CHAPTER 18

Cassava Genetic Improvement

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Introduction

A highly profitable investment in agricultural research in terms of return to research is crop genetic improvement. Increases in productivity of principal grains and oil-bearing crops observed during the 20th century have been demonstrated to be due mostly to their genetic improvement (Fehr 1987).

Cassava has also benefited from technological contributions, particularly from genetics (Kawano et al. 1998; Kawano 2003), which has enabled the development of new varieties that more adequately meet the needs of farmers and consumers. Colombia possesses one of the few cassava genetic improvement programs found in the world, thus greatly favoring cassava growers in this country. This chapter describes the methodologies used by this program and its most relevant achievements.

Advantages and Disadvantages of Vegetative Reproduction

Genotype refers to all the genetic characteristics of an individual. To a great extent, plant breeding consists of identifying genetically desirable individuals, that is, those that have a superior genotype.

Cassava is reproduced vegetatively. Each and every propagule obtained through vegetative reproduction is genetically identical, and constitutes what is known as a *clone*. This implies that when a desirable genotype is identified, the latter can be multiplied and perpetuated, generation after generation, without *genetic segregation* occurring. In this regard, cassava and other crops with vegetative reproduction such as sweet potato, potato, yam, and fruit trees offer a great advantage over those that multiply only through botanical or sexual seed.

From the genetic viewpoint, cassava varieties are, in fact, hybrids between two selected parents. Cassava improvement starts with thousands of crosses and continues with an elaborate and expensive evaluation process (described in more detail below) to finally identify a few individuals that are genetically superior. Outstanding hybrids result from a cross that produces a unique and specific combination of genes that confers on them the *hybrid vigor* that characterizes them.

An optimal combination will give good hybrid vigor and result in a successful variety (i.e., a cultivar that farmers will plant). Very few combinations of progenitors stand out, which means that thousands of crosses must be evaluated every year. Once a genetically superior cassava plant is identified, it can be multiplied vegetatively to deliver that genetic superiority to farmers.

For many other crops, where reproduction is not vegetative, farmers may also plant hybrid materials (e.g., maize, sorghum, and carrot). These hybrids result from combinations of 2 to 4 progenitors that have been specifically identified because when they are crossed, they produce outstanding material, similar to what occurs with cassava. The seed resulting from such crosses is what farmers plant.

These hybrids are identified genetically by the term *F1* (i.e., first filial generation). Thus, the vigor observed in F1 of a commercial hybrid cannot be transmitted adequately to later generations. If farmers plant seed

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harvested from F1 (technically identified as *F2* or second filial generation), "degeneration" can be observed. In other words, the high yield, uniformity, and other desirable characteristics that farmers receive from the purchased hybrid seed are gradually lost in subsequent filial generations. Hence, farmers must purchase hybrid seed year after year. The scientific basis of this "degeneration" is genetic segregation, mentioned above.

Technically, what occurs with genetic segregation is that the genes present in an F1 hybrid are shuffled, in the same way as a pack of cards are before being dealt. When an individual of any species undergoes sexual reproduction, the genes present in the individual are reorganized. From the evolutionary viewpoint, this is critical because it enables the creation of new genetic forms or recombinations that constitute the foundation of evolution. From the agricultural viewpoint, however, this is sometimes inconvenient, as the shuffling of genes, which creates new genetic forms, destroys that specific, difficult-to-obtain, yet desirable combination of genes once a successful hybrid produces F1 seed. Once a superior hybrid is identified in crops such as maize, the problem of how to perpetuate it then has to be solved, because the option of vegetative reproduction is not available. Hence, cloning would provide great advantage for such commercial hybrids.

To multiply a hybrid and produce seed for sale to farmers, the parental lines must be "fixed" by producing highly endogamous lines and later crossing them. This procedure complicates farmers' access to hybrid materials and makes them much more costly. In contrast, with cassava, once the genetically superior plant is identified, it can be reproduced vegetatively in such a way that farmers do not need to purchase hybrid seed year after year. However, as described later, the use of inbred parents allows for much more refined processes of improvement. Non-additive genetic effects (dominance and epistasis), responsible for heterosis, can be exploited more efficiently. It also enables the implementation of improvement methods such as backcrossing, which has been, and still is, widely used (Allard 1960; Blair et al. 2007).

Vegetative reproduction nevertheless presents some drawbacks: the rate of vegetative multiplication in cassava is very low—one plant produces only 6 to 10 cuttings or stakes—whereas, in sexual reproduction, the rate is usually much higher. For example, a maize cob normally produces about 400 seeds, so that the multiplication rate would be 1:400.

Another disadvantage of vegetative multiplication is the frequent accumulation of diseases, especially viral, in planting materials. Once the plant acquires a pathogen (particularly a virus), it is highly unlikely that it can free itself of that pathogen. Hence, all the stakes extracted from that plant will contain the pathogen. Good plant health management of cassava seed is therefore essential, and farmers must be encouraged to make minimal efforts to maintain the health of their planting materials. In contrast, botanical seed, resulting from sexual reproduction, is usually free of viral pathogens.

Another adverse aspect of vegetative multiplication is that stakes or trimmed stems require much more care than botanical seed. The conditions under which planting materials are stored will affect plantlets vigor and thus influence the crop's general performance.

For the above reasons, this paper gives special attention to the management of cassava vegetative seed to achieve an optimal physiological and sanitary state that maximizes returns to farmers.

A final drawback of vegetative multiplication is the volume that planting materials occupy. A 10-ton truck can load enough cassava seed for about 10 ha. The same truck could transport enough maize seed for about 400 ha.

Factors for a Successful Plant Breeding Program

The success of a genetic improvement program for a crop depends mainly on:

1. Continuity over time
2. Appropriate definition of objectives
3. Implementation of a good improvement scheme
4. Availability of representative environments for evaluations

Continuity over time

This is particularly important for cassava because of its prolonged selection cycle, which typically requires more than 5 years. The rest of the chapter describes this issue in detail, as do other publications such as Ceballos et al. (2004, 2007a) and Morante et al. (2005). In contrast, a selection cycle for grains or legumes can be completed in less than one year. Crop genetic improvement is a continuous and gradual process that requires several cycles to achieve its objectives. As a result, a fundamental need is to ensure that resources

will guarantee the continuous execution of the activity. Objectives for the process should be more or less stable, and changes introduced gradually and only when their need has been convincingly confirmed.

Adequate definition of objectives

Plant breeders should adequately define the objectives of their programs. Usually, for most crops and, in this regard, cassava is no exception, the goal is to increase yield per unit area; also to (1) maximize stability of production so that farmers will have adequate food security from their harvest, and (2) maintain or improve product quality so that this better meets the needs of end consumers. Stability of production is important and is achieved when the developed material is genetically tolerant or resistant to the main biotic and abiotic production constraints.

Because cassava is usually grown in marginal environments, it is highly susceptible to natural disasters such as drought or prolonged winters. A successful variety must necessarily have qualities that enable it to bear these and other stresses. Each environment where cassava is grown has its own list of factors that limit production. In the North Coast of Colombia, for example, the absence of rains and availability of water form the principal abiotic constraints to productivity. With regard to pests and diseases, mites (*Mononychellus tanajoa*, *M. caribbeana*, *Tetranychus urticae, T. cinnabarinus, Oligonychus peruvianus*), thrips (*Frankliniella williamsi*), and the stemborer (*Chilomima clarkei*) pose the most common problems.

In Valle del Cauca, in contrast, the availability of water is not such a major problem, which means that, instead of mites, the principal pest are whiteflies (*Aleurotrachelus socialis* and, to a lesser extent, *Bemisia tuberculata*). (In other regions of the world, *B. tabaci* is the main disease vector, including diseases such as the African cassava mosaic disease or cassava brown streak). In some areas of Colombia, cassava bacterial blight (*Xanthomonas axonopodis* pv. *manihotis*) and superelongation (*Sphaceloma manihoticola*) are also economically significant constraints.

All these observations are integrated into the process of genetic improvement for each ecoregion, so that resulting materials will have good levels of tolerance or resistance to these stresses. This activity is fundamental, both to maximize production and guarantee its stability, the latter of which is essential for the farmer's economic survival.

