strategy for farmer participation research. Even better is when this can be followed by a detailed, countrywide or regionwide characterization. The COSCA studies in Africa laid the groundwork for sharply focusing farmer participatory research, not only in breeding but other areas as well. For example, these studies demonstrated that, generally, in sub-Saharan Africa, there is a high turnover of varieties, with new materials usually exchanged between villages. Government sources introduce less than 5 percent of new varieties. Working through and enhancing the effectiveness of existing channels to

introduce varieties may therefore be a more productive option. Farmers' reasons for abandoning varieties give clues as to the types of materials to present to farmers for testing: bulking period (late varieties) (20 percent); low yield (16 percent); low ability to compete with weeds (12 percent); root deterioration (10 percent); pest susceptibility (8 percent); difficulty or undesirability for processing (peeling, grating, milling, tasting, etc.) (7 percent); inappropriate branching (too much or too little) (6 percent); high cyanogenic potential (5 percent); poor cooking quality (2 percent); and others (14 percent) (Nweke, 1992).

Obtaining farmer input in the evaluation of cassava varieties is certainly not a new concept. Nearly every breeding programme has some form of direct or indirect farmer input. There are two main problems with the way input from farmers is incorporated into conventional breeding programmes. Firstly, farmer input usually begins only when one or a few varieties have already been identified by the breeder for recommendation, after a lengthy selection process of up to ten years or more. Rejection of a variety at this stage is obviously very serious, because of investment in time and money and because developing suitable alternatives can take equally long. Secondly, there are few examples where the input from farmers is sufficiently standardized to compare their reactions usefully across regions or years.

Farmer participation in variety development can involve intervention at any of a number of entry points in the continuum from basic germplasm management, to parental selection, crossing, selection in preliminary trials, advanced evaluation, multiplication and on-farm trials. Most work has been carried out at the levels of preliminary and advanced selection, which are the levels that are focused on for the remainder of this discussion.

2. METHODOLOGY

Beginning in 1986, CIAT and Colombia's National Research Programme, ICA (Corpoica), worked collaboratively to develop, refine and apply a methodology for utilizing farmers' expertise and knowledge on their variety needs. This is presented here as a working model that can be adopted or modified by national research and extension agencies. The examples used will be mainly from the experience in Brazil and Colombia, but the overall concepts should be applicable anywhere.

From the experience in Colombia it has been observed that one of the most difficult concepts for many scientists to absorb is that the purpose of farmer participation in variety evaluation is not to promote new varieties, but to obtain unbiased opinions upon which to base further recommendations and feedback to the selection programme. Especially in cases where the extension service is heavily involved in these trials, the tendency is to structure farmer interviews like field days where new technology is being promoted rather than evaluated. If optimum feedback is to be obtained, farmers must be made to feel comfortable to evaluate openly and critically all aspects of the experimental material.

Who should consider farmer participation in variety evaluation? Probably almost every cassava breeding programme needs to be involved in farmer feedback, either directly or on a collaborative basis, for their programme to succeed. The form and extent of the breeders' involvement will vary depending upon research structure. Other disciplines or agencies almost certainly need to be involved, but the breeders' participation in the planning and in the feedback is crucial.

A distinction needs to be made between on-farm research and farmer participation in research. Research carried out on farmers' fields need not (and often does not) involve any input from farmers; farmer participation in research may take place in their own fields or at the experiment station. The methodology described here involves participation of farmers in evaluation of cassava varieties in their own or in neighbours' fields. A modified methodology might be applied by inviting farmers to evaluate experiment station trials.

The following sections define a series of basic steps in a tested methodology for participatory cassava breeding.

2.1 TARGET AREA AND PRELIMINARY RESEARCH NEEDS

The target area for development of new varieties will, in a general way, define the area for evaluation of experimental varieties with farmers. However, it is often also desirable to test these varieties outside the range of the target area to observe the limits of adaptation and farmer acceptance. Trials can be distributed throughout the target area according to regional priorities, e.g. a greater concentration of trials in parts of the target area with greater need or where greatest impact is projected.

Much of the literature on participatory research seems to assume that, unless breeders define their goals and methodologies at the outset with a formal participatory methodology, their programme is necessarily misguided and doomed to failure. On the contrary, a skilled breeder will have a good idea of appropriate breeding goals prior to a formal participatory research undertaking, through literature searches, contact with a range of local/regional agencies and personal communication. A first run at diagnosis of research needs precedes farmer participation in variety evaluation by a considerable time period. As a basic foundation to a breeding programme, a diagnosis determines, at a minimum, whether new varieties can potentially fulfil a role in improving the farmers' and/or consumers' livelihoods and the principal traits those varieties should have in order to contribute to that goal.

2.2 PARTICIPATING INSTITUTIONS

For a comprehensive coverage of the target area and to draw on varied expertise and resources, institutions other than the one directly responsible for the breeding programme will need to be involved. Additional support from a combination of research, extension and training agencies, along with private industry, will make a well-rounded effort. Each can add a unique perspective to conducting trials with farmers. Participation of organizations with close linkages to processors and consumers will strengthen validity of the results.

Given probable diverse styles and interests of participants, the coordinator plays a key role. The more extensive the network of trials and the more institutions involved, the more critical becomes the role of the coordinator. There should be formal mechanisms for input by participants, in the form of planning and discussion meetings. Large amounts of data are generated in participatory trials and the proper compilation and analysis need to be carefully coordinated. The breeder need not be the coordinator and possibly it is advantageous that he or she is not, in order that the strategy and results remain free of this possible bias. Naturally, the representatives of any discipline or interest group will introduce their own specific biases as coordinator. Generally, management and analysis benefit from decision-making on the basis of group consensus, but not at a level of detail that would inhibit efficiency of operations. Consensus should be the basis for developing broad strategies, with individuals responsible for implementation.

2.3 PLAN OF ACTION

The main points to consider in developing an institutional plan of action are the specific interests and regions of responsibility of participants and especially of the participating farmers. While a certain standard set of activities for all participants should be agreed upon, flexibility for institutions to obtain additional information for their own specific interests should be encouraged, as long as it does not jeopardize achieving the goals of participatory research. Adequate coverage of the target region and of the target socio-economic groups are the main criteria.

Institutional responsibilities should normally be divided primarily on a geographical rather than a disciplinary basis. That is, a given institution should take responsibility for complete management of a set of trials in a region rather than various institutions dividing responsibility for distinct activities within trials. The latter option could cause confusion for the farmer as well as for the researchers, with too many people involved in overseeing any given trial.

One option that seems to work well is to have each institution propose how many trials and in which regions, it is willing and able to supervise. From this beginning point, duplicated efforts are corrected,

or gaps filled. It may not always be possible, with available resources, to achieve optimum geographic or socio-economic coverage. The number of trials an individual can manage varies widely depending on time available, access to transportation, accessibility of the sites and others. As a rough guideline, a researcher with 10–15 percent of his or her time available for this activity should be able to supervise between five and seven sites, if no site is more than a few hours from the work base.

2.4 FINANCING

Covering the costs of this fairly extensive research effort will need to be planned from the start. Many entities will be involved and some sharing of costs can certainly be achieved. In most situations, the costs of planting, maintaining and harvesting the trials can be borne by the farmers themselves, as they will benefit from the possibility of obtaining new technology. They can also be given the product of the harvest for their use or sale. If evaluation is undertaken under the same conditions as the farmer normally employs, there is little additional cost as compared with his commercial crop (except possibly for underutilization of the land from use of alleyways). Principal costs are for travel of participating scientists for on-farm evaluations. As much as possible, institutions having personnel within close proximity of the trials, or their own funding to travel, should be given some priority for participation. Alternatively, an overall project budget can be developed and proposed to a donor agency for financing. In this case, a minimum three-year project period should be contemplated.

2.5 IDENTIFYING PARTICIPATING FARMERS

What incentive does the farmer have to participate? Most farmers are naturally interested in testing new technology they believe can potentially lead to higher income or better food security for their family. However, the incentive should be from the possibility of higher income that could result from later adoption of a new variety and not from paying the farmer to participate, either directly or indirectly. Limiting the sample to farmers who want to participate because of inherent interest in new technology rather than those who will accept such a trial when paid, will generally upgrade the quality of the data obtained.

Defining prototype farmers may not be very difficult on paper, but actually identifying them in the field can be more challenging. These trials are of necessity limited to farmers interested in trying new cassava varieties. They should have some status in the community so that other farmers will willingly come to the farm to observe and evaluate the trial. They should use good agronomic practices, but not those considered out of reach for most farmers in the region. Most should be cassava farmers with considerable experience in growing the crop and in dealing with the market. They must be willing to give some of their time to interviews with researchers, as trials are only useful when the farmer is an integral part of the evaluation process. Finally, they must be willing to take a small amount of risk, in giving up some of their land to grow varieties that may or may not be better than what they already have.

In most areas, extension service personnel or others from local organizations are already aware of farmers who could meet most of these criteria and they should be the first contacts. However, extension agents may be more accustomed to working with the larger, more progressive farmers, who may not be the appropriate people to evaluate technology designed for poor cassava farmers.

2.6 A GLOSSARY OF TERMS

Farmers and scientists do not necessarily use the same terms to describe identical aspects of cassava production and products. Likewise, terminology among farmers is highly regionalized. Prior to an analysis of results of interviews, it is useful to develop a glossary in order to have a clear understanding of what particular terms mean and to combine responses for terms that are synonyms.

It should be possible to make a preliminary glossary prior to the first interviews for evaluating experimental varieties. This can be done simply by asking a sample of farmers in the region of interest how they would describe various aspects of cassava production and the cassava plant parts. For example: How is a highly branched plant described? How is pest damage quantified? What are the terms used to

describe low, intermediate or high yield? How is quality evaluated? Extension workers in a region are also often a good resource in helping interpret some of the farmer terminology that may be unfamiliar to the researchers. Undoubtedly, new terms will continue to arise in later evaluations, but this initial activity can clarify many doubts and make the later interviews more productive.

2.7 DIALOGUE WITH FARMERS ON OBJECTIVES AND METHODOLOGY

Shortly before planting and preferably before land preparation, researchers meet with participating farmers to discuss objectives and methodology. Discussion of methodology at this stage should emphasize the early season activities: land preparation, trial design, planting, fertilization and weeding.

Evaluation of new varieties is probably most valid when undertaken under the conditions in which farmers normally practise, or with modest modifications that have already been tested and accepted by farmers. Following this practice will assure farmers that any superior performing varieties are not dependent on a technology they will find difficult to adopt.

Experimental plots should ideally be planted within a commercial cassava plantation, with soil preparation and crop management being the same as the practices of surrounding fields. Some farmers may find it difficult to follow these suggestions and will want to provide either more or less care of the experimental materials, depending upon his or her attitude. During initial interviews, these types of strong farmer biases may be determined and such farmers discarded as participants.

At every opportunity, researchers should reiterate with farmers that the objective of the trial is to obtain their evaluation of the materials and not to promote any particular technology. If the participating farmers are close to an experiment station or other area where the breeding programme is underway, they may be invited for a visit, with an overview of how the breeding programme works and an explanation of the importance of their participation in the process. Likewise, scientists should take opportunities to visit farmers' fields prior to the establishment of any experiments, both as a matter of becoming socially acquainted with potential trial participants and to gain further understanding of farmers' attitudes and practices.

2.8 TRIAL DESIGN

The type of trial design for appropriate evaluation of varieties by farmers is not fixed and in fact there is rather limited experience to suggest the best alternatives. Design may be as much influenced by management consideration as by statistical rigour. The basic questions of design are: how many trials, how many and which materials, plot size and number of replications?

A first question concerns how many materials to present to farmers for evaluation. Again, the distinction needs to be drawn between the objectives of participatory research and the objective of promoting new varieties for farmer use. In the latter case, only one or a few highly selected materials are offered, for comparison to a local clone. In participatory research, the scientist wishes to have farmers' opinions on a broad range of traits and this is possible only if several experimental varieties are included. On the other hand, it is not necessary to include materials with traits that the researcher knows from previous experience will be rejected out of hand.

Thus, the maximum number will be fixed either by the number that farmers can comfortably compare among each other (and fit within possible space limitations) or the number of suitable new clones the breeder has to offer. Experience in Colombia demonstrated that farmers can make comparisons without undue difficulty among about ten different genotypes. This number allows most programmes to include materials at intermediate stages of selection. If only two or three materials are offered, there is a good chance that there will be insufficient variation for many traits and it will be difficult to make solid conclusions about farmers' preferences or the strength of those preferences.

The decision of which materials to evaluate should be based on group consensus by the institutional participants. However, the breeder is likely to have the most intimate knowledge about the materials and

will usually be in the best position to make concrete recommendations. Other participants will usually have requests for materials with specific traits.

One limitation often encountered at the intermediate selection stages is availability of planting material, especially when a large number of trials is anticipated. Although there are considerable advantages to uniform trials in a region (same materials tested in all sites), some materials can be included on a preliminary basis in fewer trials when planting material is limiting.

The best information is obtained when there is wide variation for those characters for which the breeder is least certain about the farmers' reactions. For traits where the breeder feels most confident, it is nevertheless useful to include one or two clones showing a less desirable trait expression. As examples, the breeder may not have a clear idea of how important branching habit is. If only very erect plant types are included, it will not be possible to obtain any weighting of this factor by farmers; therefore, the full range of branching types should be included. If it is well-known that yellow root flesh is unacceptable in the markets of the region, it may be useful to include one or two yellow-fleshed types to clarify the importance relative to other traits. In any case, when eight or ten clones are included, there is usually considerable variation for many traits, allowing good opportunity for farmers to compare and react.

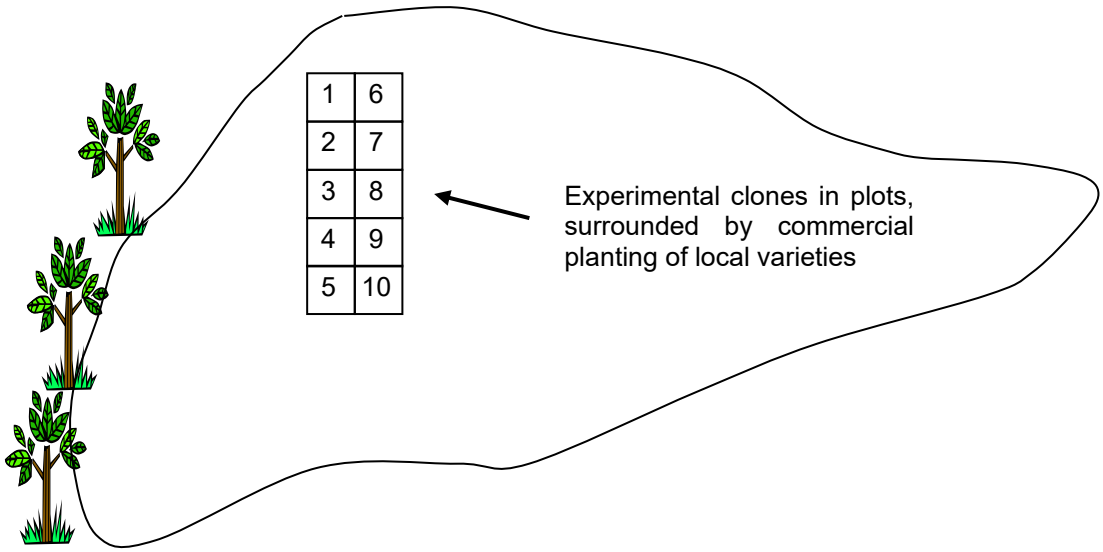
The source of planting material can have dramatic effects on varietal performance in cassava, resulting from a combination of biological and physical factors. These factors should not be confounded with genetic effects when evaluating materials with farmers. This will mean, to the extent possible, to have a uniform source of planting material, including the local check variety. Production of planting material in the target region, under controlled conditions, is the preferred option. To achieve this will probably require considerable planning ahead if the main experiment station where materials are maintained is not within the region. Having all materials centrally multiplied within the target region greatly facilitates preparation and distribution at planting time.

A minimum of ten sites is suggested to sample the agro-ecological variation of the region; several farmers' opinions can be elicited at each trial, thereby multiplying the information obtained. If resources are available, certainly more reliable information can be obtained with a larger number of trials, perhaps with 20 or 25 as an optimal number, repeated over at least two and preferably three years. Plots should be small, easily managed and not placing a burden on the farmer. However, they should be bordered plots, in order to eliminate intergenotypic competition effects. As materials may enter these trials from the intermediate stages of selection, availability of planting material is likely to be a limitation on number of sites and plot size. Plots of 20–25 plants are a reasonable minimum size. For greatest simplicity, there may be a single replication at each site. This limits the sophistication of any statistical analyses, but may be the best option in a trade-off between more sites with no replication and fewer sites with replications. CIAT's experience showed that single replications in each of many sites, repeated across years, give some very clear conclusions on materials being evaluated.

2.9 PLANTING

The farmer is the central figure in field activities. The researchers’ intervention at this stage is merely to assure that experimental materials are properly identified in the field and to indicate some of the basic requirements for planting design. The farmer and researcher together should survey the field plot to determine the most suitable location for the trial. A plot in the middle of a commercial cassava field is ideal (Figure 20.1). Usually the trial should have a nearly square configuration, as opposed to long and narrow, unless the latter design will alleviate effects of some known and apparent gradient within the field.

Figure 20.1 Principles of trial design for farmer participation in variety development



- Plots are small: 20-50 plants
- Farmers participate in all phases of planning and execution of trial
- Located within a fully farmer-managed field
- Similar management as surrounding commercial plantations (e.g. land preparation, spacing, planting date, weed control). Vary only what is being tested (varieties)
- Include the local variety within the test plots, from the same source as surrounding field
- Label plots for easy identification by any person

The farmer's normal planting density should be used and plots marked out before planting, with stakes, string or other clear markers. The farmer should personally plant in the customary manner, in terms of spacing, stake position, depth and other planting practices.

Usually the experimental materials have breeder's codes for identification and these may be complicated and confusing for the farmer to manage. Usually it is best to simply have the farmers identify the materials with a plot number and the researcher can maintain the complete identification in fieldbooks. Experience shows that farmers will often assign their own names to materials they like.

Again, at planting time, the scientist should repeat the objectives of the trial to the farmer and the importance of the farmer managing this trial as is the norm for his or her commercial plantings.

2.10 GUIDELINES FOR EFFECTIVE INTERVIEWS

Participating farmers and technicians must understand that the technology being presented is for the purpose of eliciting their opinions and not for promoting a new variety. Farmers are being asked to try some experimental varieties, to compare them with their favourite local varieties and to provide honest judgments on both positive and negative aspects of each one. Perhaps more important to the breeder, the interviews should provide detailed information on the characteristics that farmers require in new varieties, so that future selection can be fine-tuned to growers' needs.

The interviewer not only needs to explain this objective at the outset, but needs to follow an interview methodology that does not bias the farmer's response. Questions such as, "Does this not appear to be higher yielding than your variety?" may indicate to the farmer, firstly, that the interviewer believes yield to be important, and secondly, the farmer may be influenced to believe that this is the expected answer and thus lead the farmer to give an affirmative response.

How then does one introduce a minimum of bias (because it probably cannot be entirely eliminated)? Experts have suggested that an open interview is the most appropriate. In an open interview, the researcher's main role is to note farmers' spontaneous comments as they observe various aspects of each individual experimental variety. The interviewer may prompt comments about a certain aspect of a variety's behaviour, but without leading the response in any particular direction.

If the scientist can adjust his or her mentality to consider the farmer not only as a potential client of new technology, but as a partner in the process of evaluating that technology, then many of the procedures for participatory research fall easily into place. The expectation of a mutual learning experience between farmers and scientists is a prerequisite for obtaining best results. This may not happen easily with either of the parties involved, being inhibited by a long history of a traditional mind-set about what farmers and scientists may or may not know.

A complete treatment of the art and science of interacting with farmers for technology assessment is beyond the scope of this chapter; however, ten summarized guidelines suggested by Ashby (1990) are especially useful. The full document is highly recommended for an in-depth understanding of the subject.

Remember that the technical evaluation of a proposed innovation is quite different from its evaluation with a farmer, namely:

- researchers need to ensure that the obligations of everyone involved (researchers, extensionists, farmers) and what they hope to get out of the evaluations are explicitly stated and understood;
- the need to establish with farmers (not just once, but repeatedly) the research staff's neutrality and objectivity with respect to the success of a technology;
- the farmer should be treated as an expert;
- it is asked "For whom is the technology being evaluated?"
- courtesy and respect should be shown to farmers;
- farmers should be listened to;
- ensure farmers' reasons are understood well in an evaluation;
- check and recheck interpretation of farmers' preferences;
- ensure that scope exists for farmers to take the initiative in setting up and carrying out evaluations of technology.

In addition, Ashby (1990) provides a useful check-list of things to avoid in farmer evaluations:

DO NOT carry out a technical evaluation at the same time as the farmer is making his or her evaluation;

DO NOT start an evaluation without explaining the objectives and clarifying mutual expectations;

DO NOT be a technology salesperson; do not teach or make recommendations in an evaluation;

DO NOT evaluate technology with farmers who are unlikely to be future users, or have no relevant experience;

DO NOT impose your own criteria for evaluation on the farmer; do not criticize his or her criteria; do not argue with or contradict the farmer;

DO NOT be discourteous by rejecting hospitality or devaluing the farmer's time; do not oblige busy farmers to carry out evaluations when inconvenient;

DO NOT interrupt or hurry the farmer in an evaluation; do not allow asking questions to take up more of your attention than listening;

DO NOT leave an evaluation with a description of a technology by a farmer instead of his or her reasons for preferring specific features, or one alternative over another;
DO NOT interpret farmers' opinions and preferences without verifying your interpretation;
DO NOT stifle farmers' initiative and creativity by rigidly controlling what technology to evaluate, or when, where and how to carry out evaluations.

2.11 INTERVIEW TECHNIQUES

The basis for farmer evaluation of new technology needs to be an openness on his or her part to share both positive and negative perceptions, without adopting any bias of the interviewer. Bias may be introduced directly by a researcher soliciting opinions on specific points, or in subtle ways by the researcher's body language and intonation. This does not mean that only trained sociologists should undertake interviews of farmer; the attitude of interviewers is as important as their skill and experience at interviewing. It is this attitude that will guide which questions are posed and in what manner.

The CIAT-ICA experience in cassava variety evaluation led participants to build further upon the recommendations of Ashby (1990) to broaden the information obtained. Having a large number of trials in diverse environments presents an excellent opportunity not only to obtain farmers' spontaneous reaction but also to study how that relates to the observations of the breeder and other technicians on the same trials. The CIAT breeding group proposed a two-phase interview procedure. In the first stage, the interviewer takes note only of spontaneous observations and the clarification and amplification of these, as the farmer observes one variety at a time. After all materials are observed and notes taken, farmer and researcher return to the first plot and the researcher asks directed questions about those traits of his interest that did not come out in the spontaneous remarks. The two types of response are distinguished on the fieldbook forms by noting a code for each (for example s = spontaneous and d = directed) (see following section on fieldbook design). The use of this distinction in data analysis is described later.

2.12 FIELDBOOK AND QUESTIONNAIRE DESIGN

There is no universal best design for fieldbooks or questionnaires for farmer evaluation trials. The model presented here is based on several years of trials in Colombia (Figures 20.2a and 20.2b). It is comprehensive, flexible and easily managed by technicians at all levels of experience. The basic format for these forms is the notation of two types of information from the farmer. Firstly, is the response by the farmer spontaneous (S) or is it the result of a directed question from a scientist (D)? The S or D is noted in columns 11, 15, 19, etc. of the forms shown in Figure 20.2. The trait itself (e.g. sprouting, early vigour, root yield, etc.) is evaluated only on the basis of whether the farmer gives negative, neutral or positive comments (noted as -, -/+, +, respectively, in the fieldbook). Procedures for evaluation and analysis are described in further detail in later sections.

In addition to these forms designed for specific use in participatory trials, one can use the same fieldbook formats as those used in the breeder-managed trials (see, for example, Figures 8.3a to 8.3e).

This particular model includes components for both researcher and farmer evaluation.

2.13 TRAINING OF PARTICIPANTS

The coordinator of the on-farm trials will have the responsibility of organizing training sessions for those scientists involved for the first time, or in updating participants on new and modified procedures. An inexperienced interviewer should always be accompanied by one with experience.

2.14 PERIODIC EVALUATIONS

Throughout the growing season a range of factors impinges upon the farmer's perception of a variety's acceptability. Ability to compete with weeds, suitability for intercropping, influence of architecture on field operations (e.g. weeding, chemical applications), pest and disease incidence and possibly others may be noted by the farmer during the growing season. For this reason, it is highly desirable for the researcher to make a minimum of two or three visits to trials at critical periods during the growing season. In addition to obtaining timely information, these interviews can serve to condition the farmer

to the type of interaction that is anticipated, thereby making the critical evaluations at harvest conform better to expectations.

2.15 ORGANIZING OF THE HARVEST

The main focus of farmer-researcher interaction is the harvest, when the full range of varietal traits come together in the yield and quality of the roots and planting material. Consequently, the effective organization of the harvest is critical for the results to have appropriate interpretation. A scientist trained and experienced in all the techniques should manage each harvest.

More than one harvest may be planned if one of the objectives of the breeding programme is early maturity. In such a case, an early harvest and a standard harvest, using a split-plot design, can be used. The harvest date should be planned in advance with the farmer and other participants, with the farmer's preference given strong weighting.

Other participants should be limited to those who can contribute to the information to be obtained, or who can use the experience as a training exercise. Inviting a large number of farmers to a typical field day/demonstration type of activity is strongly discouraged. A group that is small enough to interchange opinions and come to a consensus on the effective evaluation of traits is needed. There is no fixed optimum or maximum number, but usually more than eight or ten farmers are difficult to manage appropriately. If, as is possible, many farmers show up simply out of their own interest (as opposed to being invited), they might be organized into groups with distinct activities.

The typical sequence of harvest activities will vary from one region to another. Local practices may be followed, or it may be advantageous to modify these for purposes of optimizing information. Each activity should be accompanied by an interviewer so that any pertinent opinions can be recorded. This will begin, in most cases, with entry into the plot for uprooting. Reaction, if any, should be noted, for example with regard to difficulty of access to the plants (results of plant vigour and branching habit), quality of planting material and pest and disease incidence. Uprooting plants may elicit comments on difficulty of harvest, size and shape, external colour and others.