Any genetic material produced should also adequately meet the needs of users or end consumers. These can be described as four major destinations for cassava in Colombia (as with the rest of the world), each of which defines specific requirements from the crop. Aspects of quality are becoming extremely important and are described in more detail in the next section.

Developing a good improvement scheme

Once defined, the improvement program's specific objectives should be implemented. For this, a good improvement scheme is necessary. Because this issue is complex, it is treated in more detail as this chapter develops.

Representative environments

Finally, to identify superior cassava varieties that adapt to the environments for which they are directed, evaluations in representative sites of the targeted environments must be conducted. Here, a compromise must be made on the number of environments that can be handled with the objective of maintaining the widest diversity of situations in which, in practice, this crop is found.

For Colombia, six relevant and distinctive environments can be determined for cassava: (1) subhumid Caribbean (Department of Atlántico), (2) humid Caribbean (Córdoba), (3) Orinoquía (Meta), (4) inter-Andean valleys (Valle del Cauca), (5) high-altitude areas of about 1800 m above sea level (Cauca), and (6) humid lowlands (Putumayo). In these six environments, most of the conditions under which cassava is cultivated in the country are represented. They are also representative of most cassava-growing environments around the world.

Specific Requirements for Cassava as Demanded by Different Industrial Uses

As already mentioned, several industries base their activity on processing cassava roots. These industries need raw material at competitive prices, in constant supplies, and typically possessing good levels of dry matter. These requirements are constant for all industries. However, specific requirements for certain qualitative characteristics exist for different industrial uses (Table 18-1).

Table 18-1. Differences in some of the specific requirements for cassava according to its final use. Values in parentheses indicate the relative significance of each characteristic on a scale of 1 to 3, where 1 = very important; 3 = not so important.

Parameter	Final use		
	Starches, bioethanol, and animal feed	Fresh-root consumption	Food processing
Yield	(1)	(2)	(1)
Cyanogenic glucosides	(3) Bitter cassavas are preferred as they do not require so much surveillance	(1) Only "sweet" cassavas are acceptable	(1) Only "sweet" cassavas are acceptable
Parenchyma color	(3) For starches, it must be white; for balanced feeds, an orange color (indicating high carotene content) is suitable	(1) Usually white is preferred, although in some regions yellow roots are acceptable	(2) Currently, only white roots are processed; yellow roots, however, offer advantages
External appearance of roots	(3) An undesirable presentation implies that less surveillance is needed	(1) The more a root looks like variety 'Chiroza' (dark coffee-colored peel and pink cortex), the better its price will be	(3) Industry does not need "markers" to recognize good cassava, as it works through contract
Tolerance of root diseases and pests	(2) Only where they affect yield	(1) If presentation is affected, even if it is only a "cosmetic" problem, the price will be greatly affected	(1) Root smallpox disease is only a cosmetic problem, but it affects prices to industry
Dry matter contents	(1) The higher the content, the higher the price	(2) Varieties for fresh consumption usually have intermediate levels of dry matter content	(1) High dry matter content is usually preferred; the proportion of sugars can be significant
Culinary quality	(3) Poor quality material is even preferred as field surveillance will not be needed	(1) The basic criterion for this type of use	(2) Quality of the processed product is more important; hence, cassava of intermediate culinary quality can be excellent

Starch production and energy source for animal feed

For these industries, the principal objective would be to produce varieties with (1) high yield potential to enable the production of raw material at competitive prices, and (2) high dry matter content to facilitate starch extraction or the drying of roots. Yellow roots would be more suitable for animal feeds, while white roots are preferred by the starch industry. *In planta* modification to produce varieties with special starch characteristics offers opportunities and advantages over the chemical and/or physical modification currently carried out to generate starches with special functional properties (Ellis et al. 1998; Davis et al. 2003).

Fresh-root consumption. This is the traditional market for fresh roots, which are sold in both open-air markets and supermarkets. For this end use, cassava should be "sweet" (low contents of cyanogenic glucosides), with usually intermediate dry matter content, and, especially, excellent culinary quality. The root appearance (e.g., form, peel color, and parenchyma or pulp color) is fundamental. Productivity in this case has a smaller relative weight than for cassava destined for either starch or balanced feed industries.

Food-processing industries. This growing sector is represented by pre-cooked and frozen croquettes and fried cassava chips. In these cases, productivity is essential, and root characteristics should be adjusted to industrial requirements. For croquettes, for example, varieties should be "sweet", with little fiber and levels of dry matter that are usually higher than for fresh consumption. Sugar levels in roots affect the quality of fried cassava chips.

Table 18-1 describes the principal selection criteria according to uses given to cassava in Colombia. Other

cassava products for human consumption include *gari* (toasted fermented cassava meal) and *fufu* (boiled cassava pounded into a paste and eaten with stews and soups), which are consumed in Africa; and *farinha* (toasted cassava meal) and *casabe* (a flatbread), which are typical in several South American countries (Cock 1985). Another highly distinctive use of cassava, which is uncommon in Colombia but adopted in countries such as Cuba (García L and Herrera 1998), is the harvest of young foliage. For this use, cassava is planted at high densities and foliage is cut about every 4 months.

Bioethanol. This is a growing industry, thanks to price increases in oil and oil derivatives on the one hand and technology developments on the other. Research on the economic and competitive hydrolysis of maize endosperm (before fermentation is begun and distillation carried out) has directly benefited similar processes conducted on cassava roots.

Adding value to crops

Traditionally, the production chain for the food sector has been fractionated so that little or no interaction exists between its different components. Thus, crop breeders interacted only with farmers who purchased their products. Communication was usually very limited between plant breeders, the sector purchasing and selling agricultural products, processors, and, in the final analysis, end consumers.

Recently, however, recognition is growing of the need for greater integration among the different components of a given production chain. This principle is influencing policies of agricultural research in Colombia (CONPES 2000), including the case of cassava within the poultry and pig-raising chains. Thus, suppliers of genetic resources are interacting more closely with, for example, merchants of grains and other agricultural products, livestock producers, food processors, and food wholesalers and retailers to discover the specific needs of the different actors.

In other words, crop genetic improvement has been reoriented so that its objectives aim more precisely at the needs of end users. Thus, in well-developed markets, cash crops seek to better meet the needs of merchants, processors, and consumers of agricultural products. For less developed markets or household consumption, the end user is usually the farmers themselves. In this case, specific participatory research methodologies have been developed to seek better satisfaction of farmers' needs as they themselves define them (CIAT 1991).

A better comprehension of the needs of different consumers enables breeders to identify the opportunities a given crop offers, and thus make it more competitive or profitable. Such knowledge, in its turn, permits a definition of research objectives not only from the viewpoint of genetically improving crops, but also from other aspects that must accompany and complement the release of new varieties. These principles also are valid, and are being applied, for the cassava crop.

Kleese (2000) suggested several factors for consideration when value is to be added to a given crop. These include:

The needs of end users should be understood. Through such understanding, areas with potential for exploitation can be identified. In some areas of economic activity, barriers may exist to free exchange of information, stemming from aspects of intellectual property, common in many highly competitive markets.

Substituting values versus creating new added values. The most obvious opportunities for adding value to a crop occur when the latter can meet needs covered by other ingredients. For cassava, the use of yellow roots (high carotene content) reduces the need for exogenous supplements of carotenes and/or colorings in poultry feeds. It can also be useful in a basic strategy to reduce the awful effects of vitamin A deficiency in humans (Echeverri 2001).

Another example that stands out is that, sometimes, added value results by removing a given product, as in the case of inositol in maize. This product links with phosphorus in such a way that it cannot be absorbed by monogastric species. Hence, the phosphorus found in chicken and pig manure from major operations is becoming a true ecological problem. A strategic alternative, in this case, would be to improve the crop to reduce inositol content (and, hence, the quantity of phosphorus linked to it), or, instead, add enzymes that will degrade it. The end beneficiary of these potential solutions would be the environment itself.

The need to capture a new added value. Two fundamental aspects of introducing crops with added value are whether the market would pay for the new value, and how the different actors of a given

production chain would share the additional profits. With respect to the latter aspect, it should be remembered that, in each case, an added value to a specific crop would compete with other alternatives available on the market. Policies being implemented should guarantee that any additional profitability is adequately distributed among the different actors of the production chain to prevent monopolization by one or another.

Redefining an added value. For farmers, yield has been the most prevalent way of determining the value of most cash crops. Ideally, the adding of value should be done without it being at the expense of a crop's productivity. For cassava, higher dry matter content and increased carotene content in roots are examples of where value can be added without necessarily reducing productivity. Assuming the simplest case where parity with productivity exists, how and who defines the magnitude of increase in the crop's value? A need must be recognized for which the consumer or processor is willing to pay extra for a product that will be more useful in meeting that need.

If this incentive does not exist, farmers will not necessarily adopt a new variety, as happened in the case of quality-protein maize (QPM)[3]. This maize was improved so that, while obtaining yields and grain quality similar to normal maize, it also offered greater availability of two essential amino acids: lysine and tryptophan (Vasal 2000). However, this maize was not extensively adopted because the food industry was unwilling to pay higher prices for it, even though it better met the industry's needs.

Can the technology function? It is clear that crop genetic improvement can improve a crop's nutritional quality. Maize with its variants of high oil content (Dudley et al. 1974) or high protein quality (Vasal 2000) demonstrates this clearly. Iron and zinc can be added to beans (Beebe et al. 2000) and carotenes to cassava (Chávez et al. 2000). But, would such modifications be sufficiently large to be reflected in changes in the crop's commercial value?

In some specific cases, answers to these questions are even more difficult to obtain. For example, in the marketing of transgenic crops (maize and cotton) that carry the gene from the entomotoxin of *Bacillus thuringiensis* (*Bt*), numerous factors intervene to influence the final commercial value that these crops have. Production by farmers benefits from the reduced need to apply agricultural chemicals to control insects that are controllable by the gene *Bt*. The environment also benefits from fewer indiscriminate interventions by farmers. However, public opinion has been manipulated in such a way that, in many cases, the polemics of transgenic crops move away from the specifically technical and scientific to the more philosophical and political, distorting the true value that these products may have.

Transgenic crops possess enormous potential for the possibilities offered, in different crops, through the addition of value. For example, the quality of oil in a given oleaginous crop could be modified to benefit human health by decreasing the proportion of the saturated fraction. Transgenesis could genetically alter the rice crop to increase carotene and iron contents ("golden rice") (Ye et al. 2000); and reduce amylose content in cassava starch (Munyikwa 1997) to produce waxy cassava that would have enormous potential in the starch industry. But, as well as the undeniable increase in the crop's value from a biological viewpoint, aspects, including psychological, must be considered as they intervene in the definition of the final value that such a crop would have for society.

Preserving the added value. This is also relevant. For those products whose added value is easily detectable (e.g., the yellow color of cassava roots with high carotene content), management is simple. However, some characteristics are difficult to detect, as in the case of beans with high levels of iron and zinc. In such cases, an independent marketing chain may be required to preserve the product's identity as having added value. However, this same need would increase marketing costs.

Freedom to operate. This refers, mainly, to the growing quantity of legal restrictions that stem from intellectual property rights for different products such as genes, procedures, and enzymes. Sometimes, a vacuum exists in the legislation of numerous countries on the use of genetically modified organisms. In other cases (Argentina, Canada, China, and USA), the regulation of its production and use is lax. In yet other cases (mainly Europe), the production, marketing, and use of food derived from genetically modified crops are highly restricted. Ironically, this situation contrasts with the production and use of drugs and medicines that are also obtained through genetic transformation techniques, but which have not generated as much polemic as the case of agricultural products.

3. For an explanation of this and other abbreviations and acronyms, see *Appendix 1: Acronyms, Abbreviations, and Technical Terminology*, this volume.

Flowering and the Acquisition of Botanical or Sexual Cassava Seed

As described in Chapter 2 on the plant's morphology, cassava is a monoecious species that has staminate (male) and pistillate (female) flowers on the same inflorescence (Figure 2-5, Chapter 2, this volume). Crop genetic improvement requires, as an essential stage, the development of new genotypes that are superior to those already available at the time of development. New genotypes occur through crosses between progenitors that have been selected for the purpose because they present one or more desirable characteristics. Crossing consists of facilitating the deposit of pollen from one progenitor's male flower onto the stigma of a female flower of another progenitor.

In controlled crosses, flowers are handled directly to ensure greater control over pollination. Occasionally, the researcher may decide to carry out self-pollination, whereby pollen from one plant is deposited on the female flowers of the same plant. This, however, is carried out only for research purposes; it very rarely gives rise to a commercial variety.

Flowering in cassava is always associated with stem branching in such a way that those cultivars that do not branch, do not flower either. The plants flower preferably during the short days of the year (Jennings 1970). Flowering in this crop is highly variable, influenced by genetic—some varieties rarely flower, while others flower abundantly—and environmental factors—the same variety may not flower in lowlands but will flower exuberantly in mid-altitudes. As a result, the production of botanical seed in a genetic improvement program is highly variable and depends heavily on the combination of progenitors that are to be crossed, as well as the sites and timing of pollinations.

After pollination and subsequent fertilization, the ovary develops, forming the fruit, which takes about 3 months to achieve maturity. The fruit is a dehiscent capsule and is either trilocular ovoid or globate. It measures 1.5 to 2.0 cm in diameter, and has six narrow longitudinal and prominent edges (Figure 18-1). The capsule is hard and has three loculi, each of which contains a seed. Seeds are elliptical and coffee-colored or gray, with black or coffee-colored guttae (or spots), depending on the mother variety (Figure 2-6, Chapter 2, this volume).

Figure 18-1. Clockwise, fruits at 1, 2, and 3 months after pollination, and cassava seeds.

The production of sexual (or botanical) cassava seed at CIAT involves several stages and options. Understanding the plant's flowering system helps in handling the procedure with maximum efficiency. Seed production includes, in addition to parent selection, hybridization or crossing, seed collection and the seeds' correct coding, storage and treatment, and use of an elaborate planting system.

Hybridization

The relatively large size of flowers ensures that controlled pollination in cassava is easy and relatively simple. The female flowers usually open 10–14 days before the male ones of the same inflorescence do. However, female and male flowers of different racemes on the same plant commonly flower at the same time, enabling self-pollination. Flowers usually open at mid-day.

During free pollination of flowers, both self- and cross-pollinated seeds may be produced, in proportions that depend on genotype, planting design, type of insects present in the area, and timing of pollination. With some practice and judging from the flower's color and form, predicting which female or male flowers will open during the day is possible. A more effective method is to loosen an unopened tepal of the female flower. If a drop of nectar lies near the tepal's base the flower will open that day (Figure 18-2).

When two different progenitors are crossed, the resulting progeny constitutes a family, usually called *F1*. Because of cassava's high heterozygosity (the genetic heterogeneity present in an individual), each seed produced in an F1 cross generates a genetically

Figure 18-2. A tepal of a female flower carries a drop of nectar, which indicates that the flower is receptive to pollination.

different plant, which then has the potential to produce a new variety. Consequently, great genetic variability can be easily introduced and managed through these crosses, which in their turn, permit the selection and acquisition of genetically superior individuals. However, the production of large quantities of seed is laborious and, usually, expensive. Within CIAT's scheme of cassava improvement, two types of crosses are made for seed acquisition: controlled and open (or *polycrosses*).

Controlled crosses

For controlled crosses, both female and male progenitors are known, so that the progenies produced constitute a family of *full sibs*. Selected parents are organized in separated crossing blocks and pollinations are directed mostly between varieties for a single adaptation area. However, they are also carried out between varieties from different areas to transfer and recombine specific characteristics and increase the *plasticity* of the materials, so that they may adapt more broadly and/or possess better stability in production.

For controlled crosses, 10 to 20 plants per selected genotype are used as parental materials, planted in rows with distances between plants being 1 m and between rows 2 m. The latter distance is to facilitate circulation of the people who observe and select flowers ready for pollination each day.