It is useful to work through the harvest of each plot individually, noting the spontaneous remarks of farmers and eliciting their further elaboration on these remarks without introducing any of the researcher’s biases. When this is completed for all plots, sequentially work through each plot again, this time asking direct questions to fill in gaps in information that did not come out in the spontaneous remarks. The questions to be asked can be from a list of criteria previously established as those being of some importance to farmers in the region or they may be of interest strictly to the breeder.

Before proceeding towards the final comparison and evaluation of materials, it is useful to have an assessment of root quality. After all the information is gathered within each plot, sample roots are taken for quality evaluation. So as not to damage plants for further evaluation, this sample may be taken from border plants. Farmers almost always have ways of quickly assessing quality of roots for their markets. If the market is for fresh consumption, roots should be evaluated directly on site by boiling and soliciting evaluation from farm family members. If the normal market is for processed cassava, there are a few possibilities to obtain evaluations of quality. This preliminary evaluation may be undertaken at the time of harvest and taken into account at the time of final ranking of varieties. Processing a sample of roots gives a more quantitative evaluation, but this is usually not possible given the small number of roots available relative to processing procedures. Brazilian scientists use small farinha makers for the purpose of determining quality of roots in experimental plots (W. Fukuda, personal communication).

After all plots are harvested, intact plants (roots still attached) from each plot are removed and lined up side by side in a clearing where they can all be observed easily and simultaneously. There are two principal objectives to this exercise: to rank the materials from best to worst and to assign to each a subjective rating of good, intermediate or poor in acceptability.

Experience has shown that possibly the easiest manner for farmers to rank experimental varieties is to place them physically in their order of preference, integrating all the positive and negative attributes into a simple one-on-one comparison of better and worse. This will probably be an iterative process of judging, making a preliminary ranking, a re-evaluation and adjustment to determine final ranking. Results are noted in the fieldbook. At this stage it is useful to question farmers on the reasons for their ranking, the principal positive or negative factors that influenced their placing of adjacent clones one above the other.

The ranking does not indicate precisely whether the farmer considers a clone to be good, intermediate or poor, nor can it be assumed that the materials will be equally divided among the three categories. Conceivably, considering two extreme possibilities, all the materials could be considered good or all poor. More likely, however, is that the clones will cover the range from good to poor and farmers should specifically be asked to rate each clone as good, intermediate or poor, after ranking is completed.

3. RESEARCHER EVALUATIONS

To date, obtaining farmers' evaluations has been focused on. Not only researchers, but also extension organizations are also likely to want more quantitative information from these trials. This can serve two basic functions: to provide the more traditional type of support data that may be required for varietal release and promotion; and to provide a possibility for relating researcher and farmer evaluations. Such comparisons will show how well researchers' evaluations conform to the criteria farmers apply in selection. Although different terminology and methodology may be used, the researchers' evaluations should be predictive of farmers' reactions. If they are not, adjustments need to be made. In the process of making these adjustments, the researchers will increasingly approach more closely the farmers' true needs for new varieties.

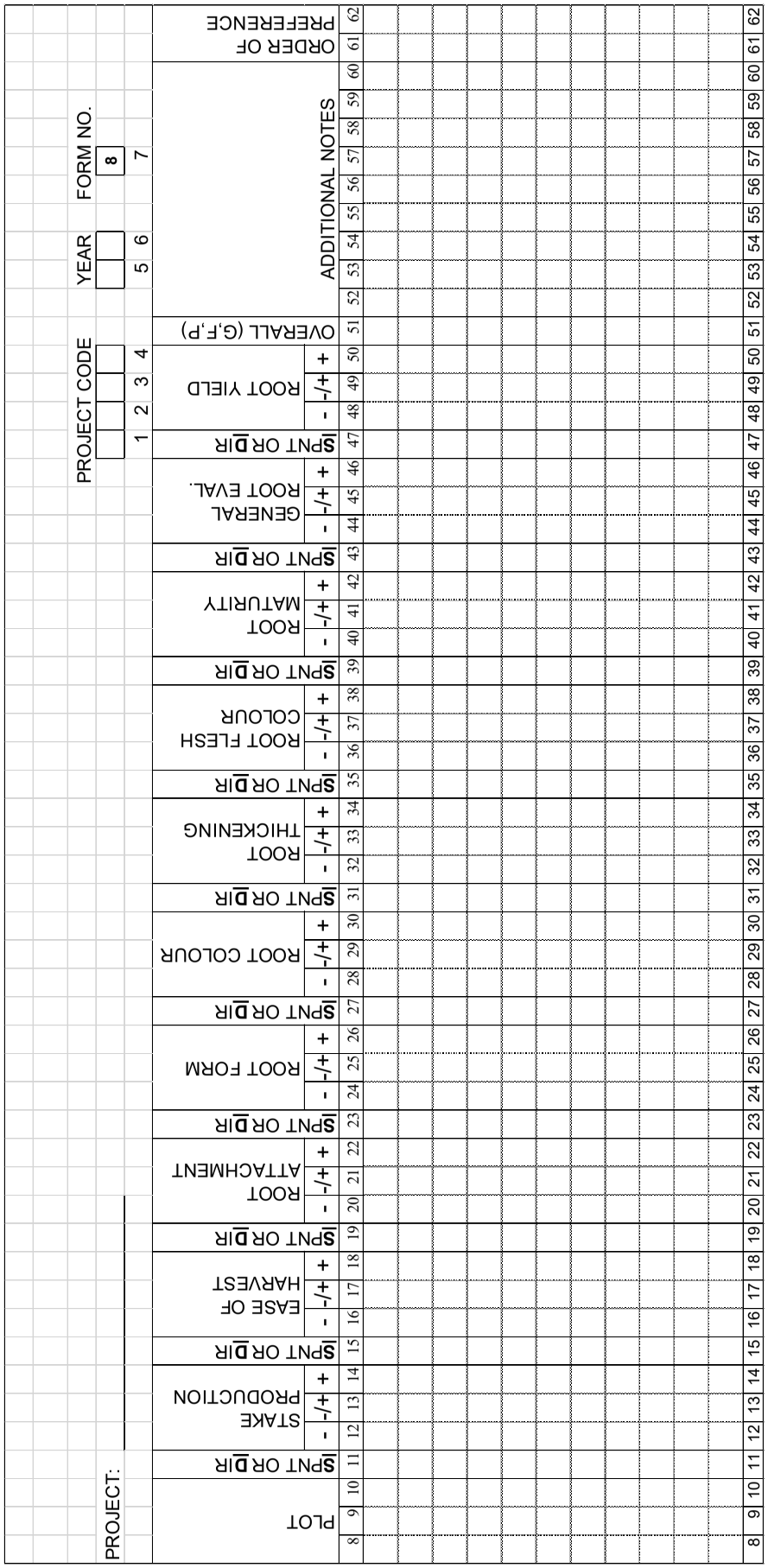
Figure 20.2a Farmer participatory trials – Form for pre-harvest evaluations

PROJECT: ______________________

PROJECT CODE: [ ] [ ] [ ] [ ] (1 2 3 4)   YEAR: [ ] [ ] (5 6)   FORM NO.: [7] (7)

PLOT			SPNT OR DIR	SPROUTING			SPNT OR DIR	EARLY VIGOR			SPNT OR DIR	PEST OR DISEASE 1			SPNT OR DIR	PEST OR DISEASE 2			SPNT OR DIR	PEST OR DISEASE 3			SPNT OR DIR	PEST OR DISEASE 4			SPNT OR DIR	PLANT TYPE			SPNT OR DIR	BRANCHING HABIT			SPNT OR DIR	GENERAL PLANT EVAL.			SPNT OR DIR				SPNT OR DIR				SPNT OR DIR				SPNT OR DIR			
			S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+	S	-	-/+	+
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62

Figure 20.2b Farmer participatory trials – Form for harvest and post-harvest evaluations



4. DATA ANALYSIS

Analysis and interpretation of some of the semi-quantitative and qualitative information in farmer participatory research can be difficult. Standard statistical tools may prove highly inadequate. Even where very strong conclusions might be drawn by a common-sense reading of results, the researcher may be hard-pressed to apply any meaningful statistical analysis. From the Colombian experience, some of the simple and useful means of summarizing and preliminary analysis of data are discussed. In this treatment, emphasis is given to relatively simple analyses that can be carried out either by hand or with just a desk calculator, but would also be facilitated by a computer, if it were available.

The first step to interpretation will probably be to standardize the information as much as possible across trials, e.g. application of common units of measure and common terminology where possible and appropriate. The next step is the aggregation of data. This should be carried out at various levels, e.g. by varieties, or by agro-ecologically defined subregions.

A simple, quick way to get to the core of farmers' opinions is to sum the ranks of each clone for each trial. There may be considerable consistency among locations in farmers' rankings for a given set of clones. Results may clearly indicate that a given clone or a few clones are uniformly highly ranked by farmers and others uniformly poorly ranked. Usually the middle-ranking clones are more likely to be most variable in acceptability. Repeated favourable ranking of a clone across two or three years, by a representative group of farmers, is pretty reliable evidence to suggest good commercial acceptability.

This alone, however, does not provide much insight into which characters are especially important for the farmer. The next step is to try to relate the variation in observed traits to variation in farmer acceptance, the essence of the feedback function for these trials. Any type of quantitative analysis requires converting subjective evaluations to some form of quantitative measure. For many characters this can be done by assigning a scale of one to three, or one to five, describing variations from good to poor in the farmers' perceptions. Most breeders are quite familiar with the use of these types of scales, especially in evaluating for disease and insect resistance and would have little difficulty with the concept or its application to other traits.

The distinction between spontaneous remarks by farmers and solicited responses resulting from directed questions was discussed earlier. For each character evaluated, these responses (both spontaneous and solicited) are classified as: very good, good, intermediate, poor and very poor. Spontaneous remarks can be weighted more heavily than solicited ones, for the same classification. Though this may not always be valid, a trait that elicits a spontaneous reaction from a farmer normally deserves a heavier weighting, either positively or negatively, than one elicited through direct questioning. Thus, a scale of positive five to negative five is derived in the following manner: 5 = very good in a spontaneous response; 4 = very good in a solicited response; 3 = good in a spontaneous response, etc. On the negative side, higher negative weight is also given to spontaneous responses, so that -5 = very poor in a spontaneous response; -4 = very poor in a solicited response; and so on.

Trends in the data may be so obvious that essentially no analysis is required; if all yellow-fleshed clones are accepted and all white-fleshed clones strongly rejected, accompanied by farmers' comments on this trait, one could probably feel pretty confident in making a recommendation to research, not to bother with white-fleshed clones in that region. Results are rarely that straightforward. The subtler preferences are often hard to detect or to assign weights in selection. Each clone will have a combination of positive and negative traits, whose combination determines an overall evaluation by the farmer. Multiple regression analysis can determine importance of traits, based on the evaluation scale of as many traits as desired, as the independent variables and the overall rating of acceptability as the dependent variable. The interpretation will depend on having an adequate range of variation in traits of importance within the sample. Again, to use the example of root flesh colour: this trait may be extremely important (and in most situations is), but if the varieties tested are uniform, this will not emerge with any weighting in a multiple regression analysis.

5. FEEDBACK TO RESEARCH

If results from farmer evaluations confirm the directions already taken by a breeding programme, no adjustment in methodology is needed. If inconsistencies between breeder objectives and farmer expectations are encountered, a process of re-evaluation of the programme needs to take place. Given the long-term nature of plant breeding, every breeder wants to be quite certain of the validity of results before making major adjustments. If results consistently and strongly demonstrate that farmers want something other than what a breeding programme is producing, breeders are advised to take note, even if it runs strongly counter to their pre-conceived notions.

Mahungu (1999) notes an example where there seemed to be a clear reason for researchers to discourage farmers from selecting a particular variety: ". . . farmers selected a locally improved variety (*Kiryunikwe 13*) which was very susceptible to CMD [cassava mosaic disease], but with good root yield. Researchers had to explain to farmers why such a variety could not be selected, as it may play the role of CMD spreader in farmers' fields. Thus, in participatory research, there is always a need for varieties selected to match both farmers' and researchers' objectives."

The methodology is not without certain pitfalls. Farmers will normally be weighting criteria on the basis of their current situation, growing conditions, cultural practices and markets. While obviously these needs must be met, the researcher must also project well into the future and anticipate changing needs. Aligning a research programme solely along the lines of farmer criteria could entail an excessively conservative approach to varietal development.

6. VARIETAL ADOPTION AND DISTRIBUTION

Participatory research has the effect of exposing a large number of farmers to experimental materials. At most, it is probable that only a few of these would ever reach the stage of later formal release. It provides a chance for farmers to experiment with and later adopt unreleased materials, essentially outside any organizational control. Whether this is seen as positive or negative depends on the philosophy of varietal release of the national programme. Some programmes are very sensitive to the unauthorized and uncontrolled distribution and adoption of new varieties. Others, recognizing the deficiencies of existing mechanisms could see it as a low cost and effective way of enhancing the normal farmer-to-farmer channels for distribution. Each research and extension system needs to confront these issues individually.

In the north coast region of Colombia, where this approach to farmer evaluation has been ongoing since 1986, there is notable continued testing and adoption beyond the controlled trials. National and regional research and extension personnel generally see this as complementary to official efforts to promote new varieties, rather than it having a negative impact. There is insufficient experience to suggest any typical patterns of adoption that might emerge from this unconventional form of cassava varietal evaluation. The advantages in terms of making selection more effective seem clear in the limited experiences to date, but the implications for adoption and distribution depend on specific policies and strategies of individual programmes.

7. PARTICIPATORY RESEARCH IN PERSPECTIVE

Work on participatory research has brought social scientists into cassava breeding to a greater extent than at any time in the past, or on any other major research initiative. The results, while on balance are positive, have been mixed. Without a doubt, breeders have gained greater appreciation of the criteria farmers apply to choosing varieties. However, there has been a tendency for social scientists to devalue the expertise that breeders often bring to the table, in their ability to elicit farmers' needs outside the more formal procedures of participatory research. Social scientists have also sometimes underappreciated the value of breeders' formal training and have suggested that farmers can readily,

with minimal training, take over some of the complex tasks that breeders carry out. For example, Saad *et al*. (2001) presented results of a workshop in which farmers were "trained" to make crosses, manage the resulting seeds and develop a selection programme to produce new varieties. The results are partially described as, "In spite of being new, the concepts were not too complex for participant farmers as some of our colleagues had warned. As a result of the workshop, the participants can now implement a full cassava breeding cycle understanding phenotype, genotype, dominant and recessive traits, variability and segregation" (Saad *et al*., 2001). While the authors make a point that farmer skill-building in cassava breeding enhances "farmers' skills, knowledge, awareness, control, independence, etc.," it is unclear how these positive outcomes actually contribute to the development of better cassava varieties.

Perhaps the most extensive PPB project to date was carried out in northeast Brazil, coordinated by CNPMF in collaboration with CIAT and with regional rural extension services, NGOs, farmers' associations and individual farmers (Fukuda and Saad, 2001, 2002). These reports summarize the findings after seven years of experience in PPB. CIAT and the Colombian National Programme had developed a PPB methodology specifically for cassava in the late 1980s and early 1990s (Hernandez, 1993). This success gave the Brazilian National Cassava Programme a motivation to adapt the methodology to the local situation and needs. With a modest early start with nine farmers in Bahia State, breeders were able to learn that the varieties developed in experiment stations tended to be unstable in farmers' fields and often inferior to local varieties. Traits such as cortex colour and presence of root peduncle were decisive criteria for farmers, but had not been considered by breeders. Prior to this contact, farmers had been largely unaware that new, better-performing varieties could be an option for their farming systems.

Between 1993 and 2001 the initiative had supported a remarkable 305 participatory trials with 1 500 families in 70 communities of four states in the northeast. These trials mainly involved testing of advanced selections by farmers, but also, to a lesser degree, testing of unselected segregating populations. EMBRAPA released eight new varieties and more than ten others appeared to have a good level of acceptance. One of the most important outcomes has been the reformulation of selection criteria to better meet farmers' varietal needs (Table 20.1).

The project achieved a number of other successes as well, namely:

- opening of communication channels between farmers, breeders, extension workers and other professionals;
- changes in orientation of traditional improvement, by including extension and farmers in evaluations, establishing trials in farmers' fields and providing feedback and constantly including farmers' opinions, observations and criteria;
- changes in attitudes. Breeders obtained a better understanding of production systems and selection criteria. Farmers showed greater willingness to test new varieties offered by plant breeding programmes;
- stimulating the interest of farmers and farmer associations in conducting research;
- reducing the period between generation and adoption of cassava clones;
- increasing the genetic diversity of the region (diffusion of introduced germplasm);
- spontaneous diffusion because farmers had confidence in new varieties;
- farmers were more willing to try other types of production technology.

Fukuda and Saad (2001) cited some challenges that remained. There is a need for formal adoption and impact studies, since much of the impact to this point had been measured through informal observations and interviews. Another challenge is how to appropriately recognize farmers' contributions in officially-released varieties.

Participatory breeding certainly became broadly popular among the donors to tropical agricultural research in the 1990s. Breeders were usually anxious to participate in these projects, both to explore the potential benefits of closer farmer–breeder interaction and to tap into funding sources at a time when funding shortfalls became critical for many programmes. Participatory breeding in cassava, has now

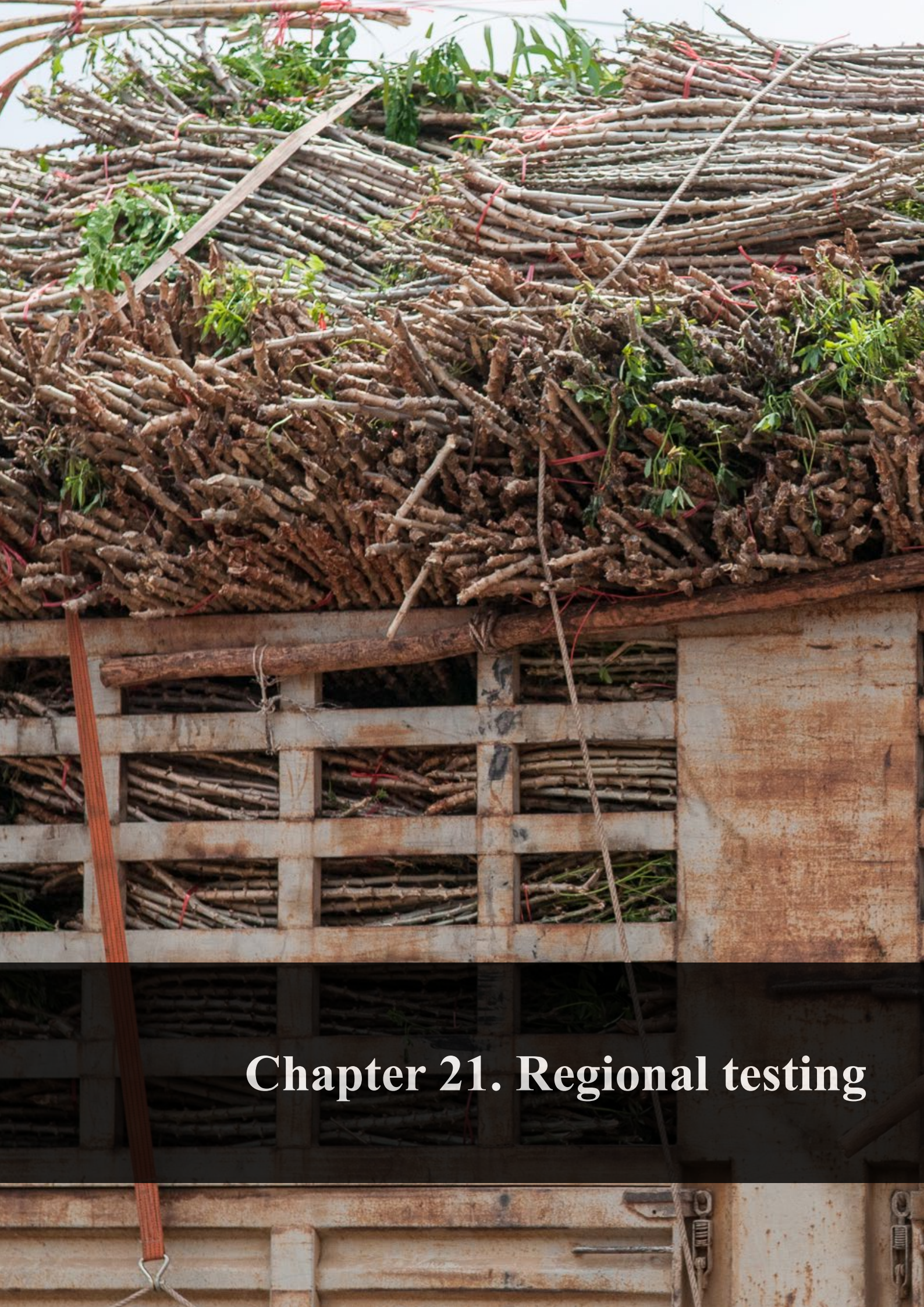
seen some 20 years of experience. Possibly the greatest contribution has been to generally raise the awareness of breeders to the myriad of criteria that producers and consumers evaluate in new varieties before considering adoption or purchase. Many programmes have experimented with some form of participatory research, but few have incorporated intensive farmer participation in an ongoing, routine manner.

One of the issues that needs to be addressed is how the successful experiences of farmer–breeder contact in a PPB project influence the need for continuing interaction. In theory, if there is a quick learning curve by breeders in the PPB process, the intensity of the interaction could quickly decrease. At least in the functional approach to PPB (as opposed to the empowering approach) it should be possible for breeders to become very well-informed about farmers' needs for varietal characteristics within just a few seasons of intense formalized interaction. Thereafter, it may be appropriate to have a lower level, continuing communication to be certain that the selection programme remains on-track.

Of course, if the goal is to empower farmers with greater decision-making input, then there will necessarily be a need to develop a longer-term, continuing relationship, with appropriate funding support. The lack of longer-term support seems to be a major roadblock to those programmes that have tried more participatory approaches. Few breeders are willing to divert already limited funds from a basic breeding programme, to support PPB.

Table 20.1 Selection criteria of the farmers in northeast Brazil

Selection criteria	Traits	Justification for their use
GENERAL		
Sprouting	Rapid; high sprouting index	Good competition with weeds; takes advantage of the rains; high productivity of roots per unit area
Content and quality of starch and farinha	High	High starch and farinha yield
Number of thick roots	3-4	Reduced labour for grating roots
Stake production	Short internodes	High yield of planting material and product for animal feed
SPECIFIC		
Ease of harvest	High	Reduced time and labour for the harvest; few roots wasted
Ease of removal of the cuticle of the root	Easy	Ease of peeling; good quality of the farinha
Constrictions in the root	Absent	Ease of peeling
Root peduncle	Absent	Facilitates harvest
Colour of the root cuticle	Light	Good quality of the farinha
Colour of the root flesh	White	Good quality of the farinha and starch
Cyanogenic potential of the roots	Low	Apt for fresh consumption
Plant architecture	Erect/high branching; low to average	Facilitates management of the crop
No. of stems per plant	2-3	Facilitates management of the crop; optimum for root yield
Yield of the aerial part, with good leaf retention	High	Alternative for animal feed
Source: Adapted from presentation by Wania Fukuda (CENARGEN/CNPMF) at Cassava Project Annual Review, CIAT, Cali, Colombia, December 2003		



Chapter 21. Regional testing

Most breeding programmes include comparisons among advanced selections across the intended target area, as the final, or near-final, stage of pre-release evaluation. These trials may go by any number of names, but are referred to here as regional trials. As a strategy of regional trials is so universally adopted across crops as part of the variety development process, there is a wealth of experience upon which to draw in designing a specific programme. On the other hand, the unique features of cassava and cassava farmers indicate some special considerations for cassava regional trials.

1. OBJECTIVES

The main objective of regional variety trials is usually to test promising materials side-by-side with locally prevalent varieties under environmental and management conditions that match the range for the projected farmer clientele. If objectives are simple, trial design and management can likewise be simple. If more complex objectives are included, management will likewise increase in complexity. While there is nothing inherently wrong with complex objectives, the trial coordinator needs to balance the resources available against what is technically feasible. Normally these trials should focus on the primary objective of comparing experimental varieties. Frequently, extension services find that these trials are also a convenient opportunity for promoting new varieties to farmers. This is premature, because the materials are still being evaluated for their performance and the breeder is not yet prepared to narrow recommendations to specific clones. If this objective is included, it must be managed in a way that gives an institution sufficient confidence to stand behind a recommended variety.

Regional variety trials may also seem to be a convenient mechanism to include other variables for testing, such as agronomic practices or pest control measures. Any additional objectives will often require significantly increasing trial size and complexity of management. For example, a trial where agronomists want to test genotype reactions to three fertility levels and entomologists want to include comparisons of pesticide protection and non-protection, will increase six-fold in size if all combinations of treatments are included. Normally this kind of complexity cannot be considered in more than one or a few sites, if at all. Historically, regional variety testing has been most successful when focused solely on comparing varieties. The other management factors in the trials must be carefully considered, but usually should be tested in other types of experiments.

2. COORDINATION

Regional trials may be managed by breeders, extensionists, agronomists or others. Often this stage is a transition from complete control of varietal testing by the breeder, to joint control by two or more institutions. This sets the stage for new ideas and diverse points of view to interact. It also opens more possibilities for conflict. There is no single best management approach. This is often dictated by institutional structure and political considerations. The breeder should in any case, be closely involved in design and implementation, because it is he or she who will be most familiar with the materials and can benefit tremendously from feedback on their performance. Overall coordination is critical to optimizing the output from these trials. A uniform set of materials, pre-determined management criteria and suitability for valid statistical analysis usually rely on good coordination.

3. PLANNING AND MANAGEMENT

This section gives some concrete examples of how regional trials can be managed. While certainly not the only options available, these examples are drawn from various programmes that have learned both from success and from failure in regional trial management.

3.1 WHICH MATERIALS

Selection of entries in the regional trial network will depend upon design of the variety development process leading up to this stage of evaluation. They may be the selections from advanced yield trials or selections from farmer participation trials. Some scientists may multiply and test clones directly

introduced from other programmes (national or international) in a regional trial network. Usually, however, it is preferable to have a preliminary stage of evaluation for these materials, because some can probably easily be discarded on the basis of far less extensive and costly evaluations. As a general rule, materials included in regional testing should have successfully passed at least two years of testing at less intensive and less extensive stages, such as the advanced yield trials of the breeding programme.