Pollination is carried out in the morning after female flowers, likely to be receptive that day, are chosen. They are then covered with cloth bags, measuring 20 × 25 cm, to protect them from undesirable pollen after opening. Those male flowers that are close to opening are themselves collected on the same day in the morning and deposited in plastic or glass bottles, identified with the variety's name (Figure 18-3). The flowers will open in their bottles at mid-day.

Pollination is carried out by first finding suitable female flowers and then gently rubbing their stigmas with pollen-bearing anthers (Figure 18-4). As many as three different female flowers can be pollinated by a single male flower. The pollinated flowers are then

Figure 18-3. One of the plastic bottles used to store male flowers after their collection in the morning until their use at mid-day.

Figure 18-4. Procedure used for controlled crosses in cassava.

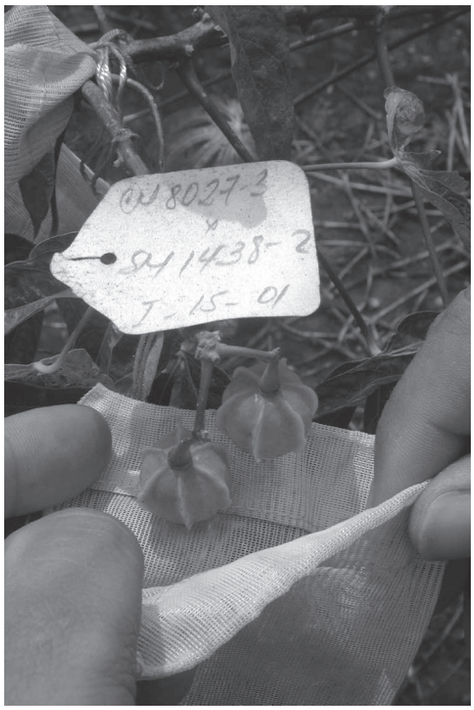

Figure 18-5. Tags used to identify different pollinations and the bags with which cassava flowers and fruits are covered.

identified, using tags on which the crosses are detailed (thus noting the origin of the pollen, together with the date and number of pollinated flowers) (Figure 18-5). Those of the raceme's female flowers that had not opened by the time of pollination are eliminated to prevent later confusion with those that were pollinated. The information of all crosses within one day is tabulated and later transferred to either a card system or directly to an electronic system. Thus, crosses can be organized first by the female parent and then by the male parent, following an alphanumerical order for each clone that was used as a progenitor.

Pollinated flowers can be covered immediately with either a cloth or paper bag, but they can also be left uncovered, as, apparently, exposed flowers are rarely, if at all, contaminated by pollen from other sources. However, flowers that are covered after pollination frequently have a low percentage of formed fruits, possibly because the temperature inside the bag increases sufficiently to "burn" the flower. After 3 weeks, the formed fruits must be covered with cloth bags to prevent attack from fruit fly (*Anastrepha pickeli*) and to collect seeds when dehiscence occurs (Figure 18-4). Fruits reach maturity at about 3 months after pollination.

Polycrosses

For open pollination, only the female progenitor is known, as the pollen may come from any of the surrounding plants. In this case, the possibility exists that, occasionally, self-pollination will occur. Progeny that results from open pollination of the same female progenitor constitutes a *half-sib* family, as the identification of the male progenitor remains uncertain. Within such a family the plants may have some phenotypic, but fewer, similarities than do full siblings from controlled crosses.

Open-pollinated seed can be collected from any cassava planting, but on the condition of having a mixture of numerous and genetically different materials. Different methods exist for increasing the possibility that the source of pollen is the desired one. The most commonly used is to plant a mixture of selected clones in isolated blocks, where they can cross exclusively with each other, while avoiding undesirable varieties. In this case, seeds from individual plants are collected, and records of female progenitors are kept. Such a system is called *polycrossing*.

CIAT uses a system of constructing blocks of polycrosses in which a spatial arrangement is designed to favor homogeneous pollination among the varieties involved. The field plans of the polycrossing plots follow Wright's methodology (1965). With this design, the same possibilities exist for crosses among selected varieties. Planting distances are 1.5 m between plants and 2 m between rows. To ensure that crosses are carried out among the varieties to be crossed, these blocks are surrounded by 8-m-wide barriers of male-sterile plants, planted at 1 m between plants and between rows. These barriers reduce pollen flow from one block to another and reduce the possibility of pollen from undesirable plants intervening in the pollinations.

Fruits generated by this system are collected when they are sufficiently mature physiologically, at about 2 months after fertilization. At this time, the fruits lose their natural shine, becoming opaque green in color. The peel then loosens readily from the fruit capsule, and dehiscence begins, with the edges separating until they are totally freed. At this moment, the peel takes on a coffee color (Figure 18-1).

Seed collection and coding the crosses

The dried and sectioned fruits in the bags, which were put in place earlier, are collected from the field and

seed is selected after all residues are removed from the peel. The seeds are organized; for controlled crosses, first according to the female progenitor and then to the male progenitor; and, for open-pollinated crosses, to the female progenitor, which is the only one known. The seed is then tested for density in a solution of 2% sodium hypochlorite to eliminate those with low-density or are non-viable and also to disinfect seeds of possible pathogens adhering on their exterior.

To facilitate data management, a code is assigned to each cross according to the progenitors involved. CIAT uses a code of two letters to indicate the type of cross and the progenitors involved. When the cross is controlled, different letters have been used over time such as "CG" or "CM". Currently, the letters "GM" are being used. Following the letters is a code of up to four numbers that represents a consecutive record of the crosses done at CIAT. This number identifies the family of the full sibs that possess progenitors in common. For example, the family CM 6740 identifies all progeny derived from the cross between M Col 1505 and M Pan 51. The same cross can be made over several years. However, if the same progenitors are used, the progeny produced will always have the same code, regardless of the year in which the cross is made.

As each individual of a full-sib family is genetically different from its siblings, the individuals of a single family are distinguished by a hyphen followed by a consecutive number. Thus, CM 6740-7 identifies the clone that was recently released as 'Reina'. In addition to CM 6740-7, numerous individuals within that family were produced, but only the seventh one was superior enough to be released as a new variety. Another interesting example is that of the family CM 3306, which produced excellent progeny that resulted in the release of the variety ICA-Negrita (CM 3306-4). More recently, the Colombian Corporation of Agricultural Research (CORPOICA) released CM 3306-19. In addition, the clone CM 3306-9 showed exceptional performance in Guajira, despite the severe drought conditions that are typical of the region.

For seeds resulting from open pollination, the letters "SG" were first used but are now replaced by the letters "SM". These letters are also followed by four numbers that represent a consecutive index that identifies a given polycrossing plot (i.e., a single group of progenitors), and the mother from which seed was obtained. For example, SM 1219 identifies the whole progeny derived from the mother CG 1450-4 that participated in the polycrossing plots of 1987. The code thus identifies not only the female progenitor from which this half-sibling family is derived, but also the group of individuals among which the male progenitor is to be found.

As in controlled crosses, the code for the half-sibling family is followed by a hyphen and a number that distinguishes the different individuals composing it. Thus, clone SM 1219-19 stands out among its half siblings for its superiority in mid-altitude valley environments. Unlike what happens for controlled crosses, the different individuals of an SM family may have different male progenitors.

Storing and treating botanical seed

Botanical cassava seed, stored at room temperature, maintains high viability for about one year after harvest. For medium-term storage (i.e., several years), they are conserved at 5–10 °C and 60% relative humidity.

Seeds are packed in small envelopes carrying the names of the cross and its parents, the source of seed, date of harvesting the fruits, and the number of seeds in the bag. During storage, seed is treated with fungicides and insecticides. In addition, drying in an oven at 55–60 °C for 10 to 14 days is sometimes recommended to eliminate potential risks of pests and pathogens from the seed. Such treatment also helps break seed dormancy, which normally lasts 2 months after harvesting.