3.2 HOW MANY?

The probability of success in identifying superior clones increases as number of entries increases. The upper limit for increasing this probability is reached when the breeder cannot identify additional clones having the minimal characters for success. For example, if there are only six selections that combine the required yield potential, pest resistance and root quality traits, this will probably define the number of entries. To include additional clones having unacceptable root quality, in this example, would not increase probability of success, but would only increase costs. Of course, one does not know for certain which clones are acceptable or not; were it so, the regional trials would be unnecessary. The tendency should be to include clones that in the breeder's judgment and hopefully supported by some farmer/consumer input, have at least a fair chance of being accepted commercially.

Two overriding considerations determine the appropriate number of materials to evaluate: how many are identified as promising; and resources available. As trial management, especially planting material, is more difficult than with many seed-propagated crops, the tendency is to evaluate a smaller number of entries than might be included in regional trials for maize, rice or grain legumes, for example. There are no stringent guidelines to follow, only some rules of thumb and practical experience from the past. The average that most programmes seem to manage is between about 6 and 15.

3.3 SITE SELECTION

Most of the criteria for site selection that were described for the earlier phases of breeding (Chapter 7) continue to apply for regional trials. At this stage however, there is more opportunity for farmer involvement, even to the point where farmers collaborate in design and are in charge of most of the management. The first reason to emphasize farmers' fields is that they probably will be more representative of conditions for which varieties need to be adapted, as compared with experiment stations. The second reason is that few countries have enough experiment stations in cassava-growing regions to meet the needs of a full-fledged regional trial network. Nonetheless, experiment stations can certainly also be included in the regional trial network. In fact, this is an excellent way to test how well a station represents the surrounding farmers' fields.

One consideration for site selection may be its potential to have a multiplier effect. This can come about in a number of ways. Even though a regional trial is not generally intended as a mechanism to promote new varieties, there are other benefits to appropriate selection of the type of farmer or organization with whom to establish trials. A progressive farmer can stimulate community interest and a spirit of collaboration that will positively influence the general attitude of farmers towards research and extension. This will notably affect any later efforts to promote new varieties in the post-release phase. Farmer organizations can be an even better means of developing community rapport and of creating interest by exposing a large number of farmers to the process of evaluating new varieties. Other community institutions such as vocational schools, processing and marketing groups, or commercial enterprises can all be options for capitalizing on social and economic institutions to improve long-term possibilities for success in varietal improvement.

In the preliminary and advanced trials leading up to regional trials, it was possible to include only a few sites that would broadly represent the target area in terms of climate, soils and pest pressures. The regional trials should sample much more of the environmental diversity, to include the extremes for these major environmental factors, but with emphasis on the predominant conditions. How many sites are needed to cover this diversity depends upon the uniformity or diversity of the target area, topics covered extensively in Chapter 7. Typically, a programme should try to establish five to eight sites in a relatively uniform target region and 8 to 15 in more variable environments.

3.4 HOW MANY YEARS?

The breeder is well aware by this stage that evaluations from a single year are not sufficient for making recommendations to growers. The number of years to repeat evaluations in a regional trial will depend in part on the design of other phases of the breeding system. At one extreme, some programmes introduce exotic clones, multiply them and place them in regional trials with no prior evaluations. In this strategy, a minimum of three years' evaluation should precede any recommendation for varietal release. At the other extreme, if the experimental materials have already passed through an extensive series of evaluation stages, across sites and years within the target region, a single year of regional trials may be sufficient to recommend certain varieties. The norm lies somewhere between these extremes. Typically, a programme can develop reasonable confidence in a clone after two years of regional trials (if it has undergone preliminary evaluation in the same region) and possibly combine a third-year regional trial with extensive pre-release stake multiplication. With this strategy, the third year's trials give additional confidence to results, but do not delay time for making material available to growers.

If some materials perform poorly after one or two years, as is very likely to be the situation, they can be discarded rather than wasting resources on further evaluation. At the same time, a set of newly promising clones can be introduced, thus giving a continuous flow of materials into and out of regional trials.

3.5 MANAGING THE CHECK VARIETIES

The objectives of check varieties may or may not be the same in regional trials as in previous selection stages. At a minimum, the most common local variety should be included and this may vary across the region. It will be the benchmark by which farmers will assess the potential of any new clone. The breeder may also want to include a uniform, well-known check that improves his or her ability to evaluate some performance criteria. For example, if all experimental entries in a trial are resistant to an endemic disease, the best means of assessing the potential benefits of that resistance could be to include a susceptible check. Or if root quality is a particular concern for the region, a check variety could be included, the quality of which is especially influenced by environmental variations.

Typically, planting material for local checks is collected locally and that of other entries is produced under more controlled conditions and shipped to the testing sites. This can introduce substantial bias into interpretation of results, either in favour of, or against, the tested clones. If stake quality is a particular concern, both locally-grown stakes and introduced stakes can be compared for the local check, and included as separate entries in the trial. If local multiplication of planting material for all entries is feasible, that will aid in eliminating possible interaction between source of planting material and variety performance.

3.6 SELECTION

Even if the breeder's goal has been to develop varieties with adaptation across the range of conditions in the target region, it is likely that G–E interaction effects will be significant in the regional trials. The breeder is then faced with a decision of whether to select or discard varieties showing specific adaptation. In part this decision may be driven by the degree of success achieved in developing varieties that are successful across all sites. If several such clones emerge, then the additional costs of promoting more narrowly adapted varieties will not be justified. Conversely, if it seems difficult to select broadly adapted types, a revised strategy for regionalized selection should be considered, where sets of experimental varieties are grouped by adaptation to subregional conditions or demands.

4. INTERNATIONAL STANDARDS FOR EVALUATION

Two principal benefits can come from adopting international standards for evaluation of advanced selections. Firstly, the process of discussion and coming to agreement on these standards means breeders will have gained insight and experience into the way other breeders manage their programmes. These insights inevitably lead to a general improvement of some programmes. Secondly, agreed-upon standards allow more effective communication of results across regions and countries.

CIAT and IITA, by virtue of their international nature, have taken the lead in promoting discussions on standardizing evaluation criteria. For example, the Pan American Breeders' Network, in its 1992 meeting at the CNPMF in Cruz das Almas, Bahia, Brazil, adopted the standards noted previously in Table 8.1.

5. ANALYSIS

If regional trials follow a standard statistical design, analysis may be straight-forward. Uniform genotypes across all sites, same plot size and design and similar agronomic management are the simplest example, but relatively rare in the real cassava world. Availability of planting material, different approaches to management by various persons responsible for individual trials and constraints on land and labour generally introduce variations that complicate analysis. In fact, most programmes choose not even to attempt statistical analysis across sites and instead report only site and variety means from regional trials. This is perhaps not a major barrier to success in identifying best genotypes, but does not allow a good interpretation of variation within and across sites and years, or interaction among parameters. By recognizing the value of some of these simple statistics for long-term success, breeders can give appropriate attention to trial design, or using alternative statistical procedures when the design is not straightforward.

6. GUIDELINES FOR MULTIPLICATION

Multiplication enters the picture at both ends of the regional trial scheme: assuring adequate planting material to go from advanced breeding trials to a regional trial network, and multiplying selected clones for distribution to farmers in the first phase of release of a new variety, after selection in a regional trial.

6.1 FROM ADVANCED SELECTIONS TO REGIONAL TRIALS

If the final stage before regional trials includes a large enough number of plants per clone, planting material may be taken directly from these to plant regional trials. As a hypothetical example, an advanced yield trial planted in four sites with three replications per site and 25 plants per plot (300 plants) might produce about 3 000 planting stakes. If a regional trial that derives planting material from these 300 plants, is planted in ten sites, with 64 plants per plot and four replications (2 560 plants), no further multiplication would be needed. This assumes that all plants in the advanced trial are available for producing planting material and that little planting material is needed for other purposes.

In reality, many programmes find that the advanced yield trials cannot completely supply the planting material needs of a regional trial network. Generally, one of two strategies is followed: (1) include an intermediate phase for stake multiplication; or (2) include new materials in only a few of the regional trials during their first year of evaluation.

6.2 FROM REGIONAL TRIALS TO VARIETAL RELEASE

One of the persistent headaches to plague cassava breeders and extension personnel is the proper planning of stake multiplication. At the moment of varietal release, large quantities of planting material should be ready to distribute to farmers. However, reaching these required quantities will entail a multistage multiplication scheme. To avoid undue delays between time of release and availability of planting material, several clones will need to undergo preliminary multiplication, later to be discarded.

There is no completely satisfactory solution to this inherent inefficiency for a crop with such low multiplication rate as cassava. This topic is covered further in Chapter 22. Most programmes find that regional trials alone are not an adequate source of planting material for a varietal release event. On the other hand, these trials can be used effectively to distribute planting material to farmers surrounding the regional trial site.

7. OVERVIEW

Local varieties are generally acceptable to growers; otherwise they would not be cultivated. Some, of course, are more successful than others. Sometimes changes occur that demand a rapid change of varieties (e.g. a devastating new disease), but more commonly, growers are quite attached to the varieties they are accustomed to and may have grown for many years. When regional trials show local varieties as failures, breeders should seriously look at both the criteria to evaluate varieties (do the criteria correspond with those of farmers?) and the location and management of the trials (do they represent the reality of farmers' growing conditions?).

Regional trials should be well-managed. They should employ good agronomic practices that will be economically viable and otherwise appropriate for local adoption. This being the case, it is common to obtain yields well above national yield averages, including those for local materials. It is not clear whether one can judge from yield levels whether trial management is or is not appropriate. If yields of the local varieties in the regional trials far exceed farmers' yields (for example, two to three or more times the regional average), one might suspect that the practices adopted in the trial are beyond the reach of most cassava farmers. In these situations, experimental materials often yield three or four times the national yield average and one has to look carefully at the relevance of these data in terms of helping select the best materials to release. There are many examples of materials that have performed with outstanding yield in regional trials, yet with very low acceptance by growers. This highlights the need to systematically establish evaluation criteria as discussed in Chapter 20. Regional testing must take a critical look at acceptability of advanced selection to meet producer demands. From this point onward, the breeder will be investing heavily in advancing individual clones through the next steps towards release, adoption and impact.

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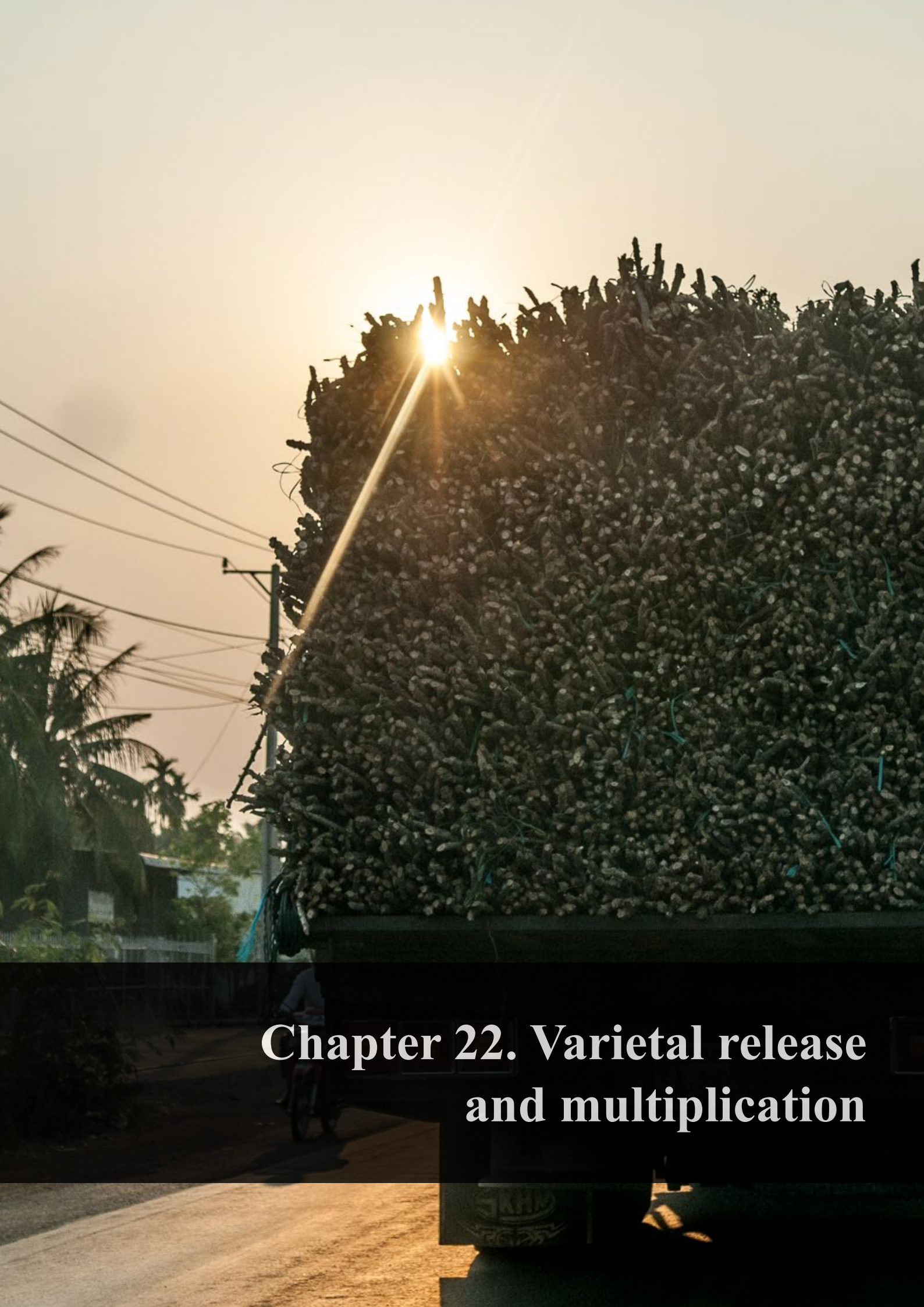


Chapter 22. Varietal release and multiplication

Release of a new variety symbolizes the successful culmination of a long series of steps in crop genetic improvement and the beginning of the rigorous test of farmer and consumer acceptance on a broad scale. While farmer and consumer input will normally be part of varietal development from the earliest stages, it is usually only after release that large numbers of farmers have access.

In English, the term release implies freeing the new variety to fly on its own. Probably the Spanish word is more precise, *lanzar* (to launch), giving the definite impression of an active rather than a passive event. Varietal release as used in this chapter encompasses all the activities related to preparation and promotion of a new variety for use by growers.

While varietal release in cassava is not a rare event, it is also not a frequent event for most countries. It is uncommon enough that many countries either have not established norms, or the norms may be unworkable because they are based on experiences with seed-propagated crops that are better-endowed with research and extension support. Successful varietal release in cassava requires some modifications to the conventional process.

There is no set formula for achieving commercial success through the process of varietal release. In fact, in many cases, varietal success has had nothing to do with official release. Probably 60-70 percent of the world's cassava is grown to traditional varieties, never released through any official organization, or released after a farmer-selected variety is already widely grown. Although several countries have experienced relatively rapid adoption of new varieties, adoption is normally a measured process, growing slowly over several years.

Cassava varietal release should combine elements of successful strategies from other crops, along with a full appreciation of the traditional modes of introduction and spread of new varieties. Evaluation, release and multiplication are intricately interwoven processes. They may blend into each other so subtly that they can hardly be distinguished as separate activities.

1. ADOPTION IN NEW AND TRADITIONAL GROWING AREAS

For purposes of this discussion, cassava-growing areas will be classified as either traditional (farmers have well-adapted varieties available and long experience in cassava production); or new (farmers have limited experience in growing cassava and traditional, locally-adapted varieties are not available).

In traditional cassava production areas, the normal procedure for farmer introduction of new varieties is for new clones to be tried out initially by a few farmers, over a limited area. If these clones prove to be superior in some way to those already available, the farmers will increase the area planted, perhaps to the extent that the natural multiplication rate allows. Neighbouring farmers may observe the success of the new variety and also begin to try small numbers of stakes. If the variety shows continued superiority, it will slowly diffuse throughout the region, gradually displacing previous varieties.

This process of slow variety replacement has been frequently observed, not only in cassava, but in many crops in traditional farming systems. One of the key elements in this style of technology adoption is risk avoidance. A variety is planted on only a limited area until the farmer is fully convinced of its merits and especially of its stability of performance over time. With these dynamics of adoption, there is very little danger that an unsuitable variety will be adopted and grown in any significant area. Although diffusion is slow, when it does occur there is a rather high probability of significant impact, because the farmers themselves have been the key decision-makers throughout the entire process.

In new production areas where cassava is just beginning to be introduced as a crop, the dynamics of adoption are quite distinct. In these situations, large-scale introduction of a new variety often seems to be the best alternative. Here, the plant breeder, extension service and others need to have carried out appropriate testing to minimize the risk of failure of a variety, because the farmer has to commit such a large percentage of his resources to material he may not even have tried previously. Unless he has some

basis for trusting the judgment of the breeder and the extension service, he will be hesitant to adopt the new variety.

There is no way to avoid the inherently higher level of risk involved in large-scale versus small-scale introduction of new varieties, but appropriate breeding and varietal release schemes can, in both cases, improve the chances of success.

2. BASIC STEPS IN VARIETAL RELEASE

Release of a new crop variety may take many forms, but at the heart of these are some common key steps: (1) identification of a superior genotype in regional and on-farm trials; (2) presentation of performance data to the organization responsible for authorizing release (often including a document with supporting data, a seminar and a field day); (3) establishment of a multiplication scheme, possibly including plans for longer-term, continuing multiplication for basic and certified seed; (4) preparation of a farmer- and extensionist-oriented bulletin describing the variety; (5) a field day for presentation to farmers, extensionists and seed producers; and (6) follow-up on distribution and performance.

An agency responsible for release may have very specific requirements for evaluating the suitability of a variety, or it may be very flexible and open-ended. The breeder should at least be able to demonstrate clearly the benefits to be derived from the new variety, for what soil and climatic conditions it is suited, appropriate accompanying agronomic practices and market-related characteristics. Weak points of the new variety should also be described.

Adequate multiplication prior to release is clearly a frequent constraint. Research administrators are often reluctant to authorize multiplication of a clone until there is assurance of release. The reluctance comes from the need to multiply several possible candidates for release, only to have most of them later discarded. This multiplication of several pre-release clones can seem wasteful of resources, but is in fact the most efficient approach in the longer term. When the data are available to support release, there usually is reluctance to delay it, on the basis of inadequate stake supplies. There is often a delay of a few or even several years between release and significant adoption of new cassava varieties, owing to shortage of planting material. Only a few of the best-funded programmes seem to be able to multiply newly released varieties in adequate supplies.

A variety release bulletin is usually a simple folder, describing the new variety in layperson's terms. It is partly promotional and partly informative. It should be written in a style that attracts the readers' interest, while being completely factual. The presentation should be attractive; photographs and professional design are helpful. The bulletin may be restricted to a description of the variety or it may include also a brief overview of recommended cultural practices, especially if these differ in some way for the new variety. For cassava it is rarely possible to be specific about yield expectations, for the many reasons repeated throughout this book and most of all because the crop is grown under such a range of conditions even within a region. Therefore, yield might be expressed in relation to a well-known local variety, under a range of difficult to favourable conditions. The morphological description should be made in terms that are familiar to farmers and ones which they can easily observe. Highly technical terminology is inappropriate.

3. TRADITIONAL VARIETAL RELEASE

There are many reasons why traditional release schemes have been practically non-existent in cassava. These are related primarily to the crop's vegetative propagation, low multiplication rate, lack of experience of most national programmes in producing new cassava varieties, the tradition of farmers selecting and multiplying their own planting material and a general lack of appreciation by growers of the importance of quality planting material to achieve high, stable yields.

Stake multiplication at commercial levels in cassava requires a major commitment of human and material resources. As multiplication rate is low in comparison with seed-propagated crops, relatively large areas need to be planted to produce stakes, in the order of one ha for each 10 to 20 ha of commercial production. Although there are methods of increasing rates of multiplication (discussed in later sections), these have only been practical, with a few exceptions, for preliminary multiplication. The final stage of multiplication, for commercial plantings, is almost always from more traditional schemes not directly involving rapid multiplication.

Varietal release in cassava has to be a low-risk proposition. As replacement of varieties is usually a slow process, a farmer cannot risk having a variety fail if it is planted on a large percentage of his land and then have nothing to fall back on for the subsequent planting.

As stability of performance across years is so critical, any varietal release scheme should avoid rapid, extensive distribution of a new variety. Even where a breeding programme has been properly structured to take into account temporal stability, varieties released for commercial production will be subjected to an even wider range of environments, pest and disease problems and the peculiar dynamics related to extensive areas planted. Although excessive conservatism is also unwarranted, varietal release in cassava probably needs to be done with somewhat more caution than in many other crops.

Cassava farmers in most parts of the world only rarely purchase stakes. This does not necessarily imply that commercialization of stakes is not a viable option in specific situations. It does, however, suggest that varietal release schemes should generally carefully consider alternatives that involve only small stake purchases, or none at all, in the diffusion process. Instead of expecting farmers to purchase stakes for their entire commercial production, a more realistic expectation would be for them to purchase small amounts for trial use and later on-farm multiplication.

Cassava stakes are bulky and highly perishable. The logistics and expense of distributing them indicate that their production should normally be highly decentralized and managed within localities rather than within large regions. A varietal release scheme should therefore be closely tied to a decentralized structure for stake multiplication (see Figure 22.1).

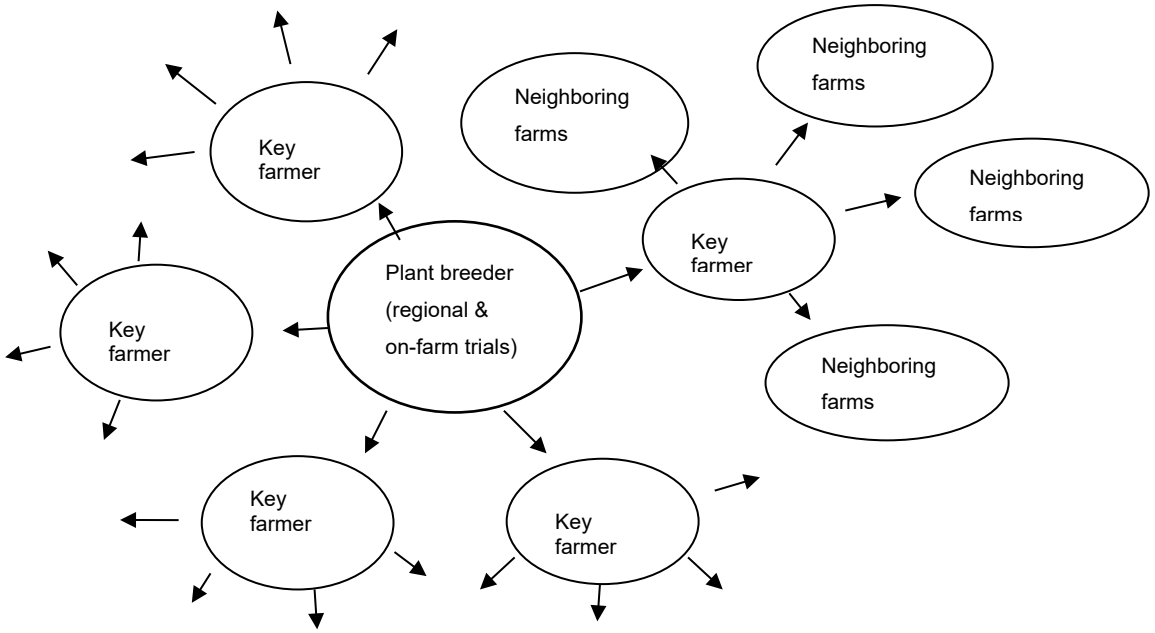
A formal infrastructure for stake multiplication is not common for cassava. Any release scheme should take this into account and develop a simultaneous plan for stake multiplication, rather than assume this will take care of itself once a good new variety is produced. Later sections present various alternatives for stake multiplication.

4. ALTERNATIVE VARIETAL RELEASE SCHEMES

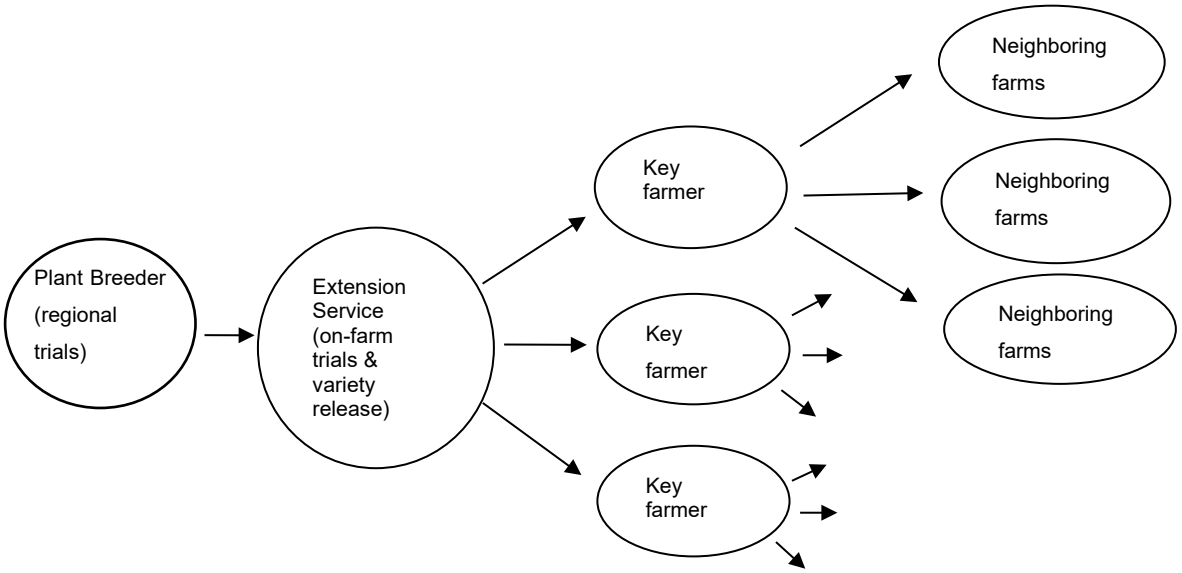
One release option is to carry out the final stages of varietal testing on farmers' fields, such that varieties to be released are already under a sort of disperse multiplication throughout a region. In fact, when trials are planted on farmers' fields, farmers will often select and multiply those that they prefer, whether or not any formal release is made. However, depending solely on the natural process of farmer-to-farmer diffusion will probably be too slow to satisfy most programmes. Research and extension agencies should promote and monitor the whole diffusion process. Researchers can make use of information on varietal performance over many sites, which the farmer will not have available. The breeder should synthesize all the information available on a given variety before making a release decision. Follow-up investigation of varietal distribution and performance is essential for continued upgrading of new varieties.

Figure 22.1 Pathways of varietal multiplication and distribution

Model 1: Breeder works to effectively use traditional farmer-to-farmer distribution system



Model 2: Extension and other local agencies have mandate and resources to distribute planting material to individual farmers



It is probably desirable to have more than one variety released simultaneously to give farmers various options, but most programmes do not have the capacity to produce multiple new varieties annually. The need for a few, or several, varieties to be released simultaneously is especially critical in new areas for cassava production. Here there is more possibility for a single clone to become dominant, to the point of creating a risk of genetic vulnerability to pests, diseases or fluctuations in the weather.

5. PLANTING MATERIAL CLASSIFICATION

Stakes pass through a series of stages between the breeder and the farmer. The standard categories of seed defined in seed-propagated crops are not all relevant to cassava. The categories of stakes that a programme defines and develops depend on the specific varietal release and multiplication scheme. The names used to describe these categories may differ from one programme to another. The following is a general classification that could be broadly applicable. (The term seed is sometimes used in this discussion, to be more consistent with broad seed industry terminology, but refers to vegetative planting material).

Breeders' seed is the definitive source of a released variety. It is a genetically pure reference and a permanent reserve. Normally, it will be a single uniform clone; however, it is conceivable that a released variety could be a planned mixture of clones. In this case, each component of the mixture would need to be individually identified when maintained as breeders' seed. As such, the definitive source must be preserved in a very secure manner and kept free of damaging pests. All chances of contamination of the genetic integrity must be minimized. At present, the most appropriate maintenance of breeders' seed is similar to that for basic germplasm conservation, a combination of *in vitro* and field collections. The amount of material need not be large; the function is primarily as a secure reserve of clean material, which would only need to be accessed in case of some problem with other sources. Fifty to one hundred plants *in vitro* and 200-500 plants in the field can serve as a base of breeders' seed.

Experiment stations maintain basic or foundation seed as a source for the registered seed distributed to seed growers. Basic seed can continue to be renewed from itself so long as it meets phytosanitary, genetic purity and other requirements established by the government. If problems occur, it may be necessary to return to the breeders' seed as a source for renewal. Experiment stations normally cannot and should not be in the business of supplying farmers with seed for commercial production. In a system in which all the phases of seed production are in place, each experiment station should not have to maintain more than a few hectares of foundation seed. The exact amount however will depend on careful planning based on expected demand and the specific system of distribution involved.

The plants grown from basic seed will be used to renovate themselves, as well as to produce registered seed. Ideally the basic seed lots should supply seed producers, either in the private or public sector. If no formal commercial seed sector exists (and usually it does not), the registered seed produced from these multiplications can be distributed to key farmers with whom agreements have been made for following specified seed production practices and for further distribution to other farmers. Registered seed is usually received each year from the experiment stations' basic or foundation seed lots, but in some circumstances may be regenerated from itself.

Certified seed may be produced from registered or from certified seed. Seed growers (who will probably also be cassava farmers) produce certified seed under specified standards, for distribution to farmers for commercial production. The key characteristic of certified seed is that it has been produced under supervised growing conditions and has been certified to meet guidelines for phytosanitary status and genetic purity by an authorized agency, normally governmental.

The previous sections describe an ideal, but in reality, very few national programmes have this full array of production steps in place for planting material. Rather than trying to simultaneously implement all the described phases of stake production, initial focus should be on those aspects observed to be a bottleneck in the given situation. In many cases, there simply is an inadequate amount of foundation

seed produced by the experiment stations and they need to take the first responsibility to correct this bottleneck. If budgets are constraining, as they usually are, selling the stakes rather than giving them away should be considered as a way of partially financing the effort.

Many different alternatives can be made to look good on paper, but they will have little chance of success if they require abrupt changes in the way farmers traditionally obtain their seed and grow their crop. For this reason, many programmes will find that a system that builds upon farmers' habits of managing their own stake production, has the best chance of success. At the same time, it is to be expected that a cassava seed sector will develop with larger or more intensive systems, especially for industrial markets.

6. VARIETAL CHARACTERIZATION

A basic requirement when a new clone is considered as a candidate for release, is to establish its distinctiveness. For this purpose, the releasing agency describes the morphological, agronomic and market traits, with special attention to those features that distinguish the variety from existing ones. Although no standard has been developed in cassava for varietal description, the IPGRI-recommended list of descriptors is an appropriate baseline description (see Chapter 5). The description must be sufficiently precise such that a person with no experience with a variety is able to identify it with a high degree of reliability from its description alone. Biochemical and molecular markers (e.g. isozymes and DNA fragment markers) provide a higher degree of reliability for positively establishing distinctiveness, but would not normally be part of a varietal description managed by extension programmes or farmers.

It is not uncommon for varietal mixtures to occur even with well-known local varieties. Farmers may confuse a new variety with another that is morphologically similar, resulting in clonal mixtures on-farm. An example of this occurred in the Caicedonia region of Colombia. Farmers reported that the highly favoured variety *Chiroza* was declining in performance over years. Further studies showed that there was in fact a progressive mixture with a morphologically very similar clone, with much lower yield potential. By simply practicing individual plant selection for planting material, the variety could be purified at an acceptable level of confidence (J.C. Lozano, personal communication).

The converse is also possible. In Colombia's north coast region, farmers felt their variety *Venezolana* had been contaminated with a mixture of clones having a different branching habit. Field trials and biochemical markers showed there was no mixture of clones, but probably a high sensitivity of this clone to some environmental factors (C. Ocampo and C. Hershey, unpubl. data).

Private enterprise has so far played a limited role in the development of cassava multiplication or distribution programmes, but there are examples that demonstrate how effective this can be. Although farmers sell stakes to neighbours, or entrepreneurs sell fairly large numbers of stakes at special prices, there is only a nascent involvement of formal private seed industry in cassava. This might be expected to change on a limited scale as farmers become more convinced of the importance of clean seed. Cassava producers themselves will also begin paying more attention to seed production, rather than buy stakes while they throw away their own planting material.

For cassava farmers, it is not a question of producing their own planting material versus selling it as an edible product, as can be done with seed-propagated crops, or with potatoes, for example. The cassava grower does not have an alternative market for the planting stakes he produces, so there is high incentive to manage them well and possibly generate additional income.

7. NAMING A NEW VARIETY

Most countries establish guidelines for the naming of new varieties, but usually the breeder responsible for development also has some input. The following are adapted from the generalized guidelines of Fehr (1987), to apply to cassava; they do not necessarily correspond to any country's specific regulations and are given only as a hypothetical example:

- a variety must be given a name that is unique for the country where released. Insofar as possible, the name should also be distinct from released varieties in other countries;
- a cassava variety, however, may be given a name that is used for a variety of another crop species;
- once assigned to a variety, the name remains exclusive. It cannot be re-used in the future even if an older variety has been out of production for many years;
- a company name may be used in a variety name as long as it is part of the original, legally assigned name;
- descriptive terms may be used as part of a variety name as long as such terms are not misleading. For example, *Mantequeira Amarela* would only be appropriate if the variety with yellow-fleshed roots had many features similar to the well-known variety *Mantequeira* (IAC, Brazil);
- a variety name should be clearly different from existing names in spelling and sound.

Apart from these fairly technical guidelines, the breeder should also consider some of the aesthetic aspects of variety name. The name may convey something positive about the variety (e.g. yield, quality, resistance) or describe some unique and easily recognized feature (e.g. root shape, leaf colour, branching habit). Complex codes or names will probably be difficult for farmers to remember and this can even affect success of adoption. Names that honour a person, place or event can be appropriate if it is a name generally appreciated by farmers.

8. IMPLICATIONS FOR TRANSGENIC CASSAVA

To date transgenic cassava appears to be at least a few years from any commercial release to farmers. While a few transgenic clones have reached the early stages of field testing, these are sources chosen mainly for their ease of transformation, rather than for adaptation to areas where the new traits will be especially valued. While the protocols for introducing and monitoring transgenic clones are established in a few countries (e.g. Colombia and the Republic of Tanzania), release procedures of potential new varieties are not defined.

At a minimum, breeders need to confirm level of expression and stability of the trait during a series of field trials, over several vegetative cycles. This stability has been accomplished for potatoes, so there is a precedent in a vegetatively propagated crop. Beyond these basic requirements for farmer acceptance, regulatory requirements could require extensive food safety trials and tests on level of intercrossing with local varieties (on the off-chance of propagation from a hybrid true seed). Local communities should have a voice in whether or not to accept transgenic crops.

One of the goals of several projects working on transformation is to be able to introduce key traits into locally adapted local varieties. When this is successful, the transgenic clone and its wild-type parent could presumably look identical or very similar in the field. Perhaps the difference may show up under pest pressure (where a resistance gene is introduced), or show different root quality traits when laboratory-tested. A release programme would need to find ways to maintain the identity of the sister clones (possibly isogenic lines) at the field level, where they could hypothetically become mixed over time and fail to perform as intended. There may be no easy solution to this, so long as farmers continue to produce their own planting material. While there are many examples of isogenic transgenic/non-transgenic lines in maize and soybeans, these are under the control of private companies and growers are required to buy new seeds each growing season. (In any case, for hybrid maize, seed-saving is not a viable option).

It could be possible to include marker genes in the transformed plants that permit some easy way to distinguish between transgenic and non-transgenic clones. This would need to be an agronomically neutral trait and the options may be limited, such as petiole or stem colour. However, transforming clones to include marker traits would be just as difficult as transforming them for economically important traits and it is unlikely any project would want to incur this additional cost unless absolutely essential.

Another solution could be to eradicate a local variety before introducing its transgenic counterpart in the region. This could prevent any mixing. However, this strategy will only work in a very few situations where: (1) growers might be willing to give up a local variety because they have others to rely on, (2) in the case where the new transgenic clone does not perform to their expectations, or (3) stakes are in short supply. This strategy would also require a massive, well-coordinated multiplication, such that there would be enough planting material available to quickly replace the eradicated local clone.

The first step probably needs to be education – to work with farmers to develop careful procedures to separate transgenic and non-transgenic clones in the field. Farmers need to understand the importance of this separation so that varietal performance continues to meet expectations year after year. In order for a system of separation to work, procedures need to be developed in a participatory mode with farmers.

9. OVERVIEW OF VARIETAL RELEASES BY NATIONAL PROGRAMMES

Nearly every important cassava-growing country has released varieties. The number of releases is not necessarily a direct measure of success of a breeding programme, since different programmes have different criteria and standards for release. Some countries have the philosophy that farmers deserve to have many released varieties to evaluate and from which to choose for adoption. Other countries believe that only those clones that have been proven over several years and many locations should be released. The release and promotion of new varieties are normally not reported in the international literature, so comprehensive and up-to-date information is difficult to obtain.

In Africa, Manyong *et al*. (2000) cited 206 varieties released up to 1998. They estimated that these new varieties covered over 9 million ha, with over half of the total in just two countries, namely, the Democratic Republic of the Congo and Nigeria. In Latin America, CIAT (2003) lists 29 varieties released up to 1998 and 33 in Asia, but this only includes those with some clear input of CIAT-developed germplasm (Table 22.1). It is not always clear whether such lists include release of landrace varieties. In Asia Tan (1994) noted landrace varieties released throughout Asia (Table 22.2). It seems to be typical that most countries began the varietal release process with selected landrace varieties.

10. RECORD-KEEPING AND POST-RELEASE FOLLOW-UP

Precise records should be kept on all phases of multiplication and release. Documenting the experiences of any programme not only helps that programme continue to make its research more effective, but also serves other programmes in the process of establishing a protocol for varietal release. Activities under control of the breeder can easily be documented. On the other hand, as materials begin to move to commercial seed producers and to farmers, keeping track of the flow of materials is far more difficult. Early planning of a post-release survey and monitoring strategy will help tremendously in information feedback. The breeder is unlikely to be directly involved in this process, but may need to take the initiative to motivate the appropriate scientists.

Table 22.1 Summary of cassava variety releases to 1998[a]

West Africa			Latin America/Caribbean		
	Benin	8		Brazil	11
	Côte d'Ivoire	2		Colombia	7
	Ghana	4		Cuba	2
	Guinea	16		Dominican Republic	2
	Nigeria	15		Ecuador	2
	Sierra Leone	6		Haiti	2
	Togo	14		Mexico	2
Central Africa				Panama	1
	Cameroon	13			
	Chad	30	**Asia**		
	Democratic Republic of the Congo	14		China	6
	Gabon	14		Indonesia	3
Eastern Africa				Malaysia	2
	Kenya	3		Philippines	8
	Rwanda	8		Thailand	7
	Uganda	9		Viet Nam	7
Southern Africa					
	Angola	9			
	Malawi	4			
	Swaziland	2			
	United Republic of Tanzania	24			
	Zambia	3			
	Zimbabwe	8			
Total		206			

[a]*This list almost certainly significantly under-reports the number of releases, especially selections from local landrace varieties*

Sources: Africa: Manyong et al. (2000); Asia and Latin America: CIAT (2003)

11. ALTERNATIVES FOR MULTIPLICATION OF PLANTING MATERIAL

Adequate and timely production of high quality planting material (stakes) is inextricably linked to successful varietal release. A range of options is available.

11.1 TRADITIONAL ON-FARM MULTIPLICATION

When area planted to a given variety remains stable, there is generally little difficulty in achieving adequate stake production. Problems may occur if a farmer harvests over an extended time period, leaving only the later harvests for stake production for the following cycle. If these last plants to be harvested are less than the optimum number, a stake shortage or poor stake quality may result. In situations of a stable production area, farmers generally have developed methods to assure adequate seed supply from one planting cycle to the next. This often includes storage of stems under semi-controlled conditions.

Table 22.2 Release of landrace varieties in Asia

Country	Variety
China	SC 201, SC 205
India	M-4, M-6, Sree Prakesh
Indonesia	Faroka, Pandemir, Bogor, Muara, SPP, Ketan, Mentik, Ambon, Gading, Valenca
Malaysia	Black Twig, Medan
Myanmar	Yezin, Hinthada, Mon State (Red)
Philippines	Golden Yellow, Lakan, Vassourinha, G29r-3
Sri Lanka	MU-51
Thailand	Rayong 1, Hanatee
Viet Nam	Hung Loc 24
Source: Tan (1994)	

11.2 TRADITIONAL SCHEME OF MULTIPLICATION BY RESEARCH/EXTENSION AGENCIES

The common procedure for stake multiplication is to have one or a few sites where stakes are multiplied by planting cassava under conditions of special care. When multiplied to the desired level, stakes are sold or donated to growers. If the multiplication fields are intended as the source of material directly for commercial plantings, enormous areas are required for multiplication, in the order of one-twentieth to one-tenth of the commercial planting area. This is rarely possible. Therefore, the more common practice is to widely distribute small quantities of seed, for subsequent multiplication by growers.

The more centralized the propagation fields, the more complicated are the management and distribution of material. The high perishability of cassava planting material makes it imperative that distribution be rapid and under conditions that do not cause deterioration of quality. Centralization of propagation fields also concentrates risk, for example, of a pest or pathogen affecting large quantities of planting material, possibly to the point of destroying it.

These factors argue for some degree of decentralization of stake production fields. The only potential disadvantages might be some lack of uniformity of management across different regions, or that some increased level of personnel might be necessary to handle the same total level of propagation. The advantages generally outweigh these disadvantages.

11.3 SITUATIONS REQUIRING RAPID MULTIPLICATION

The three principal situations where rapid multiplication may be required are: (1) where a production disaster has caused a stake shortage in an area; (2) after cleaning from pest infestation and reintroduction of a variety; and (3) for introduction of a new variety.

Natural disasters or civil strife can wipe out supplies of planting material and require rapid multiplication to re-establish a crop and avert severe hardship. These situations often require government programmes

that can mobilize resources and move large amounts of material to affected farmers. These programmes often need to be established quickly from relatively little local research or practical experience.

The justification and methods for cleaning cassava clones from pathogenic problems are covered in a later section on Improvement of Stake Quality. The point to be made here is that cleaning of varieties is carried out with a very limited number of individuals and these must then be rapidly propagated to replace uncleaned field material. To rely on the normal propagation rate of cassava would allow increased time for reinfection from yield-reducing organisms.

Varietal release may or may not generate the need for rapid multiplication to ensure dissemination of the new variety in the target area. If an experimental variety has undergone extensive testing prior to release (which should be the normal procedure), there may be enough planting material available from trials and semi-commercial plantings to supply post-release needs. Careful pre-release planning (usually three to four years prior to actual release) should allow development of a testing and traditional multiplication scheme that adequately supplies planting material to farmers. Rapid multiplication may be justified if there is a special urgency to move a new variety into a region, but most programmes will find that the expense and the logistic challenges of rapid propagation at this scale are serious impediments.

11.4 RAPID MULTIPLICATION METHODS

Four basic methods of rapid propagation are currently available in cassava. Each has a number of variations. Two of these – multiple rooted shoot production and rooted axillary bud production – have already been extensively used by research programmes. The third possibility, *in vitro* rapid propagation, can be successful under careful management with appropriate facilities. A fourth method is the use of mini-stakes, a simple and common means of increasing the multiplication rate by several fold.

11.4.1 Multiple rooted shoot production

The method is based on the fact that the nodal units of woody stem cuttings produce multiple buds, which become successively active as growing shoots are cut off. Young shoots can be cut and rooted, becoming the foundation for new plants to transplant to the field (Wholey, 1974). The new shoots can in turn be cut and rooted and so on.

Multiple shoot propagation does not require any sophisticated equipment. A healthy, mature plant is required to produce the mother cutting used in this propagation system. Woody parts of the stem are cut into two-node cuttings. These are planted in a propagation chamber (a bed of soil protected by a plastic enclosure that can be adjusted for ventilation) and kept well-watered. When new shoots reach 5-10 cm, they are cut off and placed in vials or beakers with sterilized water, which are placed in the rooting chamber. This can be a simple plastic enclosure over a table. Full sun should be avoided to keep temperatures inside the chamber at normal growing conditions. After about one week, callus forms on the basal cut and roots begin to form. When these roots are still less than 1 cm long the shoots can be planted in pots or plastic bags, or may be directly transplanted into the field. These plants may then be used for another cycle of rapid propagation or for standard stake propagation. Normally plants from rapid propagation methods would not be used directly for commercial production because of the high cost involved; technically, however, this is quite possible, as plants from rapid propagation yield are comparable with those from stakes, when given good growing conditions.

The main limitations to this technique are: (1) the process can only be started when woody lignified tissue is available; and (2) the new shoot production only continues while nutrient and carbohydrate reserves exist in the original woody cuttings.

The multiple shoot method can theoretically produce 12 000-24 000 commercial stakes in one year from a single mother plant, assuming close to 100 percent success rate in rooting and maximum use of shoots produced. Practical experience has shown that much lower levels are generally obtained.

11.4.2 Rooted axillary bud propagation

The principal advantage of the axillary bud system over the rooted shoot method is that green, slightly lignified stems can be used as the parent material, allowing a rapid turn-around time. Axillary bud propagation requires a rooting chamber, whose most important feature is a misting system. Small trays are filled with coarse sand or gravel (previously sterilized) and placed in the chamber. Mother plants of almost any age can be used. Best efficiency (rapid turn-around time and high yield of buds) is obtained with plants three to four months old.

With a sharp sterilized knife, each leaf, along with the accompanying axillary bud and small heel of stem tissue, is cut from the plant to form the propagules. The leaf lobes are then cut so that the leaf forms a rosette. Latex is washed from the cut surface, the propagules are placed in furrows in the trays and kept under permanent misting.

After one or two weeks small roots form on the cut surface of the heel and the petiole abscises. Propagules may be transplanted directly to the field, but experience has shown that better success is normally achieved with planting first in pots or plastic bags.

The axillary bud system has the theoretical potential to produce a remarkable 100 000-300 000 commercial stakes per year beginning with a single three to four month-old mother plant. These numbers, however, are extrapolated from experimental data and in practical application it is unlikely that such high multiplication rates could be achieved. Nevertheless, even at a rate of only a fraction of this theoretical rate, it is far more rapid than the traditional method of multiplication from 20 cm lignified stems, which can produce in the order of 100–400 commercial stakes per year beginning with a single mature mother plant.

11.4.3 *In vitro* multiple shoot cultures

The apical dominance of an *in vitro* plantlet can be overcome by altering the composition of the culture medium (Roca, 1984). At optimal 6-benzylaminopurine (BA) concentrations (5.0 μM), rosette cultures are formed, comprised of 10–20 nodes each, depending on the variety. Further growth of these buds occurs when the concentration of BA is reduced. The growth of axillary buds on rosette cultures gives rise to multiple shoot cultures. Up to 20 apical and nodal cuttings can be harvested weekly from each multiple shoot culture and transferred to a rooting medium for recovery of plantlets.

Rapid *in vitro* propagation techniques could be used effectively within strategies of international germplasm exchange, to move more quickly to field-level trials. The method would also be valuable in situations of varietal cleaning via *in vitro* methods. In southern China, Liu *et al*. (1990) reported on locally developed methods to produce plantlets from *in vitro* culture for commercial-scale production fields, with 90–100 percent survival. After rapid *in vitro* multiplication, they followed these basic steps:

- take vigorous plants from test tubes and wash agar off roots; keep plantlets with water in plastic trays five to seven days for hardening;
- place plantlets in nutrient media to promote root growth 20-25 days;
- pack plantlets in paper cartons, with root system wrapped in absorbent paper, ready for transplanting;
- transplant directly in the field; cover each plant with a ventilated bag to protect from raindrops.

11.4.4 Mini-stakes

The easiest way to increase multiplication rate is simply to cut shorter stem pieces, followed by normal field planting. The minimum requirement is that each planting piece has a nodal unit with a viable bud. This means that stem pieces can be as short as a few centimetres, depending on the spacing of nodes on the stem. Usually sprouting and rooting are not difficult to obtain under favourable conditions. If field conditions are well below optimum, higher success can be obtained by planting mini-stakes in plastic bags in more controlled conditions, with later transplanting.

12. IMPROVEMENT OF STAKE QUALITY

Evidence continues to accumulate on the importance of stake quality in productivity of a variety. Although it may not be directly the responsibility of the breeder to manage programmes of stake production, he or she should be aware of the importance of incorporating a plan for maintenance or improvement of stake quality into any cassava varietal release programme. The following sections consider both field and *in vitro* methods.

12.1 FIELD METHODS

The most basic of methods for improving stake quality is systematic selection of stakes from commercial production fields. Quality of planting material depends on several factors, including the type of material used, sanitary conditions and storage. The quality of the stakes *per se* is determined by the age of the stem used, the number of nodes per cutting, the thickness of the cutting, varietal differences in sprouting ability, phytosanitary status and the extent of mechanical damage the cutting may suffer when it is being prepared, transported and planted. Recommendations in all these areas are well-documented. Studies at CIAT showed that yields can be increased by visually selecting stakes appearing healthy and well-developed as compared with unselected stakes as sometimes used by the farmer.

An even greater improvement in yields is often possible by planting separate stake production plots, with management designed specifically to maximize the quantity and quality of stakes produced. Additional care in land preparation, weed and pest control and fertilization are the key elements of this management. Not only would these plots provide high quality planting material, but roots could also be harvested for sale or home use. Agronomic practices recommended for stake production are also generally favourable for root production. Separately managed stake production fields may provide benefits well in excess of what farmers might first imagine.

12.2 *IN VITRO* CLEANING

Meristem tip cleaning of cassava is based on the fact that most viruses grow and multiply less rapidly than the meristematic tissue of growing plants. There are often a few layers of tissue in the meristem tips that are virus-free, even when the remainder of the plant is virus-infected. By excising these virus-free meristem tips and culturing them in sterile *in vitro* conditions, virus-free regenerated plants can be produced. Bacteria and fungi are considerably easier to remove through meristem tip culture. Viroids, however, are generally found in the meristem tip cells and cannot easily be removed simply by excising meristem tips. For viruses that are difficult to eliminate, thermotherapy or chemotherapy are possible additional techniques.

It is not surprising that the elimination of symptoms from clones showing disease stress would result in yield increases. Many examples of such cases have been reported. More recently, however, it has become evident that improvement of vigour and yield may sometimes be achieved by meristem tip processing of clones showing no disease symptoms. The most plausible explanation is the presence of latent viruses. Information on the extent of infection of cassava clones by latent viruses and yield depression caused by these viruses, should be forthcoming as detection techniques evolve.

In vitro cleaning does have drawbacks along with advantages. Firstly, it is a time-consuming, relatively high-resource endeavour. Rather sophisticated laboratories are required for consistent success and to properly index cleaned clones to confirm pathogen elimination. This type of work is most appropriately carried out in a centralized manner. CIAT, for example, provides this service for some Asian and Latin American countries. The process is time-consuming if the goal is to replace an infected commercial variety with a cleaned version of the same variety. Farmers would have to be convinced of the yield benefits to be derived if they were to go to the trouble and expense of replacement. Also, the potential for re-infection and the length of time that a cleaned clone can be expected to remain clean is crucial information that is very difficult to obtain except with long-term trials.

Work at CIAT showed that in some instances meristem tip-derived (virus-free) material is more susceptible to root rotting organisms that standard propagated material (CIAT, 1990). This appears to be due to the elimination of beneficial micro-organisms found normally inhabiting stake-propagated plants. Considerably more work needs to be done in this field before any conclusions are made. It would seem that this need not present a major obstacle to the use of meristem tip cleaning, because the beneficial organisms could presumably be inoculated on the cleaned material.

13. CHARACTERISTICS OF SUCCESSFUL VARIETIES AND CASE STUDIES

A breeder can learn a great deal about successful varietal release by studying examples of successful varieties. Unfortunately, there is limited documentation on areas planted to specific varieties, making it difficult to classify the degree of success enjoyed by most of the several hundred released varieties. Cassava varieties typically cover narrow niches; every region has its own set of a few or possibly many unique clones. The diversity within the world's germplasm collections attests to this. The exceptions are for a few bred or newly introduced varieties that have been reasonably well-monitored and other situations where only a few varieties occupy most of a country's area. The latter situation applies especially in parts of Asia. Often, the features of a variety that confer success only become apparent when new varieties are introduced in side-by-side comparisons and demonstrate a lack of success.

In this discussion, two alternative situations will be considered. The first is where varieties are deemed successful by virtue of being the markedly predominant variety over a large contiguous area. The alternative definition considers varieties which may not predominate in any one area, but which are at least moderately successful in several countries, i.e. have wide geographical adaptation and adoption.