Little information exists as to the optimal storage conditions for seed. Under normal environmental conditions, germination drops drastically 2 years after seed is harvested, becoming non-existent by the third year (Kawano 1978). However, Martín and Ruberte (1976) found that storage under dry (in calcium chloride) laboratory conditions still produces a good germination rate, even after more than 2 years of storage. At the International Institute of Tropical Agriculture (IITA), Nigeria, germination studies (1979) of seeds stored for more than 7 years at 5 °C and 60% relative humidity found that viability in seeds between 0 and 7 years old had not declined in any way.

Sowing sexual seed and transplanting seedlings

As mentioned earlier, the management of sexual seed is not difficult, but requires special care, particularly in the first stages of seedling development. The first consideration for sowing seed is the time at which this is carried out. Normally, sowing in trays should be 6 to

8 weeks before the crop is normally planted in the area, so that transplanting to the field coincides with that time. If several planting times are available, one should be chosen, according to convenience or relative importance of each time.

Seed is sown in trays, plastic bags, or in a bed prepared in the field. The percentage of germination from sowing directly in the field tends to be low and should be avoided if possible. The most preferable method is sowing in trays with individual compartments for each seedling. Ideally, a compartment should be about 3 × 3 cm and 6 cm deep (Figure 18-6). Trays without compartments are acceptable, but great care is needed to maintain the exact identification of each seed.

The planting substrate in the trays may be soil or an artificial mixture. It should be well drained and free of insects, pathogens, or weed seeds. For greater safety, the soil should be sterilized by steaming or fumigation. The substrate should have a good balance of nutrients and, especially, an adequate level of phosphorus. After preparing the trays or bags with soil, the seeds are systematically planted to a depth of about 1.0–1.5 cm. Seed packets should be arranged in ascending order according to the code, and the seeds sown in that order, identifying each family with a plastic or wooden marker that carries the code and faces the first row.

To germinate, cassava seed has highly specific requirements, which, if they are not fulfilled, can lead to a very low germination rate. The two most important requirements are suitable temperature and sufficient moisture in the soil or substrate. The optimal temperature regime fluctuates between 25 and 35 °C in a site where temperatures can be controlled. Otherwise, in a greenhouse or mesh house, temperatures may reach as high as 38 °C during the day. The substrate should be kept moist, but not saturated. Plants grow best under sunlight with normal intensity and no shade.

Figure 18-6. A tray is used for sowing botanical cassava seed, which is left until it germinates and the resulting seedlings transplanted to the field.

Six to 8 weeks after sowing, or when seedling height averages about 20 cm, the plantlets are transplanted to the field. The soil should be well prepared and preferably with ridges. Planting distance depends on the selection system implemented. In each transplant site, a small hole is made. Seedlings should be extracted from the tray with minimal damage to the roots, transplanting in the same order that the seeds were sown, that is, in ascending order of the number of the cross. The easiest way to transplant all the seedlings is in serpentine form, planting down the field and returning in the opposite direction. A free space is left between different crosses where a stake is placed carrying their identification.

If the soil has insufficient moisture at the time of transplanting, each seedling should be irrigated individually. During the first month, until the plants are well established, adequate soil moisture must be maintained. Also, during this establishment period, seedlings are highly susceptible to damage by cutting insects, slugs, and other animals, which means that protection requires continuous treatment and frequent checks. In areas where thrips cause damage, the plants must be protected by insecticide applications for the first months, until they are old enough to form pubescence on leaf buds (the most common form of resistance).

Throughout the cycle, the normal practices of any cassava trial are carried out. Only for the first 2 or 3 months are plants from sexual seed more delicate than those derived from stakes. Once past this period, they achieve an almost normal development.

The Cassava Genetic Improvement Scheme Used in Colombia

Below, we briefly describe the procedures for the genetic improvement of cassava for specific environments in Colombia. In this section, the reader can better understand the complexity of the improvement system and the need for continuity of adequate resources. Additional information can be found in other publications such as those by Ceballos et al. (2004, 2007a) anSd Morante et al. (2005).

Selecting parents for new groups of crosses

A major decision to make in crop genetic improvement is to choose the materials that will be used as parents to produce new varieties that have higher productive potential and better adaptation to the environmental conditions where they will be grown. CIAT has the enormous privilege to be the depository for the World Cassava Germplasm Bank. With more than 6000 varieties from Africa, Asia, and the Americas, the Bank contains a major proportion of not only the current genetic variability of *Manihot esculenta*, but also numerous wild species from which valuable genes can be extracted. The materials held in the Germplasm Bank were contributed by many countries and, together, are considered as humanity's patrimony. Use of this germplasm permits development of materials not only for Colombia, but also for the rest of the world.

Not all genetic variability is usable, as many varieties held by the Germplasm Bank lack characteristics that make them suitable as parents. However, their conservation is considered as a way of guaranteeing the crop's competitiveness or its future use in the event a new phenomenon occurs that the varieties currently disseminated are unable to overcome, for example, when a new disease or pest makes a sudden appearance. Only then will some materials, which had previously not offered any advantages, become valuable genetic resources.

A recent situation illustrates the strategic importance of germplasm banks. In several regions of the Departments of Cauca, Valle del Cauca, and Tolima, a whitefly problem had been gradually but constantly evolving over recent years to the point that it became a true constraint to production in these regions.

Responding to this growing problem, CIAT began evaluating materials in the Germplasm Bank, in the hope of finding varieties that would offer some type of resistance or tolerance to whiteflies. A variety from Ecuador (M Ecu 72) was identified as having excellent levels of resistance to these insects (Bellotti et al. 1999). In fact, the resistance present in M Ecu 72 was later confirmed to be of the antibiosis type. Accordingly, with support from the Colombian Government, through the Ministry of Agriculture and Rural Development (MADR), the resistant variety was included as progenitor in crosses for the inter-Andean valley region, where this problem was most severe. The antibiosis of M Ecu 72 is the first source of resistance reported for crops affected by whiteflies.

In addition to materials from the Germplasm Bank, cassava clones resulting from genetic improvement begun at CIAT in the early 1970s are also heavily used. For example, many of the genotypes selected as parents had high dry matter productivity per hectare (e.g., M Tai 8, SM 1565-15, and SM 1219-9). Other parents presented excellent qualities for the food-processing industry (M Per 183 and SM 1460-1), recognized combining ability to produce good progeny (SM 805-15 and SM 1565-17), or special characteristics such as resistance to root rots (CM 4574-7). An important modification, introduced in year 2000, was a more frequent inclusion of materials that possess a pulp with an intense yellow color, as a result of high carotene content. These materials may possibly have specific industrial uses, as they present low HCN levels (thus reducing the problem of drying cassava for the feed concentrate industry) while providing greater nutritional value. For the snack industry (fried cassava chips), an added advantage is that the product has a more attractive presentation.

Another new development with regard to progenitors for use in producing new genotypes is the introduction, through IITA, of African materials that possess resistance to the African cassava mosaic virus (ACMV). Fortunately, this disease does not appear in the Americas, but its insect vector (*Bemisia tabaci*) has recently been detected feeding on cassava in Brazil, Ecuador, Dominican Republic, and Puerto Rico (Bellotti et al. 1999). Hence, to introduce resistance to this severe disease before its eventual appearance is to be prudent, particularly as American cassava varieties are highly susceptible to African mosaic. Because selection cannot be made for resistance (as the disease is not present), molecular markers identified at CIAT will be used. The introduced materials were obtained through embryo rescue from sexual seed to ensure there were no risks of inadvertently introducing the disease into the country—as already mentioned, viral diseases are not transmitted through botanical seed. In addition to this precaution, special measures of plant health prevention were taken.

Acquiring plants from botanical seed and selecting the respective progeny

Once recombinant cassava seeds are produced, their progeny should be evaluated to select, from the massive number of genotypes, those few that surpass, in one or more characteristics, the best of the currently available materials. This is a slow, costly, but very important process. It gradually reduces the number of

genotypes for evaluating, while increasing the quantity of vegetative seed available for successive evaluations and/or multiplications.