13.1 SELECTED LANDRACE VARIETIES

Rayong I - Thailand

Based on area planted, *Rayong I* was by far the world's most successful cassava variety up to the mid-1990s. The Thai National Programme selected *Rayong I* from among local varieties and released it in 1975, early in the programme's breeding research efforts. From the beginning of Thailand's cassava boom in the early 1970s, for a period of over 20 years, nearly all of Thailand's one million plus hectares were planted with this variety. However, this changed with success of new hybrids developed jointly by Thailand's Department of Agriculture, Kasetsart University and CIAT.

The main features contributing to its success appear to be an ability for vigorous early establishment even under difficult conditions of drought and nutrient stress, production of high quality planting material, moderate and stable yield and moderate root dry matter. Although the variety combines a range of desirable traits, the one that may have made the greatest contribution to success, as compared with competing varieties, is an ability for good establishment under stress. This conclusion is drawn from the experience of breeders faced with the failure of many introduced materials to establish well in the harsh conditions of Thailand's northeast.

Venezolana - Colombia

Venezolana is the most popular landrace variety in Colombia's Atlantic coast region. Reliable estimates of area planted are not available, but, during the late 1990s, it occupied a larger area than any other clone for a total of some 100 000 ha of cassava in the region. Since that time, new hybrids have been spreading throughout the area. This variety is locally known for its outstanding ability to maintain high dry matter content (related also to high quality for the fresh market) and moderate but stable yields throughout the years. The main reason for the popularity of this variety became more apparent when the early efforts of improvement programmes did not achieve good adoption. Although new varieties yielded well above *Venezolana,* they were not accepted by farmers, largely because of lower quality due to lower dry matter content. CIAT and ICA thereafter gave high priority to quality; yet it has been difficult to achieve the same high level and stability of quality that *Venezolana* expresses.

13.2 BRED VARIETIES

13.2.1 Latin America

The variety *Mantiqueira* (syn. *CMC 40*, *MCol 1468*), bred by the Instituto Agronômico de Campinas (IAC), São Paulo, Brazil, has been moderately successful as a variety in the subtropics of southern Brazil. Just as notable is the success that it has achieved in many other countries, sometimes under conditions very different from those where it was originally selected. It has been independently released as a variety in Cuba, Colombia, Dominican Republic, Haiti and the Philippines. Any given national breeding programme is probably not interested in wide adaptability across countries as a criterion for release, so this trait of itself is not likely to be the predominant factor in success. Nevertheless, it is obviously an indirect contributing factor. The outstanding feature identified across regions where *Mantiqueira* has been successful, is its ability to produce high yield at an early harvest. As this is a feature not commonly found in the species, its presence is a strong attribute. The variety also has outstanding quality for fresh consumption when grown in favourable growing conditions. The storage roots are relatively short and this helps them achieve a commercially useful thickness in a relatively short growing season.

13.2.2 Africa

IITA's root and tuber programme, begun in 1971, first focused on rejuvenating the work on mosaic resistance, carried out by Beck, Ekandem, Jennings and others. By 1977 S.K. Hahn and his colleagues were able to develop high yielding, mosaic-resistant varieties, known as the TMS series (Tropical *Manihot* Selection). IITA, in collaboration with the National Root Crops Research Institute (NRCRI), the Cassava Growers Association, the World Bank, the International Fund for Agricultural Development (IFAD), Shell BP, the AGIP Oil Company, churches and the media, aggressively diffused these varieties.

CMD limits production in much of Africa's broad cassava belt. Few local varieties have levels of resistance that noticeably limit the disease development. New varieties with resistance therefore have a single strong adaptive advantage. *TMS 30526* was one of the early varieties from IITA to combine high CMD resistance with reasonable agronomic quality. It has been strongly promoted in Nigeria and elsewhere and has achieved success in Nigeria. This is an example of where a variety's success can clearly be traced to overcoming a production constraint that seriously affects nearly all other local varieties. In fact, there are fewer opportunities for this type of success than may first seem to be the case. In spite of a large number of biotic and abiotic constraints facing cassava, there is usually not a single one of overriding importance to a variety's acceptance.

13.2.3 Asia

Thailand stands out as the premier example of successful development and promotion of new cassava varieties. It is a story of many factors coming together, including strong joint national public and private support in research, extension and development; long-term, extensive input from an International Centre (CIAT) in research, germplasm and training; market demand for more efficient production to lower costs of raw product; changing markets which demanded higher dry matter varieties and enterprising farmers willing to try a new technology package. From 1993 to 2000, more than 30 000 farmers were trained in new cultivation techniques and 40 million stems of new varieties were distributed free of charge to farmers. Cassava in Thailand is destined almost entirely for industrial use and root yield and starch content are two of the main traits that contribute to success. Chapter 23 illustrates the dynamics of adoption and impact of several varieties in Thailand.

13.3 IMPLICATIONS FOR BREEDING

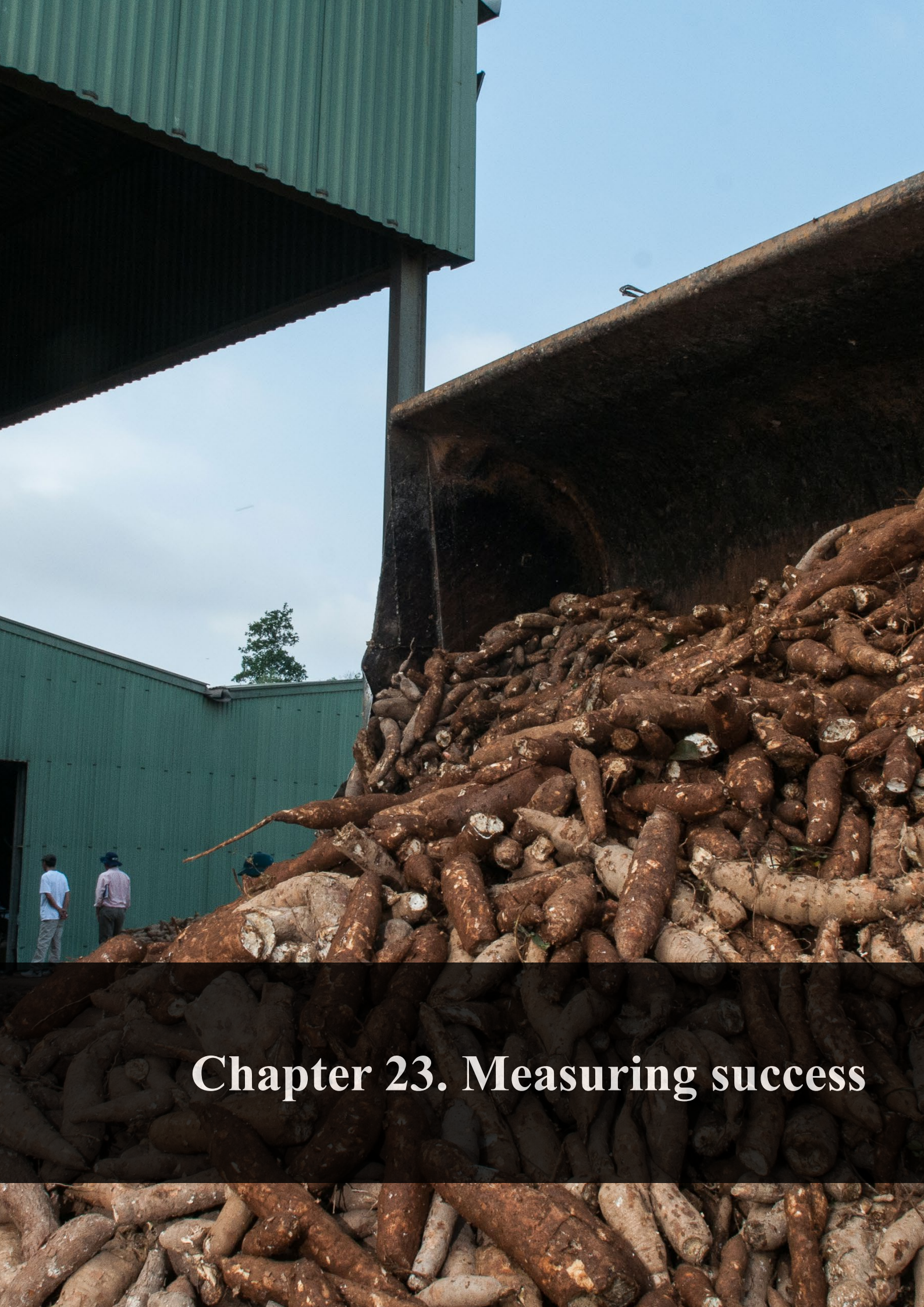
No single feature characterizes the world's most broadly successful cassava varieties, different types of traits appear to have conferred success for each example. Common among the few examples cited is the presence of a locally important trait that is relatively rare in the world germplasm as a whole. Where these types of constraints do not exist, it appears that rather than one or a few successful varieties, it has been possible to select for a range of acceptable clones.

The implications for breeding are not straightforward. However, these experiences may indicate that in most situations it will not be realistic to expect a new variety to gain very broad acceptance unless it addresses a well-defined need among growers and/or consumers. Varieties that make only slight improvement in a range of traits may have moderate, but not spectacular success. Generally, a successful new variety needs to meet farmers' needs in a way that is immediately convincing without the need to demonstrate the advantages with sophisticated statistical tests. Certainly, rigorous statistical comparisons need to be made in order for a breeder to release a variety, but farmers themselves will only be convinced to adopt it if the advantages are a quantum improvement over existing varieties. This situation is likely to change as farmers become more accustomed to evaluating and adopting new varieties. When they gain confidence in the breeders' and extension agencies' ability to provide superior genetic material, they will begin to accept varieties with lower margins of superiority.

Successful varieties do not share a common history of distribution and promotion. Some achieved widespread cultivation without any intervention by research or extension, while others relied heavily on institutional input. Farmers themselves are the principal actors in the process of deciding a variety's acceptability and its distribution to other growers. Nonetheless, most successful varieties in recent years have been strongly promoted by supporting institutions such as a department of agriculture or the extension service, the private sector, or NGOs. Aggressive promotion has been most successful in Africa and Asia. Thailand is a classic example of institutional commitment to assuring success of new varieties. In 1992, just as soon as breeders and the extension service were able to give convincing proof of improved returns from new varieties (higher starch was an important component of this), the Department of Agriculture and the Department of Agricultural Extension embarked on massive multiplication and distribution of planting material. They established a goal of replacing 240 000 ha of *Rayong I* with new hybrids by 1996 and met this goal ahead of schedule.

Success of cassava varieties in Africa has often been driven by an urgent need to resolve a major yield-limiting constraint, usually CMD. This has been most notable in Nigeria, which has the advantage of proximity of IITA's breeding programme and Uganda with its disastrous outbreak of a new variant of CMD in the mid-1990s.

The most difficult situation in which to make impact with new varieties is in stable traditional systems without new market opportunities. Clearly, farmers need to be motivated to improve their livelihoods, or to prevent erosion of their level of well-being, in order to adopt new technologies.



Chapter 23. Measuring success

1. ALTERNATIVES TO ASSESSING PROGRAMMES

The objective of breeding is genetic improvement and from a practical standpoint, the breeder needs some measure of progress in order to justify continuing investment in research and to optimize a research strategy. Many methods of measurement are possible. The more convincing ones normally are those that closely follow the acceptability criteria of client groups.

Plant breeders typically measure progress in terms of yield gains, even when it is only one of a few or several breeding goals. Usually this is a valid measure, but can rarely be used in isolation from additional criteria. Yield normally integrates the effects of genetic modification of a number of component traits. Even where other target objectives are considered (such as pest or disease resistance, adaptation to climatic or soil stresses, or plant architecture) the improvement is usually of interest to the farmer only when it gives higher economic yield. Selection for improved quality may be completely independent of root yield and is sometimes associated with yield decline, yet a market premium for high quality may more than compensate for lower yield. In the case of cassava, one of the principal criteria of quality is dry matter content. Yield comparisons should be either on a dry weight basis, or include percent dry matter content. Using both measures when assessing genetic progress avoids the pitfall of demonstrating yield gains over time at the expense of root dry matter content. However, quality is not only associated with dry matter content, but also many other factors with no direct association with yield (e.g. cyanogenic potential).

Measures of progress should reflect the full range of criteria considered in selection, either jointly or individually. A breeder is often under pressure to demonstrate genetic advances rapidly, in as little as two or three years after initiating a programme. The impracticality of this is rarely appreciated by research administrators. Nevertheless, where this expectation prevails, it often obliges the breeder to illustrate some form of short-term progress. This may be something as simple as demonstrating the implementation of a research plan, or as complex as showing changes in gene frequency in improved populations.

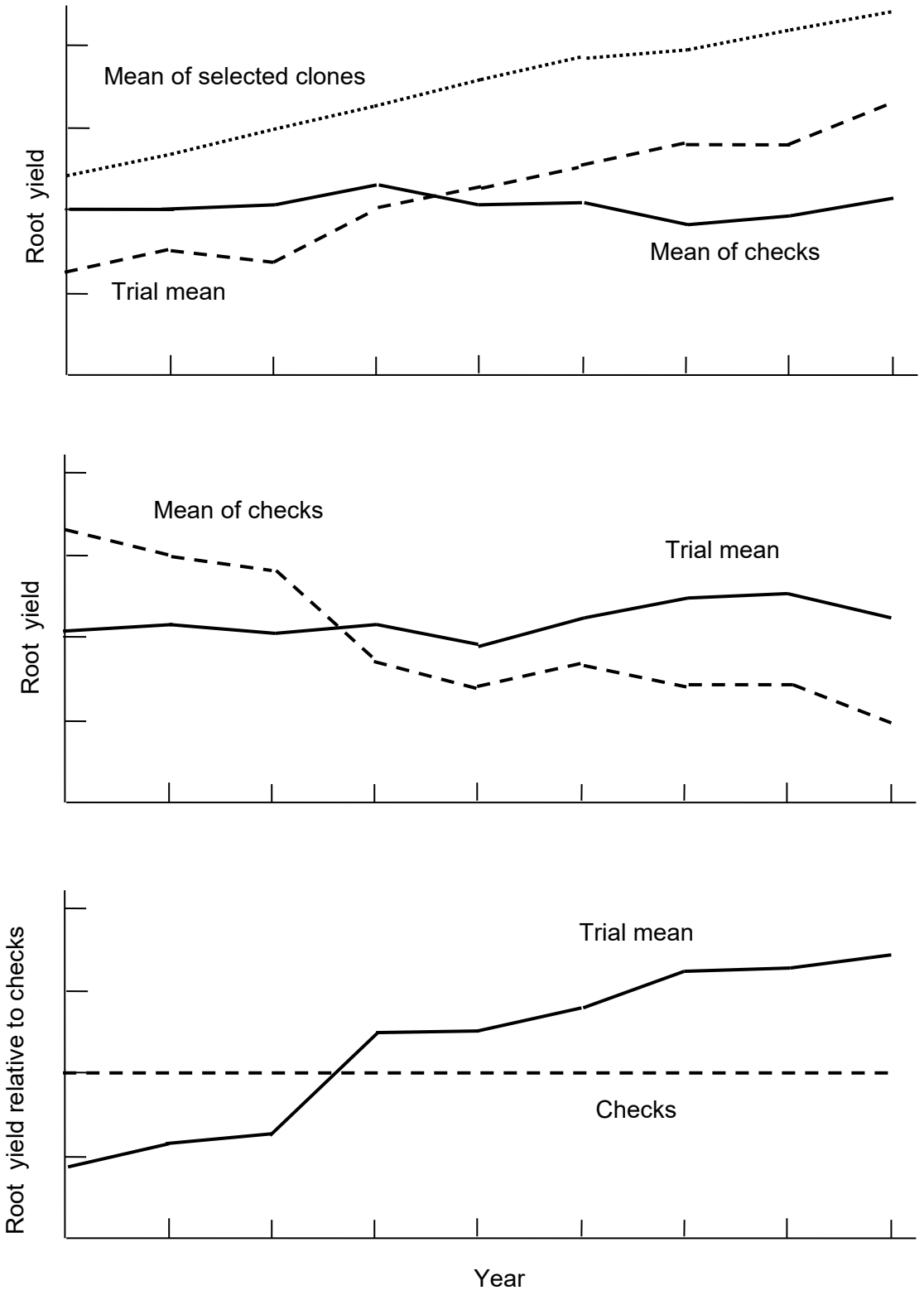
A practical method of monitoring progress is to plot the various parameters of interest as a function of time (usually a data point for each year). Each data point may represent actual performance (e.g. yield, dry matter content, resistance ratings) or values relative to check varieties. Various groupings of clones may also be informative. Change in the mean value for all clones in a trial (excluding check varieties) gives an indication of how the total breeding population is changing. The mean of the best clones each year, for example, the best five or ten, indicates the upper-end potential of materials in a programme. Plotting the single best clone each year may not provide a very realistic measure of progress in that it will normally exaggerate values by including those with high environmental error, or high G–E interaction values (Figure 23.1).

It is quite possible to have stagnant yields over time, yet make genetic progress in improving yield potential. In fact, this is not an uncommon situation in cassava. The main reason this occurs is that cassava gets pushed into continually more marginal conditions. Rather than experiencing a yield decline, farmers can realize constant yields, through improvement in stress adaptation. In this scenario, comparison with constant check varieties over time becomes an important baseline. At one of CIAT's selection sites in the Colombian eastern plains (ICA-Carimagua), advanced yield trials planted near the end of the rainy season showed relatively constant yields over a ten-year period. However, when yields were compared with constant checks, there was an increasing yield advantage for the hybrids. Actual yields reflected an increasing pest and disease pressure in the environment. The resistance was gradually incorporated into the hybrids and they tolerated continually higher pressures while the check showed a greater yield loss over time (see Figure 23.1, middle graph).

In a mature breeding programme, measures of progress can include number of variety releases, area planted to new varieties, or economic/nutritional benefits from adoption of new varieties. Breeders normally have neither the training nor the resources to conduct sophisticated impact studies, but can collaborate with economists and social scientists to assure a solid plant science basis for this work. In

recent years there has been a number of formal impact studies for technology adoption and the experiences are allowing continual refinement in methodologies appropriate to cassava.

Figure 23.1 Measures of progress in cassava varietal development



In other crops with long and intensive breeding efforts, half of yield gains are typically attributed to improved agronomic practices and half to improved genetic potential. Cassava probably has not had a level of genetic improvement on a global basis that allows us to assign a 50 percent contribution of breeding to yield increases. During the period from 1961 to 2004, the global average yield/ha rose from about 7.4 tonnes/ha to 10.9 tonnes/ha, an average increase of 1.1 percent per year. Based on localized and regional impact studies and empirical observations, one might safely assign 25 percent of yield gains to genetic improvement. The world produced 3.5 more tonnes of cassava/ha in 2004 than in 1961, 25 percent of this is equal to 0.875 tonnes/ha. On a total of 17.87 million ha planted, yield gains from breeding would be 15.64 million tonnes. If cassava is valued at US$30/tonne, the total value conferred by improved genetics in 2004 alone would be US$469 million. While there is no way of obtaining accurate figures on annual expenditures on cassava breeding on a global basis, it could be assumed that 100 breeders worldwide, together with all the administrative and support costs of their respective institutions, in addition to all the programmes that indirectly feed into a breeding programme, such as biotechnology research in advanced laboratories, each globally encompass US$0.5 million, for a total annual expenditure of US$50 million. This is nearly a 10:1 benefit:cost ratio for research on cassava genetic improvement.

2. DYNAMICS OF VARIETAL CHANGE, AND IMPACT ON NATIONAL YIELDS

Since the beginning of cassava cultivation, farmers have been evaluating new varieties and discarding old ones. The widespread availability of improved materials from breeding programmes began to change these dynamics more than 50 years ago, but only began to be a substantial force at the end of the twentieth century. Several cases of successful diffusion of new varieties, under very different circumstances, which can provide important lessons to future programmes, will be explored.

Nigeria steadily increased cassava production to become the world's top producer by the early 2000s, surpassing Brazil and Thailand. One or the other of the latter two countries had held that distinction since the beginning of record-keeping, and Brazil probably since several thousands of years ago when cassava was first cultivated. Uganda is a case where the driving force for varietal adoption was the need to solve a severe outbreak of a new variant of CMD. In Thailand, the main local variety continued to perform well, but new hybrids were able to give enough of an economic advantage to motivate farmers to replace nearly 100 percent of the local variety with new varieties over a ten-year period. In Colombia, much of the demand for new varieties derived from government and private sector initiatives to locally produce carbohydrate sources for balanced rations for poultry.

In a survey of expert opinion, Gabre-Madhin and Haggblade (2003) targeted a selected list of agricultural specialists with a single question: "What do you consider the most successful instances of improved agricultural performance in sub-Saharan Africa?" Africa-wide, cassava had the highest commodity-specific percentage of successes, followed by maize and livestock, with all other commodities well behind. Survey respondents cited both the periods of the 1920s to the 1930s and the 1970s to the 1980s, as having significant impact from new cassava varieties. In both cases, disease resistance, particularly CMD, played a key role in the success of the varieties.

Every country that sees adoption of new cassava varieties will observe a different dynamic, but the most common feature will be popularity of different varieties for different regions, for different farming systems and for different market situations. There will be very few cases where breeders develop such a prominently successful variety that displaces everything else. There is every reason to believe that breeders should take every step to assure that a number of acceptable clones are available to farmers.

2.1 NIGERIA

Nweke (2003) describes the diffusion of the TMS varieties from IITA as a key element of the cassava transformation in Nigeria, especially in the period from 1984 to 1992. Some of the main forces behind this success were: a ban on the subsidization of imported food grains; inclusion of cassava in major government-funded agricultural extension programmes; and government investment in measures to diffuse the new varieties. By 1989, 60 percent of survey villages (COSCA) grew the new varieties. The combination of new varieties and mechanized gari processing were the main contributors to a rapid rise in per capita cassava production from the mid-1980s to the early 1990s. By 2000, only 10 percent of Nigeria's cassava was processed for cattle feed (compared with Brazil, for example, with 56 percent) (Nweke, 2003). This illustrates a high unexploited potential to further increase the spread of new high-yielding varieties, as this market grows.

2.2 UGANDA

Like much of Africa, cassava in Uganda has become an increasingly important staple in the past century. After an outbreak of CMD from 1933 to 1944, the Government of Uganda instituted a campaign to require each farmer to grow 0.4 ha of a CMD-resistant variety as a buffer against famine (Bua *et al*., 2005). Cassava yields increased slowly (from very low base levels of 4–5 tonnes/ha) during the 1960s and 1970s and then nearly doubled within a few years at the end of the 1970s. Throughout the 1980s and most of the 1990s, yield steadily declined, with a somewhat sharp decline in 1993 and 1994. From a high national yield of almost 10 tonnes/ha in 1981, yields had dropped back to less than 7 tonnes/ha in 1994.

While this decline was the result of a combination of many factors, by far the main one was the spread of a new aggressive mosaic virus race. This motivated a steady rise in the adoption of improved virus-resistant varieties. About 20 percent of farmers were using new varieties in 1993 and this increased to over 80 percent in 1999. At the same time there has been some short-term success in producing clean planting material of susceptible clones and identifying tolerant local varieties (Bua *et al*., 2005).

While the dissemination of new varieties in Uganda has been a clear success in the last decade, long-term countrywide yield trends have been trending upward for many years. One can only imagine how dramatic progress could have been achieved without the need to focus research so intensively on CMD resistance. Mean national yields are now close to 14 tonnes/ha, nearly 50 percent above Africa as a whole. Instead of the new varieties succeeding only in achieving the pre-CMD epidemic levels, in addition to solving the CMD problem (where deployed), they also showed a greater yield potential than the local clones.

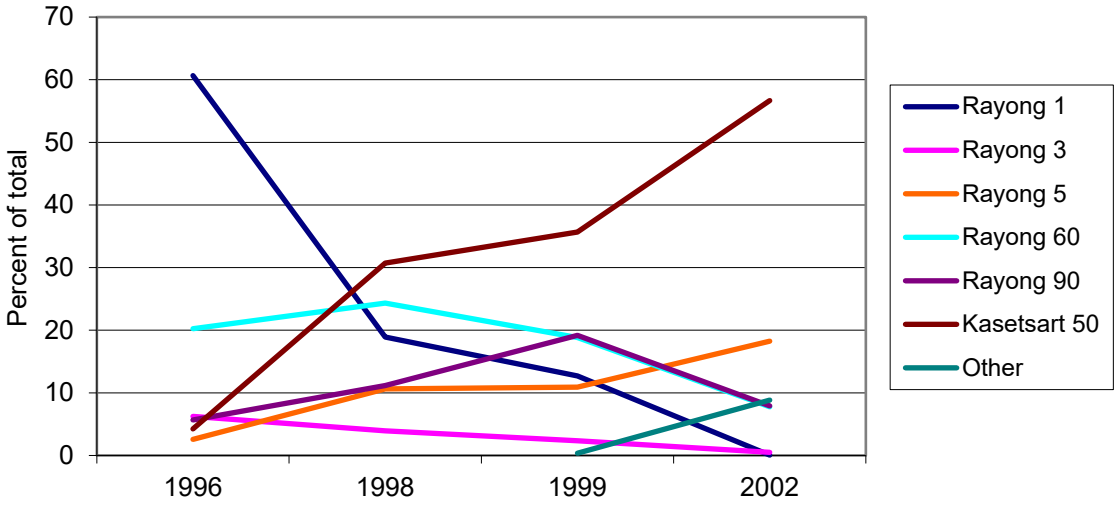
2.3 THAILAND

Thailand has long been among the countries with the highest mean yield of cassava, in spite of the fact that most of the crop is grown under difficult conditions of water stress and low-fertility acid soils (Northeast). From 1961 through the mid-1990s, yields were relatively stable at approximately 15 tonnes/ha, to slightly declining (FAOSTAT). Thereafter, yields climbed quickly and steadily to 2004 (latest available data). The flat yields during the 35 years before 1995, in spite of substantial research into improved management and a successful breeding programme, are largely owing to the spread of cassava into ever more stressful conditions, where other crops were unable to thrive. Thailand has experienced no significant threat from pests or diseases and breeders could concentrate on yield potential to a far greater extent than in Uganda. This situation meant that when new varieties began to spread rapidly in the mid-1990s, there was a direct response in national yields.

Thailand was a unique situation in that close to 100 percent of the area was planted to a single landrace variety, *Rayong 1*. This made tracing the change in varieties more clear-cut than in any other country. Figure 23.2 illustrates the dynamics of this replacement of the local variety with the most popular new hybrids and the subsequent shifts in balance among the new varieties. With the availability of a number of options, farmers will multiply or discard new varieties to fit their needs. While there seems to be a

relatively low level of interest in a number of clones, it is somewhat remarkable that the single hybrid, *Kasetsart 50*, is gaining popularity at almost the same rapid rate as the disappearance of *Rayong 1* fifteen years earlier.

Figure 23.2 Proportional area planted to Thai cassava varieties



Source: Rojanaridpiched et al. (2002)

3. COSTS AND BENEFITS OF CASSAVA BREEDING

3.1 OVERVIEW

Private-sector plant breeding companies keep very close tabs on the bottom line – company profitability – which for most is closely related to the income generated from selling seed, compared with the cost of producing and distributing new varieties. Public and private non-profit institutions often pay less attention to the market value of the varieties they produce, either because their goal is altruistic and not based on the users' ability to pay for the product, or because the funding entities (such as tax-payers) may be less demanding of this information. As there are no private companies pursuing cassava breeding as of the early 21st century, stakeholder pressure to account for research spending in terms of payoff for variety end users has been limited or indirect. This is changing, however, as tax-payers and donor organizations increasingly expect transparency in the use of funds they provide and the benefits that accrue. Research organizations are finding that they need to put more resources than ever before into monitoring economic and social impact. The types of impact that donors expect can vary widely, but in the case of cassava breeding, this generally means, at a minimum, having reasonable information about adoption of new varieties and the increases in yield that have occurred as a result. Programmes should expect also to be able to provide further information on how adoption impacts farmer income and well-being and effects (positively or negatively) the environment. More extensive impact studies can become major projects in and of themselves and generally go well beyond the purview of a plant breeder's project management area.

Breeding programmes have been reluctant and slow to invest in research on adoption and impact, because breeders feel that this money is being taken away from their ability to invest more in breeding research *per se*. They are often convinced through their own observations, which usually include extensive travel throughout the target production area, that they have a good grasp of the extent of adoption. Their extensive research data from variety comparisons, often in combination with on-farm

data, give a good basis for understanding the benefits of new varieties. While in the past, this level of information was often sufficiently precise to satisfy funding agencies, most programmes can now expect to be required to show results from more independent surveys and evaluations and to be able to quantify costs and benefits reasonably precisely.

Defining the direct costs of a breeding programme is generally not very difficult. Every programme has a budget and needs to account for its expenses. The indirect costs can be rather complex to define and may in fact be a substantial part of the total costs of varietal development and adoption. Costs for inputs from collaborating institutions, for the time and land resources of farmers and for training are part of the total.

Benefits may be more complex to decipher. The means by which benefits are measured will depend upon the programme's goals in the first place. If the goals are to increase on-farm yields, this can be accomplished at several levels, e.g. by questionnaires, on-farm sampling, market monitoring, or by regional or national yield statistics. Each provides some segment of the whole picture regarding impact.

Probably the most common (and one of the least accurate) means of illustrating impact is to extrapolate yields from research plots to regional or national production. This is almost always seriously erroneous and should only be used to establish potential impact in a broad and theoretical way, or possibly to illustrate yields that the best farmers could obtain.

Typically, the introduction of new varieties, to replace existing ones, is not the only change occurring over a period of time in farmers' fields. If the new varieties are intended to solve a disease problem, farmers may gain nothing in yield, but they could reverse a declining yield with the old varieties. This type of impact may show little benefit when on-farm yields or market data are compared, but depends on evidence that the new varieties are responsible for yield stabilization. This type of impact has been common in Africa, where many programmes have focused on resistance to cassava mosaic disease.

In a similar way, breeding varieties for better adaptation to drought or soil aluminium stress may allow expansion into areas previously not cultivated. This type of area expansion has been typical with farmer-selection and their ability to use locally available genetic diversity to adapt materials into new environments. Breeders are only recently making progress in this type of selection.

For these reasons, one of the most difficult levels of impact to demonstrate is at the national yield level. For cassava, in particular, there are so many confounding effects between the farmers' decision to adopt and the national production statistics, that it is usually very difficult to use the latter as a measure of impact (or conversely, to claim that breeding has had no impact if national yields have not improved). For reasons to be explained below, this is now changing for some countries.

While new varieties may not require improved management practices to show improved yield over local landraces, it is typical for farmers who successfully adopt one technology component to become more open to adopting others. In the case of cassava, this often means that new varieties are accompanied by agronomic practices such as better weed control, more optimum plant spacing, greater care in management of planting material and installation of erosion control practices or fertilizer application. The different practices and the new varieties may each have an additive effect on yield, but more likely they interact with each other in complex ways that make it difficult to weigh individual benefits.

Private companies generally do not become involved in analysing impact at this level of complexity. They try instead to convince farmers that their varieties will meet specific needs and if farmers buy the seed, that is the only impact that matters. Also, for public breeding efforts, if farmers make a decision to grow new varieties, on a continuing basis, that is probably the best indicator of success. Despite what any impact assessment may show at the level of social or economic benefits, the farmers' adoption can normally be taken as evidence that farmers themselves are satisfied that there are positive benefits.

Very few thorough impact studies have been accomplished for cassava breeding. Nearly all studies are based on adoption as the single most important measure of impact and when they go to the next step of measuring yield or income improvement, this is usually based on limited experimental comparisons between new and traditional varieties. It can be expected that more precise techniques will begin to be applied under pressure from funding agencies.

3.2 A CASE STUDY

Only in recent years has the level of adoption of new cassava varieties been reaching a magnitude that allows the usage of macrolevel production statistics to measure impact in some countries. The best data are available from Asia. Thailand is the most notable case and this country has served as somewhat of a central point for the development of widely adapted varieties throughout the region.

New cassava varieties have had important localized impact on cassava production in Asia for many years. India, with one of the earliest comprehensive breeding programmes in the region, achieved steady impact, probably from the 1970s. In the period 1961 to 1995, yields in Asia increased at a rate of 0.18 tonnes/ha/year (FAOSTAT, 2005) (Table 23.1). Most of this could be attributed to improved production practices, since adoption of new varieties was just beginning to take off by the end of that period. Beginning in the mid-1990s, new varieties spread rapidly in some of the major producing countries, especially China, Indonesia, Thailand and Viet Nam.

Table 23.1 Yield trends for cassava by region (average change in yield [tonnes/ha/year])[a]

	Yield gains by decade				Avg. 1961-2004	1995-2004
	1961-1969	1970-1979	1980-1989	1990-1999		
Africa	0.04	0.11	0.11	0.07	0.09	0.08
Asia	0.12	0.29	0.13	0.08	0.17	0.42
Latin America and Caribbean	0.17	-0.26	0.11	-0.02	-0.02	0.15
[a]Based on linear regressions for each time period *Source: FAOSTAT, 2005*						

For purposes of calculation, all of the 0.18 tonnes/ha/year from 1961 to 1995 are attributed to improved agronomic practices and to date, assume a continuation of this level of impact. As average yield gains per year reached 0.42 tonnes/ha in the period from 1995 to 2004, 0.24 tonnes per year (0.42 tonnes [total], minus 0.18 tonnes [agronomy contribution]) is attributed to the impact of plant breeding. Therefore, in 2004 the contribution of new varieties would be in the order of 2.1 tonnes/ha, as an average for all of Asia, compared with 1994. With cassava planted on 3.5 million ha, total yield gains from breeding were 7.35 million tonnes. At US$25/tonne, total added value in 2004 alone was US$184 million.

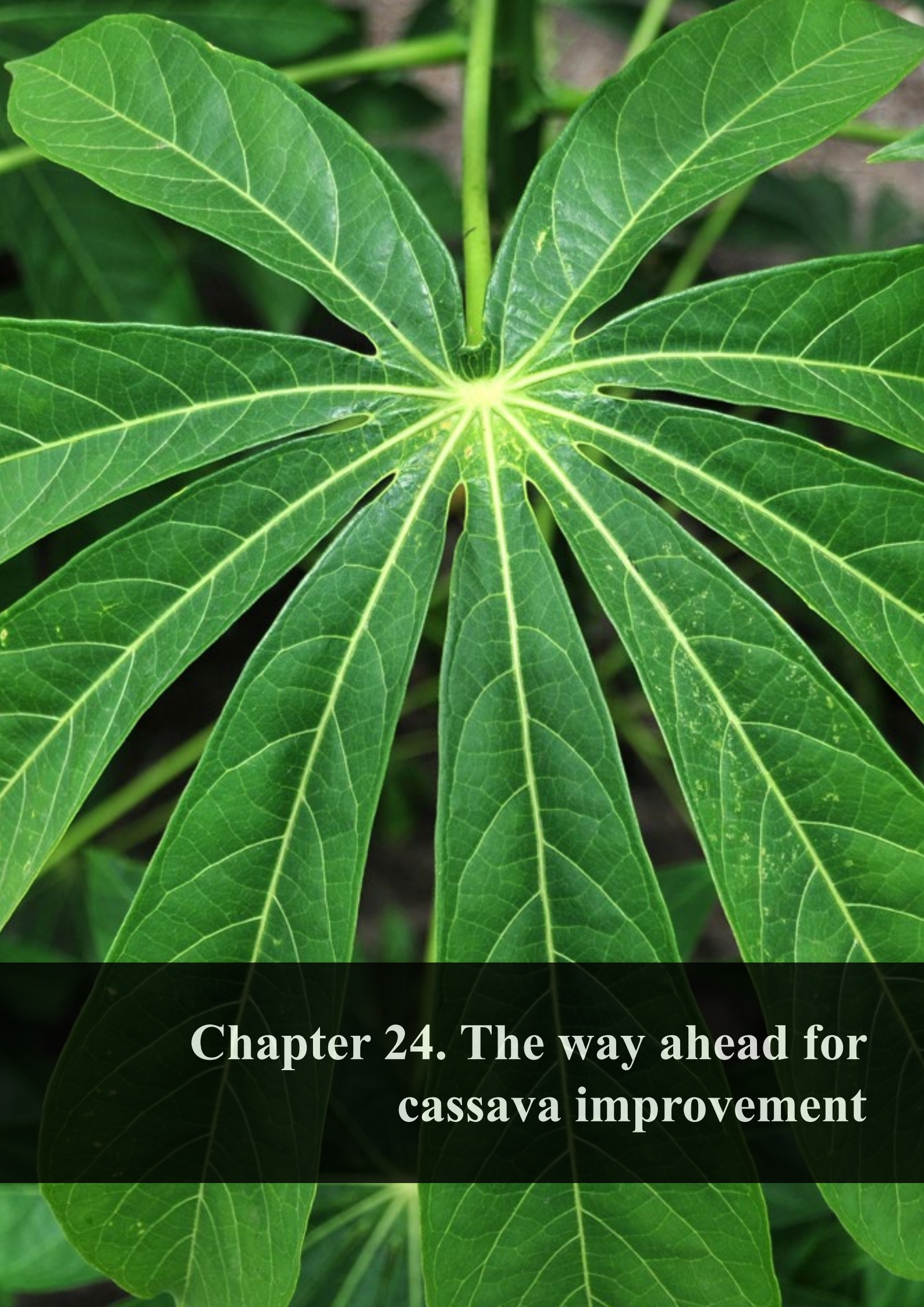
An impact assessment with over 800 farmers in Thailand and Viet Nam showed half the yield increases from new varieties and half from improved agronomic practices, especially fertilizer application (R. Howeler, personal comm.). With this assumption, the contribution from yield increase due to breeding was still US$92 million in 2004.

There is another aspect to the benefits of the new varieties that is not evident from the national statistics: the new varieties in Thailand have a 5-10 percentage point increase in starch content (from 18 percent for the old varieties to 23-28 percent for the new varieties). Cassava buyers now pay farmers based on starch content, so these improved levels correspond to an additional US$2.5-US$5/tonne. Not only does this bring greater income to the farmers, but also to the processors who can dry or extract starch more efficiently in the high-starch varieties. Thus, adding a conservative US$3/tonne to the value of production, to a yield gain of 3.68 million tonnes (half of the 7.35 tonnes total increase in production), gives another US$11 million in added value of production, for a total of just over US$100 million.

By way of comparison, using survey data, Howeler (personal comm.; project report to Nippon Foundation) calculated an increased gross income of US$272 million in 2003 as compared with the base year of 1994, with about half (US$136 million) attributed to new varieties and half to agronomic practices. Probably the higher figure is closer to the actual benefit from new varieties, given some of the confounding factors on national yields, described above. Cassava has continued to expand into marginal lands, where, without the benefit of new varieties, yields would actually decline. Benefits from cassava breeding in Asia have probably exceeded US$1 billion since 1990, with considerably lower benefits prior to that date.

Investments in breeding are difficult to ascertain with any precision. Kawano (1995) estimated direct costs for Chinese, Indonesian, Philippino and Thai national programmes at US$5.5 million for the 20 years from 1975-1994 and for the CIAT cassava breeding programme at US$11.3 million. With about 300 000 ha planted to new varieties at that time, the benefit:cost ratio was estimated at 7.6:1. These estimates do not include the extensive breeding effort in India, since they had considerably less direct collaboration with CIAT.

Total investment across all Asia in the development and promotion of new cassava varieties (including institutional overheads, inputs from related disciplines like physiology and entomology and extension efforts) probably averaged in the order of a few million US dollars per year, from 1974-2004. If US$1 billion were conservatively attributed in benefits from breeding in the period 1975-2004 and an investment made of US$60 million, the benefit:cost ratio would be 16.7:1 (US$16.7 return to growers, processors and consumers for every US$1 invested in research). In actual fact, the payback rate is logarithmic, while the investment rate is more linear and level (actually, declining in the last decade). While the additional costs to farmers of growing the new varieties is not well documented, these costs are clearly well below the additional income they generate. Hence, the benefit:cost ratio will continue to rapidly increase for some time into the future. If investments can be reestablished at adequate levels to rejuvenate cassava breeding, it is clear that strong positive returns can continue indefinitely, as they have for other major crops.



Chapter 24. The way ahead for cassava improvement

1. A REVIEW OF PROGRESS

Formal cassava breeding has been in progress for almost a century, although there were few comprehensive, long-term efforts until much more recently. The earliest programmes grew out of industrial interest in cassava starch in Asia and were aimed at plantation culture. A new era of interest and investment began in the 1970s, with the establishment of two international research centres (CIAT and IITA) and the establishment or renewal of many national research programmes. International training and the formation of regional or global networks gave a critical boost to cassava genetic improvement.

Efficient and effective breeding programmes only became possible as basic information about the crop (genetics, physiology, pest problems, climate and soil response, agronomic requirements) became available, mainly since the 1970s. These initiatives have brought clear benefits to cassava growers and consumers on all continents, through the application of basic science to the production of improved varieties.

Breeders have a vast germplasm base with which to work. The late 1960s through the mid-1980s saw the major part of the world's cassava genetic diversity established in *ex situ* collections. Cassava genetic resources, the biological base for crop improvement, consists of a comprehensive international collection at CIAT, Colombia, a regional collection at IITA, Nigeria and working national or regional collections in most cassava-growing countries. Collection was most important for the Americas, evolutionary homeland of the crop, but the adaptation and diversification within Asia and Africa also contributed to genetic variation of value to breeders. At present there is usually little distinction between the breeders' working collections and those held for long-term conservation. There are, in fact, no existing base collections, following the definition of IPGRI.

Cassava breeding has been largely a public-sector enterprise. Most farmers have a strong tradition of producing their own planting material (stakes) and there are many challenges for a private entity to make a profitable business of promoting new varieties. While the private sector provides increasing support to cassava breeding, it is still at a very low level and is mainly channelled through projects within public programmes.

There has been a tendency for cassava breeding to be strongly associated with social goals. Usually this is an outgrowth of the fact that a high proportion of cassava growers are small farmers, on some of the less productive land (often due to low soil fertility, soil acidity and/or drought stress). Although cassava was long considered a rustic crop that required little attention between planting and harvest, it is now clear that high and stable productivity is only possible with good management. Pests and diseases can be major production constraints, especially in Africa and the Americas. With the expansion of industrial markets in the latter part of the twentieth century, both internal and for export, some breeding programmes moved towards breeding for responsiveness to higher inputs and more intensive management.

Biotechnology research on cassava has made progress parallel to that in many crops. This is all the more remarkable when one considers the near-absence of private sector research and overall relatively low funding for cassava research in general. A global network (the Cassava Biotechnology Network) functions in a highly collaborative spirit, supported by government and private funding and includes several leading-edge laboratories in developing and developed countries.

2. THE NEW LANDSCAPE

2.1 AGRO-ECOLOGICAL

There seems to be overwhelming justification for breeders to continue to capitalize on cassava's special adaptation to less favourable tropical environments, especially drought and acid soil conditions. The species' comparative advantages here, evolved and fine-tuned over thousands of years, should be fully

exploited. These advantages are already well-understood by cassava growers. The long-term effects of global warming are likely to include accelerating desertification in sub-Saharan Africa. Drought tolerant crops, including cassava, will be increasingly critical for agricultural viability. At the same time, advances in breeding of other crops for better adaptation to these stresses, will give farmers and consumers (who may now be limited mainly to cassava) more options.

Cassava's high yield potential in favourable conditions has long been known, but the justification for further breeding has been limited to a few specific situations where the agro-ecological, social and economic environments combine to make this strategy viable. Trends toward free trade and improved economic conditions in many countries are creating new demand (mainly industrial) for cassava products. This growth in demand will make cassava economically competitive in some more favourable environments, where it previously was not. Although many current varieties can respond well to better conditions, intensive breeding will be needed to fully exploit genetic potential.

Most cassava farmers grow the crop for a combination of reasons, including its suitability for their cropping environment, their familiarity with its cultivation and its role as a source of food and income. However, some farmers would prefer to grow more profitable crops, or a broader range of crops and are unable to do so because the soil is too poor for anything but cassava. Northeast Thailand, for example, is one important cassava-growing area where policy-makers have been attempting for many years to diversify agriculture. The most appropriate research strategy aimed at these farmers may be to consider cassava as a transition crop, whose purpose is in part to foster investments in soil improvement to the level of allowing the cultivation of other more demanding crops. Strategies for soil management in cassava have been continually improved in past decades. A coordinated strategy involving breeding, crop management and soil management could succeed in soil improvement for a crop upgrade. Some cassava scientists may see it as a research failure, if farmers discontinue growing the crop, but quite the opposite would be true if this change is part of a plan motivated by farmer interests.

2.2 POLITICAL

Partly because cassava plays a critical role in poor households throughout the tropics and partly because there is a comparatively lower expectation that another country will gain an unfair competitive economic advantage through cassava technology development, governments have generally been open about sharing germplasm and technology. Restrictions on germplasm movement for quarantine reasons have been in place for decades in most countries. These restrictions are becoming more stringent as the risks of the spread of pests and pathogens become clearer and detection techniques are refined. However, restrictions on germplasm movement based on intellectual property rights are not yet common, with the exception of a limited number of transformed varieties at the experimental stage of development.

Thailand probably has more reason than any other country to be protective of its new cassava varieties, due to potential competition from production in neighbouring countries. However, it has freely shared germplasm as a goodwill gesture. Brazil, with the world's richest cassava genetic diversity, has provided duplicates of its national collection to CIAT, with the knowledge and understanding that it then becomes freely available worldwide. It has also been a key participant in a project to expand Africa's germplasm base (Porto *et al*., 1994). The issue of recognizing farmers' rights in germplasm development has had minimal impact on free availability of germplasm, since no there are essentially no for-profit entities directly involved in cassava genetic improvement.

Technology from advanced laboratories is frequently patented, usually because of its application to crops other than cassava and its distribution is more restrictive. Nonetheless, many of the gene constructs are, or will be, available without royalty or licensing fees to developing countries. Cassava researchers have enjoyed an unusually liberal attitude among most government and private holders of germplasm, regarding free access. The fact that cassava is not grown in the developed world tends to create less reluctance for sharing. Some technologies, such as constructs for starch modification, are being developed with support from private companies and will not be freely available.

Development of an efficient regulatory environment for transgenic crops has been painfully slow in most countries. For cassava, this has not yet been a major impediment to moving technology into the hands of farmers, since only a few traits are at the stage where field testing is appropriate. Nonetheless, this will have major repercussions on potential impact on the medium-term future, as the availability of transgenic traits expands. Without well-funded advocates to educate policy-makers and to help guide the process, a protocol for cassava is likely to remain stalled in many countries for years to come. The most likely and most effective advocates can probably be producer and processor groups whose self interest in receiving new technology should be a good motivation. Breeders, biotechnologists and research managers should work with these groups to coordinate a strategy for government education and advocacy.

Cassava breeding research depends fundamentally on government funding. Most of this funding comes in the form of support to national breeding programmes, which are almost always part of a comprehensive national agricultural research system. Another component is the pool of international funding, much of it also from politically influenced government sources, that supports both the international agricultural research centres and national research programmes, often through competitive grants. On the whole, funding for cassava breeding expanded during the 1970s and 1980s and shrank seriously in the 1990s. Most programmes survived, but often at a seriously reduced level of activity. The funding tends to be much more restricted, with research objectives more influenced by donor agencies. Breeders often have less influence over their priorities than in previous years.

There is a serious risk of further decline of support for broad-based, field-oriented cassava breeding. Funding agencies are often captivated by the theoretical potential of biotechnology research to improve cassava, without recognizing the fact that this technology can only contribute to the crop's success by feeding into a comprehensive and effective breeding programme. For most crops in developed countries, the public sector has withdrawn from plant breeding to avoid duplication of private company research. The same trend is in progress in developing countries, but often without regard to those crops like cassava that have much less chance of attracting major private investment. Cassava breeders and research managers need to make the argument, in multiple ways and multiple times, to convince policy-makers of renewed support to a crop that plays critical socio-economic and environmental roles in many tropical countries.

2.3 SOCIO-ECONOMIC

From a socio-economic perspective, cassava production can be classified as: (1) subsistence (little linkage between producers and markets; on-farm use of the crop); (2) small, resource-poor farmers linked to traditional markets with little growth potential; (3) the same farmers linked to growth markets; and (4) large-scale producers linked to growth markets. As cassava has long been seen as a crop with a strong social equity role, breeders generally are conscious of the need to incorporate social factors into research planning and implementation. The literature makes it clear that cassava breeders generally aim to improve people's lives, rather than improve the crop as an end in itself.

Socio-economic trends in different countries will clearly have implications for breeding. At the one extreme, there will continue to be subsistence cultivation in regions like inner Amazonia and Central Africa. These areas might benefit from cassava genetic improvement, but there are generally other types of social investment with more critical long-term impact, like education and health care and infrastructure to allow access to markets. At the other extreme there will be technology-intensive production for industrial markets that demand specific traits and a specific production schedule. The vast majority of production will lie between these extremes, but with a generalized movement toward market-oriented production, for diverse and demanding markets. Cassava breeding is a key to success of market-oriented production at all levels of complexity, whether for human food, animal feed, or industrial starch.

The parameters for subsistence production can be very stable year after year; change tends to occur slowly and predictably. For example, the change from shifting agriculture to continual cultivation

usually takes place over a long time, as population pressure builds. On the other hand, many commercial markets are in constant change, in order to adapt and remain competitive. Most breeders will benefit from the input from economists to analyse market development and new market potential.

The breeder is in a nearly untenable position of having to predict socio-economic trends and varietal demands 10–15 years ahead of the time they are needed by growers. This, however, is the nature of crop genetic improvement and plant breeders have been eminently successful in meeting these challenges in many crops. It is a compelling argument for a breeding programme to establish somewhat diversified objectives, rather than to focus strictly on current problems and opportunities.

2.4 RESEARCH AND TRAINING ENVIRONMENTS

Cassava breeding is always part of an organization (e.g. research centre, university) that includes a range of research thrusts. The manner of organization within an institution has important implications for how a breeder works with collaborators. In broad terms, institutions are often organized either on a commodity basis or a disciplinary basis. In the former, an interdisciplinary team of scientists works on a crop or group of crops. In the latter, a disciplinary team (e.g. entomologists) works across a range of crops. Universities are generally organized along disciplinary lines and this provides a model for many national research programmes. CIAT and IITA initially organized along strong commodity lines, but in the 1990s, this transformed into a structure that is based on projects, which intend to efficiently utilize both commodity and disciplinary structure and expertise. While it is clear that there are various possible research structures that can be effective hosts to a cassava breeding initiative, there are some key considerations for research administrators. At the forefront is the fact that cassava breeders constitute a very small group of specialists, rarely more than one in any given institution. The ability to interact easily with other specialists (both breeders and other disciplines) is critical for success. Institutions will often be most successful when they organize their scientists as teams with common goals, motivating continual interaction among team members.

It is unrealistic to imagine that cassava breeding will attain the level of support that is seen for maize, rice, wheat or soybeans, on a global level. However, research planning can still be ambitious and optimistic. Many of the components for continued impact of cassava genetic improvement are in place, but for this to happen, there needs to be a broad commitment to strengthening breeding programmes and all the supporting disciplines. The future of cassava genetic improvement depends fundamentally on the training of qualified plant breeders who are attracted to a career in cassava. These scientists will combine a background in classical plant breeding with training in molecular genetics. The shift towards training in molecular genetics of the past several years leaves a gap in expertise with whole plant systems and plant improvement aimed at farmer adoption. There has also been a rapid shift in recent years away from public plant breeding in general, affecting most crops. This has been motivated by the success in some major crops (especially maize, soybeans and cotton) for private companies to mount comprehensive programmes. The erosion of public funding and lack of interest by the private sector to take up the slack, have to be addressed as we move forward. The research environment for cassava breeding will depend on integrating public and private efforts. Neither sector alone will be able to cover demands for new technology.

3. RESEARCH STRATEGIES TO MEET TOMORROW'S CHALLENGES

Regardless of the size or sophistication of a genetic improvement programme, the basic components remain similar: defining and managing the germplasm base; creating new genetic variation and applying selection in order to improve trait expression.

3.1 DEFINING AND MANAGING THE GERMPLASM BASE

Given increasing sophistication in conservation techniques, there will need to be some consolidation of collections into centralized national and international collections, as a way to capitalize on expert human resources and state-of-the-art facilities. Breeders will have working field collections, but will pass the

responsibility for long-term conservation to specialized laboratories. On the opposite side of the consolidation argument is the increased risk if some disaster strikes a large centralized collection. There will always be the need for some form of duplication as a means of safeguarding germplasm.

It is clear that breeding progress could continue indefinitely based on existing *ex situ* diversity. At the same time, uncollected regions, especially in the Americas, should be explored. The potential for discovery of rare genes, or characters with a different system of genetic control, is high.