Cassava is characterized by a notable genotype-by-environment interaction that results in a marked specificity of adaptation of varieties to specific environmental conditions. For example, varieties for the subhumid Caribbean usually do not adapt to humid Caribbean or Eastern Plains. As a result, selection must be made in each ecological region. In this regard, the country is seen to be highly favored by CIAT carrying out its selection activities in Colombia, as this guarantees excellent adaptation of germplasm to prevalent environmental conditions. For similar reasons, CIAT is highly favored by having possibly available such contrasting environments within a single country.

For each ecoregion, an independent evaluation scheme is carried out. Figure 18-7 illustrates the way in which these evaluations are currently conducted for each ecoregion. The sexual or botanical seed is sown in mesh houses to prevent the possibility of transferring diseases such as frogskin and then transplanted to the field at 2 months old (Stage F1).

These plantings are carried out in isolated plots to maintain the materials as free as possible of disease vectors (particularly, whiteflies) and thus reduce to the utmost the probability that these materials will contain communicable diseases. At 10 months, these plants are "harvested" to produce eight stakes or cuttings. All the stakes from one plant are packed together, suitably identified, and transported to the respective area of specific adaptation (e.g., subhumid Caribbean). On collecting the stakes, roots are reviewed to confirm that they do not have symptoms of diseases such as frogskin. The eight stakes are planted in individual furrows of eight plants in trials known as *clonal evaluation trials* (CET).

The enormous genetic variability, based on so many crosses among selected progenitors, can be appreciated in the CET. To exploit this great variability, several very large segregating families must be evaluated. Currently, between 1500 and 2000 clones are being planted in these trials. As expected, many of these clones will present different, possibly undesirable, characteristics. Hence, at this stage, selection is highly drastic, reducing the number of clones that will pass to the next stage of evaluation and selection to 200–300. As each genotype is represented by a relatively reduced number of plants (up to eight) planted with one replication, selection in the CET is based mostly on highly heritable characteristics (e.g., plant type, dry matter content in roots, capacity to produce storage roots, harvest index, and resistance to certain insects or diseases).

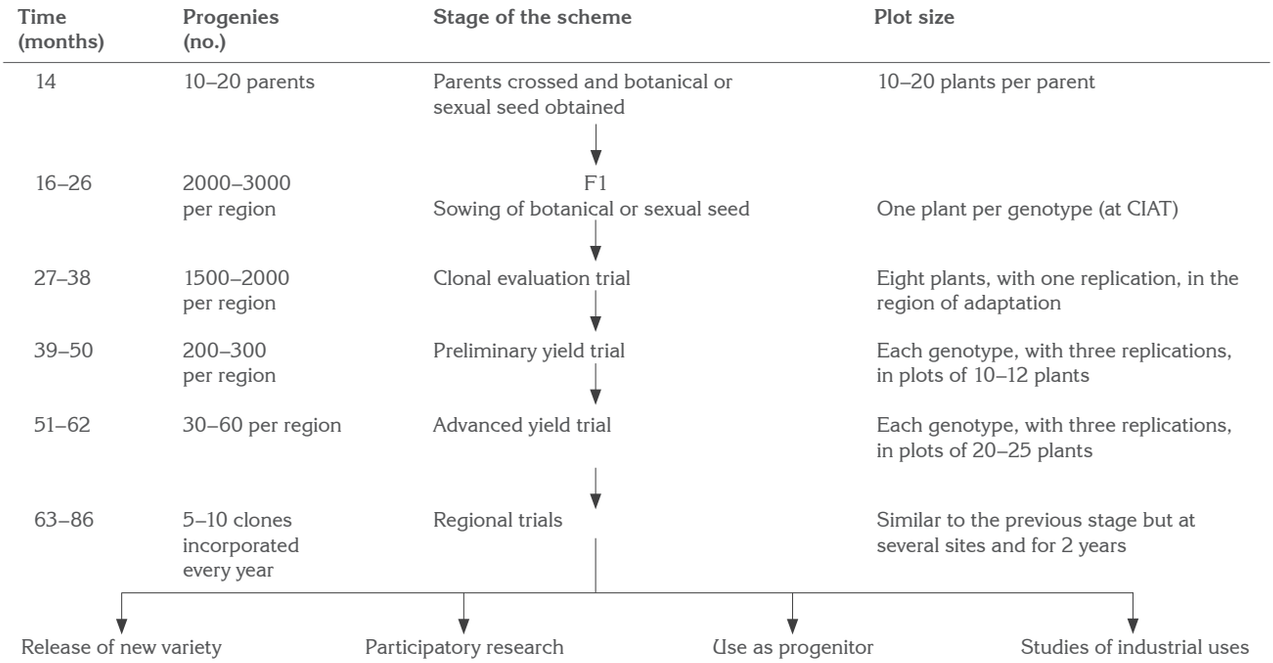

Figure 18-7. Basic cassava improvement scheme for each typical cassava-producing region in Colombia.

Figure 18-8. Clonal evaluation trial at Santo Tomás, Atlántico, Colombia, showing variation in the capacity for leaf retention.

Figures 18-8 and 18-9 illustrate the type of variation that can be observed among families evaluated in a CET. Figure 18-8 (Santo Tomás, Atlántico) illustrates how, in some families, at 5 months old, leaves tend to drop relatively quickly during plant development (whether for genetic reasons or the presence of biotic or abiotic factors), whereas other families maintain their leaves over longer periods. This capacity for leaf retention is a favorable influence (dry matter yield is about an extra 2 t/ha), as seen when the families were harvested 6 months later.

Figure 18-9 illustrates segregation for resistance to leaf diseases (bacterial blight and superelongation) typical in the Orinoquian Region. In this particular case, stakes of highly susceptible materials were planted to separate plots, which had been planted one behind the other. The plants originating from these stakes served as sources of inoculum, that is, as "spreaders", to ensure that disease pressure is relatively high and uniform throughout the whole trial.

Once this first selection in the CET is made, thus reducing the number of families to evaluate and increasing the quantity of available seed, evaluations start with larger plots and with replications. As the process advances (Figure 18-7), selection focuses more and more on characteristics of low heritability such as yield. This is because, only through the use of special experimental designs, inclusion of replications, and

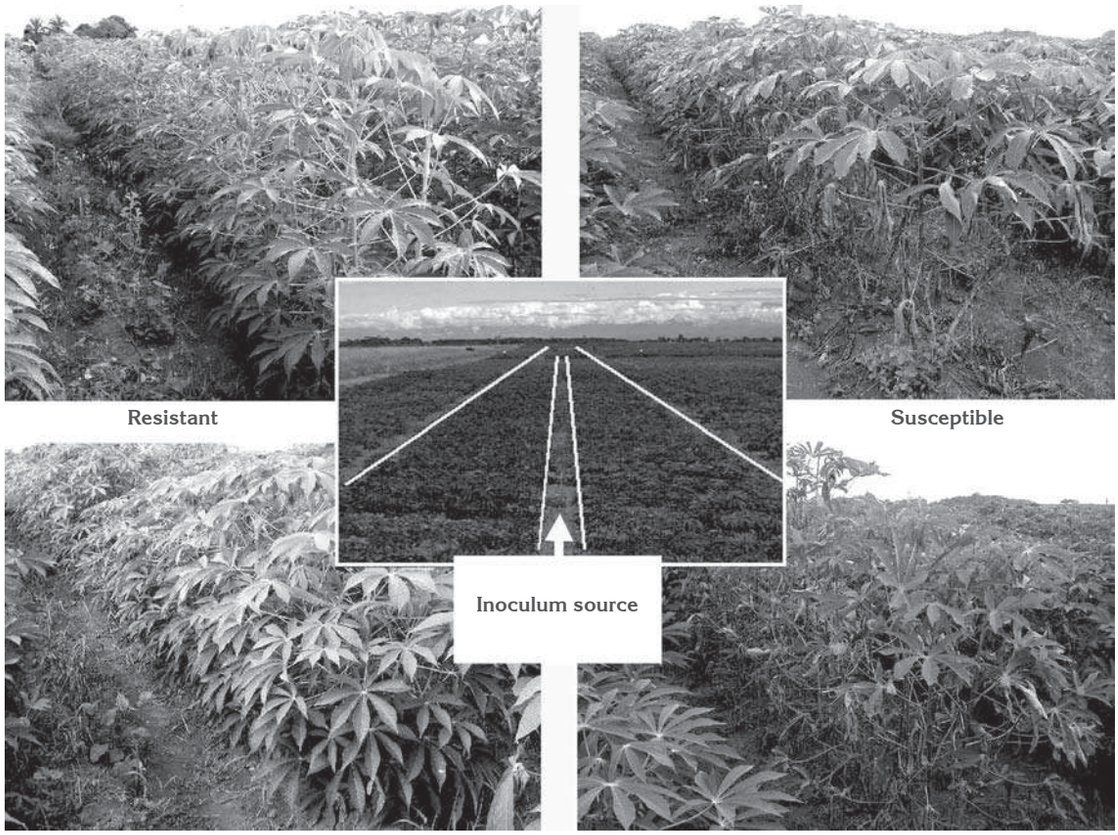

Figure 18-9. Clonal evaluation trial at CORPOICA–La Libertad, Villavicencio, Meta, showing resistance and susceptibility to leaf diseases.

evaluations across several sites, can the environmental effects influencing the expression of low-heritability characteristics be satisfactorily reduced.