Gene bank conservation is an ever-increasing concern for the long term. Vegetative samples of landrace varieties are likely to be the main form of conserving germplasm long into the future. Given the general genetic instability of protoplasts and unorganized tissue, clonal conservation will probably need to continue as differentiated tissue such as meristem tips and somatic embryos. Conservation will be more secure and less labour-intensive than present field and *in vitro* methods and will include cryopreservation and very slow growth *in vitro* methods. With cryopreservation not yet completely defined for routine conservation, current gene banks face the risk of losses that are unacceptably high. Small national programme collections are often poorly monitored and subject to losses. The international collection at CIAT needs to be duplicated in its entirety, as part of a policy on permanent and secure conservation.

Germplasm banks will be the foundation in the search for characters for new agronomic and market situations. Although cassava gene bank curators have had little recognition for their work, these banks will be as important in the future as they were at the outset of breeding activities. Every effort needs to be made to prioritize the conservation and access to cassava's genetic resources, including both wild and cultivated species. An international monitoring system for all of the world's collections is eminently feasible, given today's communications and computing capabilities. Cassava breeders need to be a key part of the team efforts that define and manage gene banks.

Breeders, as well as those in other disciplines, will increasingly pursue a more complete characterization of the major collections, especially for new or rare traits. New techniques in genome characterization will bring considerable enlightenment with regard to relationships among species and crop origin and evolution. Tagging of specific genes to trace their progress through a breeding programme will soon be routine. Characterization of germplasm for physiological and quality traits has been rudimentary to date. Many techniques already exist that can be applied to cassava and its relatives. Emphasis will continue to be on the genetic variation for attributes that allow cassava to be adapted to common environmental stresses: nutrient stress, water deficits and low temperatures. Diversification of markets will be the driving force in the search for variability of starch quality characteristics and post-harvest shelf life.

3.2 CREATING NEW GENETIC VARIATION

The ability to create new genetic variation has not been considered a constraint for most cassava breeders, with the exception of areas where the species does not flower well. Hybridization is straightforward and few incompatibility mechanisms exist that constrain genetic interchange in any desired combination. In addition, many of the wild species of *Manihot* are easily accessible to the cassava gene pool through traditional crossing, or can be crossed with special techniques.

The need for new ways to create genetic variation seems to be less urgent for cassava than for many other crops, because there is very wide variation already available using only conventional forms of recombination. However, there is a need to make the present capabilities more efficient – to produce larger segregating populations more efficiently and to shorten the breeding cycle. Work on enhancing flowering is needed, either through physical, chemical or genetic manipulations. The ability to induce early and prolific flowering would tremendously improve the productivity of many breeding programmes.

Creation of variability through conventional crossing is not usually a constraint for most selection criteria for cassava improvement. Nevertheless, the increasing capabilities for directed genetic manipulation at the DNA level will have profound impact on cassava breeding in the future, as it is

already having on other crops. In part, the impact can potentially be negative, in that resources are being withdrawn from the productive, more traditional approaches, where success is far from being exhausted. Even in well-funded institutions, there are hard choices to be made in terms of the balance of investment in biotechnology and in classical breeding.

Transformation and other molecular techniques are essentially tools that a plant breeder draws upon; they are not an alternative form of plant breeding, but are complementary to classical methods. The cassava plant must be modified in many aspects to meet the needs of the future. Molecular techniques will provide a small but crucial proportion of the needs for new variation, but most will continue to come from classical recombination techniques, well into the future. Over-concentration on sophisticated and usually expensive molecular techniques would, in most cases, be detrimental to overall progress in genetic improvement. Valuable as some of these contributions might be in the next decades, they do not reduce the need for the steady, comprehensive improvement in productivity, resistance and quality that can only come from a field-oriented breeding programme.

Although the current pool of cassava clones that can be successfully transformed is very limited, this group will continue to expand. It cannot be too strongly emphasized that there is a critical need to be able to transform nearly any cassava clone, in order to benefit broadly from transformation technologies. A single gene, no matter how valuable, will find little practical use if it is in an unacceptable genetic background. In order to move that gene from an unacceptable to an acceptable background through cross-breeding implies the full application of a long-range breeding programme and perhaps 15-20 years or more to achieve. On the other hand, inserting a gene into an existing, locally accepted clone has the potential for very rapid diffusion and adoption, since the clone’s traits are already well-known by farmers. This situation is largely hypothetical, however, since regulatory requirements, as well as the need for extensive testing for unexpected changes in performance, create a rather lengthy period between varietal development and commercial impact.

3.3 SELECTION TOOLS AND STRATEGIES

Selection opportunities are the outcome of one's ability to distinguish genetically controlled differences in the material under observation. Improved selection efficiency can result from the ability to accentuate those differences and/or improvement in measuring techniques in order to more confidently detect small differences. Both these aspects of selection will develop rapidly in the coming years resulting in an improvement in the rate of genetic advance for a range of characters.

The ability to accentuate the expression of genetic differences hinges on understanding the plant biology and its interaction with the environment. Some of the most critical areas of cassava breeding, yet many of which remain poorly understood, are related to tolerance to soil and environmental stress and root quality. Better understanding of genotype by environment interactions, involving specific environmental components will go a long way towards improving selection efficiency. Detailed biochemical and genetic studies that clarify the molecular basis of these interactions, will be long-range activities and require collaboration from advanced laboratories.

In vitro screening techniques (at the cellular, tissue, or whole plant levels) have long been seen potentially as highly efficient tools of selection. However, there has been relatively limited success, in any crop, in associating the expression of agronomically useful traits at the *in vitro* level with their expression at the mature-plant level in the field. The technique is most likely to be developed for simple contrasting traits where biochemical pathways have been worked out. Acyanogenesis and starch quality traits could be early targets of development of *in vitro* screening.

However, *in vitro* screening has quickly moved to the use of molecular markers to tag genes that have variable or unreliable expression at the field level. The main thrusts to this point have been in identifying disease resistance, especially to CMD in the absence of the causal agent and identifying root quality characters. Molecular-assisted selection will become an integral part of most cassava breeding programmes in a relatively short time.

Farmer participatory research went through a massive trial and error period in the 1990s, resulting in a wide range of approaches and opinions. This is to be expected, given the variability among crops, regions, cultures, markets and breeding goals. Breeders need to be sure that the methods they use for setting goals involve farmers and that a system of continual feedback keeps the breeding programme on track. There are however, many different valid approaches to doing this. Cost effectiveness of different approaches to farmer participation is central to the choice.

4. SOME KEY BREEDING GOALS

Cassava breeding will in all likelihood be evolutionary rather than revolutionary. Some research areas will continue: e.g. yield, plant architecture, pest and disease resistance and starch content. Others will arise or take new pathways. Some examples are given below.

4.1 EARLY-BULKING VARIETIES

Cassava's adaptation to a long growing season fits well with traditional cultivation techniques. A particular field can provide piecemeal harvests for daily use over an extended time. Clearing land to prepare a field is highly laborious, so a long cropping season extends the time between clearings. When the crop is in the ground for long periods, there is less soil disturbance and hence less erosion. Early bulking varieties, however, are needed as pressure on land intensifies. Many industrial uses demand a continual flow of raw material, which can be achieved in part by farmers planting varieties of different maturity in a region. In most farmer surveys, earlier harvest stands out as a priority.

4.2 ADAPTATION TO MECHANIZED AGRICULTURE

A crop whose success relies on cheap labour is bound to see eroding importance as the world develops and seeks ways to increase labour productivity and reduce some of the drudgery of labour-intensive agriculture. The counter-argument that labour-intensive crops like cassava play a critical role in regional employment is also valid in some situations. As globalization of economies progresses, crops with low labour efficiencies will not compete with those that can be produced more efficiently and cheaply. Whether aiming at local or international markets, cassava growers will increasingly need to adopt labour-saving technologies. Cassava has never been bred for mechanization and this will become a critical thrust. The main implications, initially, will be plant types suitable for allowing machinery to pass between rows (erect, compact plant type) and root form and position suitable for mechanical harvesting.

4.3 FOLIAGE AND INTEGRATED ROOT-FOLIAGE PRODUCTION

Cassava leaves are an important source of protein in human diets in parts of Africa, Asia and Latin America and are occasionally fed to cattle. Researchers have been interested for many years in the use of cassava leaves and young stems for forage. Experiments have focused mainly on processing and nutritional aspects, but there is also a growing body of information on production management. Genetic variability for capacity to produce foliage and the ability to optimally combine foliage and root production is receiving more attention. Most of the key cassava-producing countries of Asia and several in Africa and Latin America, have begun work on comprehensive systems for foliage production, processing and utilization. Cassava for foliage production essentially becomes a new crop and as such requires research into the total system, including soil fertility management, time of planting, plant spacing, harvest frequency, nutritional and palatability variation (for feed or food uses) and especially harvest and post-harvest management. Research on options for management of planting material is crucial. The high planting density and the total or partial destruction of lignified stems at harvest (depending on the harvest system), combine to create a huge challenge for developing a cost-effective stake management system. In summary, the genetic component of research for foliage production may not be the first priority, but will certainly be critical as other parts of the technology package are developed.

4.4 OTHER MARKET-DRIVEN TECHNOLOGY DEVELOPMENT

Cassava has the potential to satisfy very different functions in local or national economies: subsistence in marginalized, at-risk societies, where natural or social disasters are a frequent threat to livelihoods; local commerce and development, where cassava provides a combination of family food and income, local employment and inexpensive food to consumers; and growth markets in trade and industry. Much of cassava research in the past was aimed at the first two sectors. These will remain critical targets for attention. Societies will also broadly benefit from research that makes cassava more competitive in the latter sector. This will depend on developing products for specialized markets, increasing crop productivity and developing the capacity to provide a steady supply of high quality products to the market.

4.4.1 Root quality for traditional markets

Given the increasing popularity of cassava among Africa's poor, substantial segments of the population would benefit from improved nutritional value of roots and more palatable leaves. Potential for increase in protein levels may be limited, but even a small increase has a very high potential payoff. Investment in research is warranted, both through conventional breeding and genetic engineering. Conventional breeding for increased protein has seen little success historically, but recent findings give some hope in this area. Molecular techniques also may contribute to improved protein content or quality.

Increases in iron, zinc and β-carotene are eminently feasible, based on variation observed in existing germplasm. The key to success will be to identify these traits in agronomically acceptable types, in order to keep the time frame for variety development reasonably short.

4.4.2 High-value cassava for industrial applications

Economic globalization, which accelerated in the 1990s, has opened new opportunities for industrial uses of cassava, specifically as a source of energy in the animal feed industry, fuel alcohol and starches. Tropical production of maize is increasingly having difficulty competing with maize from temperate regions. This situation has prompted governments and the private sector of many tropical countries to turn to cassava as a competitive alternative to imported maize. In addition, advances in molecular biology, genetic engineering, plant tissue culture protocols and starch technologies, provide important tools that will allow the bridging of main gaps between cassava and the cereals.

Current available data indicate a wide variability for many root quality traits, but relatively low variability for some of the key traits that industry will need. Compared with the many economically useful mutations found and exploited in the maize kernel (e.g. sweet corn, popcorn, waxy maize starch and opaque 2) less variability has been reported for cassava. Experience with crop species in general suggests that there should be more variability in cassava root quality than has been discovered to date. There are some likely explanations for this. Firstly, starch mutations are more difficult to detect in roots than in grain kernels, where they are often easily identified by visual inspection. For roots, on the other hand, laboratory tests are usually needed. Secondly, the known starch mutants are usually recessive. The fact that cassava seldom undergoes inbreeding drastically reduces the chance of low-frequency recessive alleles, to express the phenotype. It is possible, in fact, that clones with valuable traits have appeared in breeding nurseries, but were discarded because of having no distinguishing visual characters. The fact that roots are not reproductive organs may offer cassava (and other root crops with the same characteristic) an advantage over seed-propagated crops. Cassava roots should be able to withstand mutations that would otherwise be lethal for reproductive organs such as the kernels of cereals (H. Ceballos, personal communication).

Industrial use of cassava will increasingly demand a longer post-harvest window for shipping and storage, via reduced physiological deterioration of the roots. While existing germplasm shows some genetic variability, any breakthrough is more likely to result from work at the cellular level, through transformation.

4.4.3 Nutritional value of roots for the animal feed industry

The modern animal feed industry relies heavily on commercially balanced rations that combine ingredients from many sources in a least-cost linear programme. For this industry, the main goal is to produce energy (starch) at a low cost. If other nutritional traits can be added to this goal, at a lower cost than can be provided by other sources, there may be reasons for expanding the repertoire of nutritional goals. The most likely scenario is that efforts aimed at improving human nutrition with higher protein, high vitamin A and higher mineral content, spin off to applications for animal feeds.

4.4.4 Cassava for the energy industry

Diminishing fossil fuel reserves have long motivated thinking about alternative energy sources, especially as a substitute for gasoline. This interest has been somewhat cyclic, waxing and waning with global prices for crude oil. Alcohol from biomass is one of the simplest technologies for renewable energy. Cassava has been used both in pilot projects and in commercial production (Brazil) as the raw material for alcohol fermentation. While the Brazil project in the 1970s proved not to be viable, mainly because there was inadequate planning on the agronomy side, there has been renewed interest with the rise in oil prices early in the 21st century (Piyachomkwan *et al*., 2005). One desirable characteristic would be a storage root that accumulates a high concentration of simple sugars rather than the complex starch molecules. Carvalho *et al*. (2004) reported the occurrence of cassava germplasm with high free sugar content as well as novel starch including a glycogen-like molecule. These results suggest that it is feasible to generate cassava clones with root characteristics ideal for the production of ethanol.

4.5 CASSAVA'S ROLE IN NATURAL RESOURCE MANAGEMENT

Breeders need to recognize the potential to shape cassava's positive role in natural resource management, as part of a broad breeding strategy. It is an exclusively tropical crop that is extremely well adapted to a wide range of conditions, without the need for undue artificial *life support*. This can mean a crop that does not require inputs with potentially harmful environmental effects, like pesticides and high levels of fertilizer. The breeder can exploit these inherent traits, or allow them to be lost through genetic drift if selection environments are more luxurious. If appropriately selected, cassava can continue to have comparative advantages for efficient use of inputs and as a crop that uses few pesticides.

5. FROM GROSS MODIFICATION TO FINE TUNING

The tendency in crop improvement has been to progress relatively rapidly by modifying some key morphological or physiological traits at the outset and evolve toward a fine-tuning approach. This can be seen, for example, in rice, wheat and maize, where major breeding goals were to reduce plant height, improve responsiveness to increased fertilizer without lodging, and increase tolerance to high planting density. Now that these goals have been achieved for many breeding programmes, varieties are being fine-tuned for adaptation to specific environmental conditions, for specific quality traits and others.

Cassava will be no exception to this trend. The difference is that cassava breeding is still in the midst of the first phase of this progression. Breeding programmes in general are attempting to accomplish some rather major changes in cassava's behaviour, as compared with landrace varieties. This means manipulating large numbers of genes through breeding – practically a rebuilding of the cassava plant. A few more decades may be required to accomplish this goal, given the long cycle of cassava and the low level of research resources given to the crop on a worldwide scale. As this gross modification progresses, breeding should become much more physiologically based, focusing on specific yield, quality or resistance-related traits. Fine tuning might also include breeding for adaptation to specific biotypes of mycorrhiza and beneficial bacteria, areas which have barely been explored at this stage.

Another area of fine-tuning will probably come in the form of adapting varieties to their best performance after inserting new genes via transformation. This is still a hypothetical scenario, but it is certainly possible that newly-inserted genes could create epistatic effects on non-target characters of commercial importance. If this were to happen, breeders may need to attempt additional transformation events of the same parent clone, or adapt the transformed clone through additional conventional breeding.

6. LONGER-TERM OPPORTUNITIES

6.1 A CASSAVA INBRED-HYBRID SYSTEM

If cassava were not vegetatively propagated, there would be very high incentive to develop an inbred-hybrid system of breeding. This includes the ability to optimize heterotic effects; to expand breeding methods to include backcrossing; and to use true seed in multiplication, interchange and, potentially for commercial production. There is every reason to believe that such a system is technically feasible. An inbred system would not assure a profitable entry of the private sector in cassava breeding and therefore there has been no private funding available for this research. The key to moving this research forward may be the development of a routine protocol for producing haploids/dihaploids to achieve homozygosity in large numbers of genotypes. The difficulty of obtaining homozygosity through conventional selfing is well-established. Though certainly not impossible, there is unlikely to be an adequate financial commitment to a long-term, extensive programme for inbred production through selfing, given the successful history of breeding cassava as a strictly heterozygous species.

6.2 COMMERCIAL CASSAVA PRODUCTION FROM TRUE SEED

Proposals for research into the possibility of utilizing true cassava seed for commercial production have been made since the early 1980s. The Central Tuber Crops Research Institute in India (CTCRI) and CIAT in Colombia have both carried out some preliminary research. There are reasons to believe that more research in cassava is warranted. Five principal known or hypothetical advantages of a seed-propagated crop can be visualized:

- virus build-up appears to be a major yield constraint in vegetatively propagated cassava. Few viruses (none of economic importance) are transmitted through true seed;
- stake storage problems are persistent and widespread in virtually all cassava-growing systems. Cassava true seed can be stored easily, for a year or more under ambient conditions and much longer at lower temperatures;
- there is a widespread interest in early maturing cassava varieties to fit into multiple cropping systems, especially in Asia. Early maturity also increases the flexibility of the crop in providing a continuous supply of roots throughout the year. Cuba, for example, promotes a multiclonal system including an early-, medium- and late-maturing variety. However, there are two major limitations: firstly, the necessity to store planting material for unreasonably long periods; and secondly, the need for a relatively long growing season just to produce sufficiently lignified stems for planting material. Propagation from true seed could overcome these constraints;
- the low vegetative multiplication rate of cassava is normally not a constraint under stable cropping patterns, but is a real constraint when area planted is being increased, or for introduction of new varieties; and
- true seed is far more easily managed and transported than stakes.

There are also many difficult constraints to overcome in aiming for a true seed technology: definition of genetic structure of a variety; seed production (genetic and management factors); seed dormancy and field germination constraints; productivity under stress; and farmer acceptance of a true seed technology. This is an area of enormous potential impact but would require a large and long-term research commitment (Table 24.1).

CTCRI and CIAT both found that seedling yield can be close to that of stake-propagated crops, but tends to be somewhat lower. Seed germination is acceptable at higher temperatures (about 35°C) but can be very delayed at 25°C (Table 24.2).

6.3 THE OPEN-ENDED OPPORTUNITIES OF TRANSFORMATION

Cassava breeders have nearly always taken a problem-solving approach to setting goals. This is reasonable, since no programmes are funded at levels that allow high-risk avenues of research, at the expense of neglecting urgent issues that growers and consumers currently face. Breeders usually

structure their programmes for fairly safe and predictable results based on steady progress from established methodologies. However, the possibilities of transformation (or genetic engineering) open genetic improvement to highly unpredictable opportunities. Traits not previously considered a priority and maybe not even a possibility, can suddenly become available as an option. This highlights the need for breeders to be in close contact with advanced laboratories, to be continually aware of opportunistic (and problem-solving) technologies that can be applied to cassava.

7. THE BROAD IMPACT OF CASSAVA BREEDING: AN ASSESSMENT

7.1 THE RISKS OF SUCCESS

Success in plant breeding brings about change. In a properly planned and executed programme, this change will be positive, on the whole. Success may carry with it some risks as well, either calculated or unexpected. The breeder should be able to anticipate and adjust for both the positive and negative changes brought about by successful breeding.

7.1.1 Vulnerability resulting from genetic uniformity

A breeding programme may have (and should have) incorporated into its long-term objectives, the production of several new varieties for any given target region. The reality is, however, that even with the breeder's best intentions, one or two new varieties may become much more popular and more widely planted than others. Even if several new varieties are planted in equal proportion, they may have certain common genetic backgrounds that could make them vulnerable to a new pest or disease, or environmental condition.

Genetic vulnerability came to the forefront of strategic planning by plant breeders after an epidemic of southern corn leaf blight destroyed an estimated 15 percent of the United States' maize crop in 1970. Virtually all the maize hybrids in use at the time had the same Texas male-sterile cytoplasm, which happened to confer susceptibility to a race of the *Helminthosporium maydis* pathogen. There are several reasons why a similar situation is far less likely to occur in cassava (Chapter 16). This does not however, rule out such a possibility, nor the need to take precautions against genetic vulnerability, either in terms of pest resistance or other destabilizing environmental factors.

Table 24.1 Comparison of seed and vegetative propagation systems for cassava

	Description	
Characteristics	**Vegetative**	**True seed**
Pest and pathogen problems	Many	Few
Seed quality	Variable	Unknown
Seed storage	Few months	Many years
Multiplication rate	1:10-20	1:100-1 000
Plant architecture	Constrained by need for production of cuttings	Branching required for seed production
Photosynthate partitioning	Dry matter partitioning to stems critical for propagation	Efficient – more to roots and less to plant support
Plant maturity	Need for adequate time to produce well-lignified stems	Unrestricted (assuming separate seed production lots)
Ease of genetic improvement	Rapid genotype fixing	Complex
Early establishment and vigour	Good	Limiting
Source: adapted from Iglesias and Hershey (1994)		

With the generally narrow adaptation of many current varieties, the chance of widespread planting of any individual clone is small. Even the newer, more widely adapted clones will probably not be grown over extensive geographic regions because of the nature of the high variability of cassava-growing environments. The trend toward market diversification for cassava products will lessen further the risks of widespread adoption of a narrow genetic base. There are important exceptions to these generalities. Thailand grows nearly all of its nearly one million hectares to a few varieties, although the current diversity, which includes several new hybrids, is far greater than in the era prior to adoption of new varieties.

Table 24.2 Yield comparison between clone CM 340-30 and its open-pollinated progeny as seed-propagated (cycle 1) and stem-propagated (cycle 2) plants

Group	Cycle	Propagule	Root yield (tonnes/ha)	Total plant yield (tonnes/ha)	Harvest index
CM 340-30	1	stem	41	91	0.48
Progeny	1	seed	43	78	0.54
LSD (0.05)			8.7	-	0.07
CM 340-30	2	stem	20	46	0.43
Progeny	2	stem	22	45	0.49
LSD (0.05)			9.4	-	0.10
Source: Bolaños (1987)					

7.1.2 Effects on ecological balance

Modern agriculture in general has often been associated with disruption of existing balances between pests or pathogens and their hosts, resulting in yield-reducing outbreaks. Although there are breeding strategies that can reduce this type of risk, crop–pest interaction models are far from adequate in predicting all the potential results of introduction of new varieties. Continued emphasis on integrated pest management techniques, as opposed to sole reliance on chemical control of pests, will alleviate the danger of ecological imbalance. For cassava, host plant resistance will remain the centrepiece of management for many pests.

7.1.3 Loss of genetic diversity

Genetic diversity is the foundation of crop improvement. Successful adoption of new varieties can, without doubt, reduce available genetic diversity by replacing diverse landrace varieties with a smaller number of new varieties. The partial loss of this diversity from farmers' fields is hardly avoidable, as it is often part of the basic objectives of the breeder, i.e. replacing traditional varieties with improved ones. Thus, it is essential that the genetic diversity be preserved in a systematic manner through well-endowed gene banks in key locations, or a system of incentives for farmers to preserve landrace varieties in their native agro-ecosystems. If this is accomplished, then the risk of permanent loss of genetic diversity due to successful adoption of new varieties is substantially reduced.

7.1.4 Spread of cassava into submarginal areas

Cassava is already well-known as a crop that can tolerate several types of stress conditions and as such is cultivated in many of the more marginal agricultural areas where other crops would require high inputs to give reasonable yields. One of the very real risks of successful genetic improvement of cassava is that the crop could move even more extensively into areas with a high risk of environmental degradation. Foremost of these possibilities seems to be steep slopes highly vulnerable to erosion, where only pastures, perennial crops or tree crops should be grown. Avoiding this type of risk may be (and probably

is) beyond the control of the breeder, except for the possibility of breeding new varieties adapted only to favourable conditions. This is not really a viable option for the large majority of cassava-growing areas. Preventing cassava from being cultivated on inappropriate soils is largely an educational issue (of teaching farmers the long-term consequences of this action), a legislative problem (of regulating land use by law) and/or a matter of creating alternative economic opportunities for farmers who feel pressured by their circumstances to abuse marginal lands.

Are these potential negative impacts of developing and promoting new varieties so high that research into developing them should be halted? Probably only in rare cases, but the breeder does need to safeguard against these possibilities. Moreover, it is important to balance this discussion with a citation of the risks of failure in developing widely-adopted new varieties.

7.2 THE RISKS OF FAILURE

Human population growth carries with it the inexorable demand for increased food production and usually more consumer goods. Cassava yields are probably destined to remain stagnant or even decline in areas where no new varieties are introduced. This is not to minimize the impact of new agronomic practices, but recognizes that improved agronomy and new varieties are generally closely linked and complementary. So quite simply, the risk of failure to provide appropriate gene-based technology for improving cassava productivity, is a decline in well-being for millions who rely on this crop for basic nutrition and income.

7.3 CASSAVA GENETIC IMPROVEMENT IN PERSPECTIVE

Science has an important, but certainly not an exclusive role, in the improvement of quality of life for cassava growers and consumers. This contribution is especially critical among the poorer sectors of human populations, where even the basic necessities of life, such as food, clothing and shelter are inadequately met. The challenges to society are substantial, in order to provide for these needs without long-term damage to the natural resource base. It is within this larger world view that cassava breeders need to define a research agenda.

Crop yield increases have slowed in much of the world. The reasons are varied and complex, yet many analysts seem to come too quickly to the conclusion that yield plateaux are universal and can only be overcome by new technologies yet to be developed. Declining investments in agricultural research, irrigation and rural infrastructure and increasing water scarcity account for much of the slowing, but there is also concern that genetic limits are being reached. While this may be true for a few intensely researched crops, it does not seem to be true for cassava. Large-area experimental yields of over 80 tonnes/ha, compared with average national yields of about 10 tonnes/ha, demonstrate a level of genetic potential that is not nearly met by management practices. Given the comparatively low level of investment in cassava breeding relative to maize, wheat or rice, there is reason to believe that continued genetic progress, long into the future, is eminently feasible and almost certain to be achieved. Many of the elements are in place for continued and accelerating success in cassava genetic improvement as a means to improve the livelihoods of millions of farmers and consumers in the tropics.

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APPENDIX I. FIELD PROCEDURES FOR COLLECTION OF CASSAVA

Collection in the form of stem pieces (stakes), the normal propagative material, is most appropriate. There may be some instances when it is inconvenient to collect stakes, in which case, true seeds may be collected. As cassava is a heterozygous plant, segregation occurs in a population of plants from true seed.

Plants to be sampled in the field should be nearly mature if possible.

Pull plant before cutting any branches to inspect roots for symptoms of frogskin disease, a systemic viral disease which should not be introduced into germplasm conservation areas.

Avoid cutting stakes from clones affected by bacteriosis, superelongation or virus diseases.

Cut a few stakes of about 50 cm each. Tie stakes together from the same clone, and label with two water resistant tags. The tags should contain information on: date, common name and field collection number.

Complete collection forms for each collection (see Appendix III.)

Treat stakes with a fungicide/insecticide mixture. A treatment used at CIAT consists of 3 000 ppm each of Orthocide® (captan) and Bavistin® (BCM) plus 1 ml/litre water of Malathion EC. Treatment is for five minutes after which stakes are allowed to dry.

Cut ends of stakes may be covered with paraffin to prevent desiccation. If possible stakes should be maintained in a shaded cool area for the duration of the expedition.

At the germplasm repository, plant the stakes from each clone in small well-labelled plots for evaluation and multiplication if necessary.

If meristem culture facilities are to be used, some of the stakes collected should be covered at the upper end with paraffin to prevent desiccation. Plant the stakes in small pots and use the sprouts as a source of meristems.

Source: Gulick, et al. (1983)

APPENDIX II. FIELD PROCEDURES FOR COLLECTION OF WILD MANIHOT SPECIES

1. Seed collection should be the primary objective, to optimize genetic diversity and ease of collection.
2. In the field, a bag is placed over an unripe fruit until dehiscence takes place.
3. The bags and seeds are collected after maturation.
4. Stakes may also be collected according to procedures outlined for cassava, in Table 8.1.
5. For those species which set no fruit and for which rooting from stakes is not possible, the whole plant must be collected.
6. The optimum collection time for wild species (stakes and whole plant) is at the end of the dormant period, i.e. at the end of the dry season/beginning of the rainy season, when the new growth begins.
7. A collection form should be filled out at the time of collection (see Appendix III).
8. A pressed botanical specimen should accompany each accession. A photograph is also useful.

Source: Gulick, et al. (1983)

APPENDIX III. *MANIHOT* FIELD COLLECTION FORM

***MANIHOT* COLLECTION FORM** (part 1 of 2)

GENUS: _____ **SPECIES**: _____ **SUBSPECIES**:_____

COLLECTORS' INITIALS: _____ **COLLECTION NUMBER**: _____

INSTITUTION RESPONSIBLE: _____

DATE OF COLLECTION (Day/Month/Year): ____/____/____

COUNTRY OF COLLECTION: _____ **PROVINCE/STATE**: _____

LOCALITY:

Nearest town/village: _____

Distance (km): _____ Direction from town: _____

LATITUDE: Degrees: _____ Minutes: _____ N S

LONGITUDE: Degrees: _____ Minutes: _____ E W

ALTITUDE: (m) _____

COLLECTION SOURCE (circled):

Wild	1	Village market	5
Farmer's field	2	Commercial market	6
Store	3	Institution	7
Backyard	4	Other: _____	8

SAMPLE STATUS (circled):

Wild	1	Primitive variety/landrace	4
Weedy	2	Improved variety (breed)	5
Breeder's line	3	Other: _____	6

LOCAL NAME: _____

NUMBER OF PLANTS SAMPLED: _____ **PHOTOGRAPH** (circled): Yes No

TYPE OF SAMPLE: (circled):

Vegetative 1 Seed 2 Both 3

HERBARIUM SPECIMEN (circled): Yes No

QUANTITY OF MATERIAL (number of seeds, stem pieces, tubes *in vitro*): _____

PRIMARY MORPHOLOGICAL DESCRIPTORS (cultivated cassava only) (circle number):

Colour of apical leaves: 3=light green; 5=dark green; 7=purplish green; 9=purple

Leaf lobe form: 1=linear; 2=elliptic; 3=lanceolate

Petiole colour: 1=yellowish green; 2=green; 3=green with slight red; 5=green with red; 7=red; 9=purple

Apical pubescence: 0=absent; 3=little; 5=moderate; 7=high

Stem epidermis colour (internal surface): 1=silver green; 2=light brown/orange; 3=dark brown

Stem periderm colour: 1=light green; 2=dark green; 3=yellow

Root surface colour: 1=white or cream; 2=yellow; 3=light brown; 4=dark brown

Root flesh colour: 1=white; 2=cream; 3=yellow; 4=pink

Flowering: 0=absent; 1=present

Storage root peduncle: 1=sessile (absent); 2=short (<5cm); 3=intermediate/long (>5cm)

Root cortex colour: 1=white or cream; 2=yellow; 3=pink; 4=purplish

MANIHOT COLLECTION FORM (part 2 of 2)

GROWTH HABIT (circled):
Tree 1 Bush 2 Vine 3 Other _____
PART OF PLANT UTILIZED (circled): Roots 1 Foliage 2
PRINCIPAL USE (circled):

Human consumption - fresh	1	Animal consumption - dry or processed	4
Human consumption - dry or processed	2	Starch extraction	5
Animal consumption - fresh	3	Other _____	6

SPECIAL QUALITIES (according to farmer) (circled):

Yield	1	Disease resistance	5
Starch content	2	Pest resistance	6
Eating quality	3	Edaphic adaptation	7
Root storability	4	Other _____	8

NOTABLE DEFECTS (according to farmer): _____
DISEASES AND PESTS PRESENT AND SEVERITY:
(Severity: 1=little damage; 2=moderate damage; 3=severe damage)

Diseases/Pests	Severity	Diseases/Pests	Severity
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

WILD SPECIES AND ASSOCIATED CROPS:
_____ _____
_____ _____
TOPOGRAPHY (circled):

Marshy	1	Rolling hills	5
Flood plain	2	Steep hills	6
Riparian	3	Mountainous	7
Flat – not flood-prone	4	Other _____	8

VEGETATION (circled):

Rainforest	1	Thorn woodland	6
Humid forest	2	Scrub desert	7
Semi-humid forest	3	Desert	8
Dry forest	4	Other _____	9
Very dry forest	5		

SOIL TEXTURE (circled):

Sandy	1	Clayey	5
Sandy loam	2	Silt	6
Loam	3	Organic origin	7
Clay loam	4	Other _____	9

DRAINAGE (circled):
Poor 1 Moderate 2 Good 3 Excessive 4
SLOPE (circled):

Flat or almost flat	($< 4^{o}$)	1
Moderate slope	(4-14^{o})	2
Steep slope	($>14^{o}$)	3

_____

Source: Adapted from Gulick et al. (1983); CIAT (1994); IPGRI (1995)

APPENDIX IV. FAO/IPGRI RECOMMENDATIONS FOR SAFE EXCHANGE OF CASSAVA GERMPLASM

General recommendations

1. Material should be collected, processed and shipped with the necessary precautions to avoid accidental movement of pests.

2. Under no circumstances should germplasm be moved as rooted plant material except for *in vitro* plantlets.

3. Cassava germplasm can be moved as seed, pathogen-tested *in vitro* material, or as cuttings from re-established pathogen-tested *in vitro* material that has been grown under containment. Each of these categories should be treated as described in the technical recommendations (below).

4. Only under special circumstances should the movement of untested, vegetative material be considered.

5. All germplasm should be collected from healthy-looking plants and when possible from areas where quarantine pests are not present.

6. Germplasm from areas where pests of quarantine concern are known to occur should go through intermediate, or post-entry quarantine.

7. The transfer of germplasm should be carefully planned in consultation with quarantine authorities and should be in amounts that allow adequate handling and examination. The material should be accompanied with the necessary documentation.

Technical recommendations: Seed

1. Seed production should be carried out in areas which are free of diseases of quarantine significance whenever possible.

2. Fruits should be harvested from healthy-looking plants.

3. Seeds of normal size should be selected from healthy-looking fruits.

4. Seeds should be treated according to the following recommendations, either in the country of origin or in the country of destination:
   a) Immerse the seeds in water and discard any floating seeds.
   b) Treat the seeds immersed in water in a microwave oven at full power until the water temperature reaches 73ºC and pour off the water immediately after the treatment.
   c) If a microwave oven is not available, treat the seeds with dry heat for two weeks at 60ºC.
   d) Dry the seeds and treat them with thiram dust.
   e) Pack the seeds in a paper bag.

5. After arrival in the country of destination, the seeds should be inspected for the presence of insect pests. If found to be infested, they should be fumigated or destroyed (if fumigation is not possible).

6. Seeds should be sown under containment or in isolation and kept under observation until the plants are well-established and normal healthy leaves are produced.

Technical recommendations: Pathogen-tested *in vitro* cultures

1. Stem cuttings should be collected from healthy-looking plants, whenever possible.

2. Stem cuttings should be grown in pots and, after sprouting, be subjected to thermotherapy in a growth room with temperatures of 40ºC by day and 35ºC by night.

3. Meristem-tips of less than 0.4 mm should be cultured and each meristem-tip derived plantlet should be given an accession number and multiplied.

4. For each meristem-tip derived accession, one plantlet should be grown out under containment and indexed for the disease present in the area of origin of the material, and/or in areas where the material has been field-grown prior to deriving meristems, according to the procedures recommended in the present guidelines. (It is not necessary to index for bacterial and fungal pathogens as these will reveal their presence in the culture medium).

5. When the indexing procedures reveal that the plants are free of the pathogens of concern, *in vitro* plantlets derived from the same meristem-tip can be transferred.

6. For the movement of *in vitro* plantlets, neither antibiotics nor charcoal should be added to the culture medium.

7. In the recipient country, *in vitro* plantlets should be examined for contamination and if found free, grown out and maintained under containment with regular inspection.

Technical recommendations: Cuttings from pathogen-tested *in vitro* cultures

1. This method is recommended only where recipient countries are unable to handle *in vitro* material.

2. Pathogen-tested plantlets produced according to the procedures described above should be grown out and multiplied in an insect-free facility with adequate measures to prevent reinfection by pathogens.

3. Stem cuttings from these plants should be washed, surface-sterilized with sodium hypochlorite and treated with appropriate insecticides, acaricides and fungicides before dispatch.

4. In the recipient country, the cuttings should be grown under containment and subjected to regular inspection.

Technical recommendations: Untested vegetative material

Untested vegetative material, either as *in vitro* cultures or as stem cuttings, should only be moved to intermediate or post-entry quarantine facilities where they will be subjected to the therapy and indexing procedures described above, before being released. When stem cuttings are moved they must be treated with the appropriate pesticides in the country of origin.

APPENDIX V: EXAMPLE OF A MATERIAL TRANSFER AGREEMENT (MTA) UNDER THE INTERNATIONAL TREATY ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

This MTA covers materials which are being transferred before the entry into force of the International Treaty on Plant Genetic Resources for Food and Agriculture. The Treaty envisages that the International Centre for Tropical Agriculture (CIAT) will enter into an agreement with the Governing Body of the Treaty, once the Treaty enters into force. CIAT has indicated its intention to conclude such an agreement with the Governing Body. This agreement, in line with the Treaty, will provide for new MTAs and benefit-sharing arrangements for materials transferred after the entry into force of the agreement.[7]

The plant genetic resources (hereinafter referred to as the "material") contained herein are being furnished by CIAT under the following conditions:

CIAT is making the material described in the attached list available as part of its policy of maximizing the utilization of material for research, breeding and training. The material was either developed by CIAT; or was acquired prior to the entry into force of the Convention on Biological Diversity (CBD); or if it was acquired after the entering into force of the CBD, it was obtained with the understanding that it could be made available for any agricultural research, breeding and training purposes under the terms and conditions set out in the agreement between CIAT and FAO dated 26 October 1994.

The material is held in trust under the terms of this agreement, and the recipient has no rights to obtain Intellectual Property Rights (IPRs) on the material or related information.

The recipient may utilize and conserve the material for research, breeding and training and may distribute it to other parties provided such other parties accept the terms and conditions of this agreement.[8]

The recipient, therefore, hereby agrees not to claim ownership over the material, nor to seek IPRs over that material, or its genetic parts or components, in the form received. The recipient also agrees not to seek IPRs over related information received.

The recipient further agrees to ensure that any subsequent person or institution to whom he/she may make samples of the material available, is bound by the same provisions and undertakes to pass on the same obligations to future recipients of the material.

CIAT makes no warranties as to the safety or title of the material, nor as to the accuracy or correctness of any passport or other data provided with the material. Neither does it make any warranties as to the quality, viability, or purity (genetic or mechanical) of the material being furnished. The phytosanitary condition of the material is warranted only as described in the attached phytosanitary certificate. The recipient assumes full responsibility for complying with the recipient nation's quarantine and biosafety regulations and rules as to import or release of genetic material.

Upon request, CIAT will furnish information that may be available in addition to whatever is furnished with the material. Recipients are requested to furnish CIAT with related data and information collected during evaluation and utilization.

The recipient of material provided under this MTA is encouraged to share the benefits accruing from its use, including commercial use, through the mechanisms of exchange of information, access to and

[7] The attention of the recipient is drawn to the fact that the details of the MTA, including the identity of the recipient, will be made publicly available.

[8] This does not prevent the recipients from releasing the material for purposes of making it directly available to farmers or consumers for cultivation, provided that the other conditions set out in this MTA are complied with.

transfer of technology, capacity building and sharing of benefits arising from commercialization. CIAT is prepared to facilitate the sharing of such benefits by directing them to the conservation and sustainable use of the plant genetic resources in question, particularly in national and regional programmes in developing countries and countries with economies in transition, especially in centres of diversity and the least developed countries.

The material is supplied expressly conditional on acceptance of the terms of this Agreement. The recipient's acceptance of the material constitutes acceptance of the terms of this Agreement.

Source: http://www.ciat.cgiar.org/pgr/mta.htm

APPENDIX VI-A. VALUES FOR PRINCIPAL CHARACTERS IN YIELD TRIAL EVALUATIONS [a]

	Foliage eval.[b] (1-5)	Root eval.[b] (1-5)	Bacterial blight[c] (1-5)	Root yield (tonnes/ha)	Harvest index	Dry matter (%)	Eating quality[d] (1-5)
CG 1139-2	3.5	2.2	1.7	27.1	0.64	35.2	3.4
CG 1450-4	2.4	3.8	2.3	20.8	0.62	39.0	1.0
CM 2166-6	2.1	2.4	1.3	27.3	0.63	36.1	1.8
CM 2766-3	3.9	3.3	1.3	22.4	0.51	38.0	1.0
CM 2766-5	2.7	3.6	1.3	17.8	0.45	36.9	1.4
CM 3320-8	4.1	2.9	2.0	23.5	0.49	35.0	1.4
CM 4484-2	3.2	3.1	1.3	23.8	0.54	37.6	1.0
CM 5620-3	3.1	2.2	2.0	24.7	0.56	36.1	2.6
CM 5789-1	2.3	2.7	1.7	22.3	0.50	38.3	1.4
CM 5898-1	2.5	2.3	1.7	24.5	0.54	33.3	4.4
CM 5898-2	2.7	3.5	1.7	29.6	0.61	37.0	1.0
CM 5902-2	4.1	4.9	1.0	20.6	0.48	37.5	1.0
CM 5948-1	3.2	3.8	2.3	16.8	0.50	37.3	1.0
CM 6049-1	2.5	4.2	1.7	18.4	0.46	32.7	3.2
CM 6061-1	2.9	3.4	2.0	22.3	0.59	37.1	1.0
CM 6068-3	3.5	2.7	2.3	20.3	0.48	36.4	1.4
CM 6070-1	2.4	2.6	2.7	17.4	0.48	35.3	1.8
CM 6082-1	3.1	1.2	1.7	28.4	0.60	33.8	1.4
CM 6438-17	3.2	3.8	2.0	22.9	0.48	37.5	1.0
CM 6630-2	2.8	2.5	2.0	27.5	0.63	35.2	1.0
CM 6664-3	2.7	3.4	2.3	26.1	0.61	36.2	1.0
CM 6691-2	2.3	2.9	1.7	36.0	0.61	35.1	3.8
CM 7050-1	3.6	3.3	2.0	24.5	0.64	38.4	1.0
CM 7086-14	4.2	2.6	2.7	26.2	0.57	37.5	1.0
SM 667-1	1.8	2.5	2.7	20.3	0.53	33.7	1.0
SM 673-1	2.7	3.2	1.7	23.5	0.54	38.9	1.4
MBra 97	3.5	4.4	2.0	25.8	0.55	36.7	1.0
ICA Catumare	3.2	3.1	2.0	16.3	0.46	40.6	2.0
Mean	3.0	3.1	1.9	23.5	0.55	36.5	1.6
SD	0.6	0.8	0.4	4.4	0.06	1.9	1.0

[a] Part of the data taken from an actual yield trial of the CIAT breeding programme, and part synthesized for illustration purposes
[b] 1 = excellent plant/root type and general appearance; 5 = very poor
[c] 1 = very low disease damage; 5 = severe disease damage
[d] 1 = excellent; 5 = very poor

APPENDIX VI-B. STANDARDIZED VALUES FOR YIELD TRIAL EVALUATIONS[a]

	Foliage eval.[b] (1-5)	Root eval.[b] (1-5)	Bacterial blight[c] (1-5)	Root yield (tonnes/ha)	Harvest index	Dry matter (%)	Eating quality[d] (1-5)
CG 1139-2	0.8	-1.1	-0.4	0.8	1.5	-0.7	1.8
CG 1450-4	-1.0	0.9	0.9	-0.6	1.2	1.3	-0.6
CM 2166-6	-1.4	-0.9	-1.4	0.9	1.3	-0.2	0.2
CM 2766-3	1.4	0.3	-1.4	-0.2	-0.6	0.8	-0.6
CM 2766-5	-0.5	0.7	-1.4	-1.3	-1.5	0.2	-0.2
CM 3320-8	1.7	-0.2	0.2	0.0	-0.9	-0.8	-0.2
CM 4484-2	0.3	0.0	-1.4	0.1	-0.1	0.6	-0.6
CM 5620-3	0.1	-1.1	0.2	0.3	0.2	-0.2	1.0
CM 5789-1	-1.1	-0.5	-0.4	-0.3	-0.7	1.0	-0.2
CM 5898-1	-0.8	-1.0	-0.4	0.2	-0.1	-1.7	2.9
CM 5898-2	-0.5	0.5	-0.4	1.4	1.0	0.3	-0.6
CM 5902-2	1.7	2.3	-2.0	-0.7	-1.1	0.5	-0.6
CM 5948-1	0.3	0.9	0.9	-1.5	-0.7	0.4	-0.6
CM 6049-1	-0.8	1.4	-0.4	-1.2	-1.4	-2.1	1.6
CM 6061-1	-0.2	0.4	0.2	-0.3	0.7	0.3	-0.6
CM 6068-3	0.8	-0.5	0.9	-0.7	-1.1	-0.1	-0.2
CM 6070-1	-1.0	-0.6	1.8	-1.4	-1.1	-0.7	0.2
CM 6082-1	0.1	-2.4	-0.4	1.1	0.9	-1.5	-0.2
CM 6438-17	0.3	0.9	0.2	-0.1	-1.1	0.5	-0.6
CM 6630-2	-0.3	-0.8	0.2	0.9	1.3	-0.7	-0.6
CM 6664-3	-0.5	0.4	0.9	0.6	1.0	-0.2	-0.6
CM 6691-2	-1.1	-0.2	-0.4	2.9	1.0	-0.8	2.3
CM 7050-1	0.9	0.3	0.2	0.2	1.5	1.0	-0.6
CM 7086-14	1.9	-0.6	1.8	0.6	0.4	0.5	-0.6
SM 667-1	-1.9	-0.8	1.8	-0.7	-0.3	-1.5	-0.6
SM 673-1	-0.5	0.1	-0.4	0.0	-0.1	1.3	-0.2
MBra 97	0.8	1.7	0.2	0.5	0.1	0.1	-0.6
ICA Catumare	0.3	0.0	0.2	-1.6	-1.4	2.2	0.4

[a] *Derived from Microsoft Excel© functions to standardize data:*
AVERAGE(number 1, number 2, . . .)
STDEVA(value 1, value 2, . . .)
STANDARDIZE(x,mean,standard_dev)
where: x is the value you want to normalize; mean is the arithmetic mean; standard_dev is the standard deviation of the distribution

[b] *1 = excellent plant/root type and general appearance; 5 = poor*

[c] *1 = very low disease damage; 5 = severe disease damage*

[d] *1 = excellent; 5 = poor*

APPENDIX VI-C: CALCULATIONS FOR THREE SELECTION INDICES AND THE TOP TEN CLONES FOR EACH INDEX [a]

	Selection index 1	Selection index 2	Selection index 3
CG 1139-2	**7.8**	**7.1**	-1.0
CG 1450-4	**13.4**	0.3	0.0
CM 2166-6	**18.0**	**27.7**	**0.7**
CM 2766-3	-3.2	0.7	0.4
CM 2766-5	-17.5	-5.5	-1.1
CM 3320-8	-16.1	-16.5	0.2
CM 4484-2	4.0	**10.3**	**0.7**
CM 5620-3	1.7	**5.8**	-0.7
CM 5789-1	4.7	**17.0**	0.0
CM 5898-1	-9.6	1.6	-2.7
CM 5898-2	**22.6**	**18.1**	**2.0**
CM 5902-2	-12.8	-16.5	0.0
CM 5948-1	-16.5	-23.8	-0.9
CM 6049-1	-32.5	-29.6	-2.8
CM 6061-1	3.8	-2.7	0.4
CM 6068-3	-15.4	-14.0	-0.5
CM 6070-1	-21.5	-18.7	-1.6
CM 6082-1	3.4	**15.5**	**1.4**
CM 6438-17	-3.3	-5.5	0.5
CM 6630-2	**11.2**	**8.9**	**1.6**
CM 6664-3	**11.2**	0.6	**1.2**
CM 6691-2	**31.0**	**32.9**	**0.6**
CM 7050-1	**15.2**	2.1	**0.9**
CM 7086-14	**6.7**	-6.2	**1.3**
SM 667-1	-15.0	-12.5	-0.1
SM 673-1	**11.3**	**14.7**	0.2
MBra 97	4.1	-9.9	**1.2**
ICA Catumare	-6.6	-1.8	-2.0

[a] Clones selected by each index highlighted in bold. Selection index formulas:
*Selection index 1 = -(foliage yield * 3) + (root yield * 8) + (harvest index * 5) + (dry matter * 8)*
*Selection index 2 = -(foliage yield * 6) – (root yield * 6) –(bacterial blight * 5) + (root yield * 8) + (dry matter * 8)*
Selection Index 3 = (root yield – cooking quality)
Note: Negative values are applied where a lower number is preferred, and a higher number is less preferred, such as general root and foliage evaluation, bacterial blight and cooking quality

APPENDIX VI-D. DATA FOR SELECTIONS FROM THE APPLICATION OF VARIOUS SELECTION INDICES (SEE APPENDIX VI-C). SHADED COLUMNS INDICATE CRITERIA INCLUDED IN EACH INDEX

	General foliage eval.[a]	General root eval.[a]	Bacterial blight[b]	Root yield (tonnes/ha)	Harvest index	Dry matter (%)	Cooking quality[c]
Selection index 1							
CG 1139-2	3.5	2.2	1.7	27.1	0.64	35.2	3.4
CG 1450-4	2.4	3.8	2.3	20.8	0.62	39.0	1.0
CM 2166-6	2.1	2.4	1.3	27.3	0.63	36.1	1.8
CM 5898-2	2.7	3.5	1.7	29.6	0.61	37.0	1.0
CM 6630-2	2.8	2.5	2.0	27.5	0.63	35.2	1.0
CM 6664-3	2.7	3.4	2.3	26.1	0.61	36.2	1.0
CM 6691-2	2.3	2.9	1.7	36.0	0.61	35.1	3.8
CM 7050-1	3.6	3.3	2.0	24.5	0.64	38.4	1.0
CM 7086-14	4.2	2.6	2.7	26.2	0.57	37.5	1.0
SM 673-1	2.7	3.2	1.7	23.5	0.54	38.9	1.4
Selected mean	**2.9**	**3.0**	**1.9**	**26.9**	**0.60**	**36.9**	**1.6**
Trial mean	**3.0**	**3.1**	**1.9**	**23.5**	**0.55**	**36.5**	**1.6**
Selection index 2							
CG 1139-2	3.5	2.2	1.7	27.1	0.64	35.2	3.4
CM 2166-6	2.1	2.4	1.3	27.3	0.63	36.1	1.8
CM 4484-2	3.2	3.1	1.3	23.8	0.54	37.6	1.0
CM 5620-3	3.1	2.2	2.0	24.7	0.56	36.1	2.6
CM 5789-1	2.3	2.7	1.7	22.3	0.50	38.3	1.4
CM 5898-2	2.7	3.5	1.7	29.6	0.61	37.0	1.0
CM 6082-1	3.1	1.2	1.7	28.4	0.60	33.8	1.4
CM 6630-2	2.8	2.5	2.0	27.5	0.63	35.2	1.0
CM 6691-2	2.3	2.9	1.7	36.0	0.61	35.1	3.8
SM 673-1	2.7	3.2	1.7	23.5	0.54	38.9	1.4
Selected mean	**2.8**	**2.6**	**1.7**	**27.0**	**0.59**	**36.3**	**1.9**
Trial mean	**3.0**	**3.1**	**1.9**	**23.5**	**0.55**	**36.5**	**1.6**
Selection index 3							
CM 2166-6	2.1	2.4	1.3	27.3	0.63	36.1	1.8
CM 4484-2	3.2	3.1	1.3	23.8	0.54	37.6	1.0
CM 5898-2	2.7	3.5	1.7	29.6	0.61	37.0	1.0
CM 6082-1	3.1	1.2	1.7	28.4	0.60	33.8	1.4
CM 6630-2	2.8	2.5	2.0	27.5	0.63	35.2	1.0
CM 6664-3	2.7	3.4	2.3	26.1	0.61	36.2	1.0
CM 6691-2	2.3	2.9	1.7	36.0	0.61	35.1	3.8
CM 7050-1	3.6	3.3	2.0	24.5	0.64	38.4	1.0
CM 7086-14	4.2	2.6	2.7	26.2	0.57	37.5	1.0
MBra 97	3.5	4.4	2.0	25.8	0.55	36.7	1.0
Selected mean	**3.0**	**2.9**	**1.9**	**27.5**	**0.60**	**36.4**	**1.4**
Trial mean	**3.0**	**3.1**	**1.9**	**23.5**	**0.55**	**36.5**	**1.6**

[a] *1 = excellent plant/root type and general appearance; 5 = poor*
[b] *1 = very low disease damage; 5 = severe disease damage*
[c] *1 = excellent; 5 = poor*

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