At typical harvest time (e.g., February and March in the North Coast) the first two plants in the furrow are harvested to measure dry matter content (Figure 18-10). This characteristic modifies considerably according to the time at which roots are harvested. As a result, it should be measured in the representative season. The remaining plants are left in the field until the rains begin, when they are then harvested and potential yield evaluated in relation to volume of roots produced. Other variables are integrated into a *selection index* (SI), which is processed and analyzed by computer to quickly and efficiently choose the best 200 to 300 of 1500 to 2000 genotypes.

Cuttings from the remaining six plants and from only selected materials are used in *a preliminary yield trial* (PYT), with three replications of plots with 10–12 plants each. In other environments, such as the Eastern Plains or inter-Andean valleys, where the dry period is not so marked, fluctuations in dry matter content are not strong. Hence, all the plants of each furrow are harvested when the crop is usually planted. This permits identification of the best clones while all plants are also used as sources of vegetative seed. Stakes from selected materials and not used in the three replications are planted in a separate nursery to serve as sources of planting material for the next evaluation stages.

The best 30 to 60 clones identified in the PYT are selected to continue on to the next selection stage, known as *advanced yield trials* (AYT). These are carried out, using three replications, but with plots of 20–25 plants. From this stage on, harvests are carried out at the optimal and typical time (e.g., February and March for the North Coast) but only for the 6 to 9 plants in the center of the plot (Figure 18-11). These plants always have all around them other individuals of the same clone and their harvest permits estimating their characteristics more precisely.

The remaining 14 to 16 peripheral plants of each plot that were not harvested are left as sources of planting material, and used at planting time (e.g., May for the North Coast). These plants, located on the outside of each plot, are not evaluated precisely, because they are competing with plants of other varieties that may, for example, be more or less vigorous or more or less aggressive. As these plants are affected by their neighbors, their performance is not representative of the variety. They can nevertheless be used as sources of planting material without problems. This distinction is not made in the earlier stages of the improvement scheme because not enough vegetative seed is available for planting plots with 20 to 25 plants.

Between 5 and 10 of the best genotypes from the AYT are incorporated into *regional trials* (RT), which are planted in various representative sites of each ecoregion and with three replications per site. As, in each year, a new selection cycle is initiated, this means that, in a given region and at the same time, all the selection stages as described above can be found growing. Regional trials are conducted continuously. They include the current best clones in each region and cover the different purposes for which they may be destined (e.g., fresh consumption or industry). They also serve as checks.

Figure 18-10. Clonal evaluation trial at Santo Tomás, Atlántico, Colombia, after the first two plants of each row are harvested to ascertain the correct measurement of dry matter content.

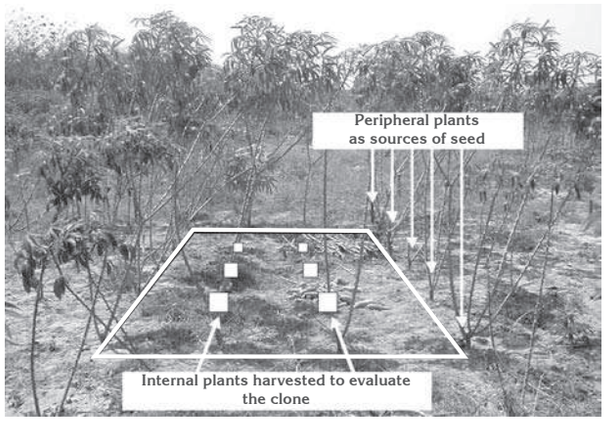

Figure 18-11. Plots with internal plants harvested and the peripheral plants left standing. These latter will be used as sources of planting material.

Those materials that do not surpass the checks are eliminated from the RT after 2 years and new promising genotypes planted after continual identification in the AYT. Ultimately, some new clones will surpass the checks in one or more characteristics in the RT. These materials are then evaluated for eventual release as varieties. This task is typically carried out by CORPOICA.

Results of the last regional trials held in the Caribbean coast, Eastern Plains, and inter-Andean valleys ecoregions

Below, we briefly describe some relevant results of the last selection stages in RT held in different regions of the country. In each case, the calculation of a selection index (SI) is included. This parameter condenses into a single number numerous variables that the breeder assesses. Its use permits even the consideration of variables that may have more weight than others (Baker and Rodgers 1986). The index is currently frequently used, as follows:

$$SI = [\text{fresh-root yield} \times 10] + [\text{dry matter contents} \times 8] + [\text{harvest index} \times 3] + [(-\text{plant type}) \times 3]$$

To remove the problem of the units in which each variable is measured (and which would influence the weight of each in the SI), each variable is standardized, following the statistical formula (Steel and Torrie 1988):

$$(X_i - \mu)/\sigma$$

where,

X_i is the average of a given variety,
μ is the average of all clones, and
σ is its standard deviation.

The coefficients of each term (10 for fresh-root yield, 8 for dry matter content, and 3 for harvest index and plant type) reflect the relative importance of each variable within the SI. These weights are subjective and may vary from one evaluation to another.

The variables included in the SI formula are the most important, but not the only ones to take into account in the selection process. The materials searched for are not only those with high yield potential (fresh-root yield), but also clones with high dry matter content (%) in roots, as this facilitates starch extraction and the drying of chipped cassava destined for animal feed.

The *harvest index* measures the proportion of the plant's total biomass that is represented by roots. This variable is particularly important in initial selection stages when not enough replications are available for each genotype (Kawano et al. 1998).

The fourth factor included in the formula is plant type. This variable is measured by using a visual scale that ranges from 1 (excellent) to 5 (highly undesirable). In this case, low values are preferred, in contrast to the first three variables where the highest values are preferred. Hence, this variable receives a negative sign in the formula.

The SI permits ranking the materials to facilitate final selection. In addition to the variables included in the index, other variables (e.g., resistance to pests or diseases) are reviewed. Sometimes, a material with an excellent SI has to be discarded for other reasons that make them totally unacceptable. In calculating, an SI close to zero describes varieties of average performance. When SI is positive, the varieties are superior (the higher the positive value, the greater will be the material's genetic superiority). Similarly, a negative SI reflects a performance that is below that of the average of the materials evaluated (the higher the negative value, the worse is the material's general performance).

Caribbean Region. Below we present data from three RT conducted in sites of the subhumid coast: Pitalito, Santo Tomás, and Molineros (Table 18-2), all in the Department of Atlántico. In this table, the clones have been ordered according to their rank in relation to the performance of their respective selection indices in each of the three sites. Of the 60 varieties evaluated, the results of the best 15 varieties are presented, as well as some checks that represent materials available to farmers.

Clone M Tai 8, known in Asia as 'Rayong 60', was the result of collaboration between CIAT and the Thai Government (Kawano 1992). Until recently, it was the best material available for industrial purposes and was planted to a large area of the country's Atlantic Coast. Yet, in these regional trials, it occupied tenth place in regional trials, thereby suggesting that a new generation of materials could very soon surpass the excellent performance of M Tai 8. Some of these materials are already being spontaneously disseminated (e.g., CM 4919-1) and others have proven to adapt well to other environments (e.g., SM 1411-5), which stood out in Lower Cauca, in Caucasia, Antioquia).

Table 18-2. Results of the 15 best of 60 genotypes, plus four local checks, from three regional trials conducted at each site in the Department of Atlántico, Colombia. Each trial consisted of three replications with plots of 25 plants each.

Clone	Pitalito					Santo Tomás					Molineros				
	Fresh roots (t/ha)	Dry matter (t/ha)	Dry matter (%)	Sel.[a] index	Rank	Fresh roots (t/ha)	Dry matter (t/ha)	Dry matter (%)	Sel.[a] index	Rank	Fresh roots (t/ha)	Dry matter (t/ha)	Dry matter (%)	Sel.[a] index	Rank
15 best genotypes															
SM 1438- 2	52.8	19.8	37.6	34.9	1	38.7	14.2	36.9	20.3	4	18.4	6.4	34.1	22.7	4
SM 1665- 2	49.9	17.3	34.7	22.9	5	47.7	15.7	32.9	23.9	1	19.2	5.5	28.7	16.3	9
SM 1669- 7	37.4	14.1	37.7	23.2	4	30.3	12.0	39.5	17.5	7	17.2	5.7	32.6	21.1	6
SM 1778-45	41.2	14.4	34.9	14.9	9	36.3	12.5	34.1	16.0	10	16.7	4.9	29.2	12.4	13
CM 4919-1	37.0	13.0	35.1	17.0	7	34.0	12.0	35.2	18.2	5	12.3	3.9	31.9	7.1	21
SM 1669- 5	31.5	11.6	36.9	8.4	16	33.8	12.3	36.5	16.8	8	15.1	4.6	30.9	15.2	10
SM 1411- 5	34.9	12.5	35.4	7.7	17	33.1	11.1	33.5	8.7	18	22.9	7.0	30.5	32.1	2
SM 1565-17	48.6	15.4	31.6	9.9	14	36.3	10.5	29.2	8.3	21	23.3	6.2	26.5	24.3	3
SM 1511- 6	34.9	12.3	35.2	7.5	18	29.9	11.5	38.5	14.6	12	15.8	4.6	29.2	14.7	11
M Tai 8	33.3	11.8	35.6	7.2	20	33.0	11.6	35.1	15.9	11	14.9	4.6	31.1	10.6	16
CM 6119-5	30.6	11.5	37.6	12.6	11	29.1	11.0	37.6	13.2	15	12.7	3.8	29.8	2.6	27
CM 3306-19	42.7	12.7	29.8	-1.5	34	33.4	10.3	30.9	8.7	19	23.8	7.6	32.2	39.0	1
SM 1778-53	34.0	11.9	35.0	3.0	23	23.5	8.5	36.3	1.2	32	19.9	5.8	29.2	21.2	5
SM 1973-25	43.6	16.7	38.1	26.0	3	36.0	13.3	37.6	16.5	9	10.2	2.8	27.4	-15.4	50
M Ven 25	32.0	11.3	35.0	0.7	31	40.8	14.4	35.2	22.3	2	12.2	3.8	30.7	1.3	29
Average	39.0	13.7	35.3	13.0	14.2	34.4	12.1	35.3	14.8	11.6	17.0	5.1	30.3	15.0	13.8
SD	7.1	2.5	2.3	10.2	10.0	5.6	1.8	2.8	6.0	8.3	4.3	1.3	2.0	13.2	13.3
Checks															
CG 1141-1	24.0	8.7	36.2	-7.2	43	22.0	7.6	34.7	-5.7	42	14.7	4.2	29.0	6.5	23
CM 3306-4	30.4	11.1	36.3	1.4	28	20.4	7.5	36.6	-10.7	48	12.2	3.7	30.8	-3.2	36
M Col 1505	28.3	9.6	33.9	-10.7	46	30.0	10.2	34.1	1.9	31	11.2	3.0	27.3	-7.0	41
M Col 2215	21.5	7.9	36.8	-11.8	49	19.8	7.3	36.4	-9.6	47	12.1	4.0	33.4	6.10	24.0
Average	27.2	9.7	35.6	-5.5	39.4	26.6	9.4	35.4	-0.4	34.0	12.5	3.8	30.2	0.7	30.6
SD	4.4	1.5	1.2	6.2	9.3	8.9	3.0	1.1	13.6	19.1	1.3	0.4	2.3	5.9	7.8
All 60 genotypes															
Average	33.7	11.7	34.8	0	30.5	26.8	9.3	34.0	0	30.5	13.1	3.8	29.1	0	30.5
SD	8.3	3.0	2.0	14.7	17.5	8.6	3.0	5.1	18.0	17.5	4.3	1.4	3.0	16.0	17.5

a. Sel. = selection.

Table 18-2 also shows the poor performance of materials in Molineros (average fresh-root yield of the 60 materials was 13.1 t/ha), compared with Pitalito and Santo Tomás (33.7 and 26.8 t/ha, respectively). This was due to a severe drought at the first site, where it had begun 2 months before the rains normally cease. It is precisely because of such variation, which frequently and unpredictably affects agricultural activities, that evaluations should be carried out in different environments and, if possible, for more than one cycle.

Across environments and time, genetically superior materials with stable production are gradually identified. In Molineros, dry matter content (29.1%) was considerably less than at the other sites (34.8% and 34.0%) because the rains began before the normal time. This meant that harvest was carried out when shoot growth was already observed in the plants. Dry matter content in cassava roots declines drastically when growth is reinitiated after prolonged drought, as the plant consumes part of its root reserves.

On average, the 15 best clones yielded across the three sites 10.3 t/ha of dry matter, while the averages for the total of the three experiments and for the checks were, respectively, 8.3 and 7.6 t/ha. This reflects the crop's enormous potential for genetic improvement. Even in advanced selection stages, not all materials were satisfactory. A fundamental aspect of this stage is the expansion to include numerous sites. We emphasize that when these materials are transferred to farmers' fields, productivity is usually reduced because of numerous factors, many of which cannot not be controlled by the farmers.

In these advanced stages of selection, when the number of materials to select has been considerably reduced, evaluations are started for characteristics that can be measured only in a limited number of progenies. For example, trials may begin on culinary quality and cyanogenic glucoside content to determine whether the cassava is "bitter" or "sweet". Thus, when the regional trials are finished, the excellent agronomic performance and stable productivity of the genotypes can also be assured, as can be the information on different characteristics that will help define the potential use of these materials (e.g., starch production, energy source for animal feed, and fresh-root market).

Another example that illustrates the importance of evaluating materials in different environments is that of the clone SM 1433-4. This material was included among the 60 described in Table 18-2 (but its performance is not shown as it was not among the best 15). This clone performed well under subhumid Caribbean conditions, where reduced precipitations limited the development of bacterial blight (*Xanthomonas axonopodis* pv. *manihotis*). In wetter conditions, however, this pathogen spreads more easily, as happened in the Departments of Sucre and Córdoba, where SM 1433-4 proved to be excessively susceptible to this disease.

Eastern Plains. The materials adapted to this environment characteristically tolerate acid soils. Bacterial blight and superelongation (caused by the fungus *Sphaceloma manihoticola*) are the principal diseases that affect cassava in this type of environment. Many of the materials developed here have performed very well in other regions of the country such as Quindío, Antioquia, Huila, and Tolima.

Table 18-3 presents the averages of three RT conducted in Restrepo, Matazul, and La Libertad, all in the Department of Meta. Because of the effects of genotype-by-environment interaction (i.e., differential performance of genotypes in different environments), identifying materials that show excellent development in the three sites was not readily possible. However, CM 6438-14 (released in 2001) and CM 6740-7 (CORPOICA–Reina) performed very well, surpassing the check material ('Brasilera'). Other clones also performed well in this evaluation, as in previous years (SM 1363-11, SM 1821-7, and SM 1143-18). Clone CM 4574-7 also performed well and showed resistance to root rots.

Clones CM 4574-7 and CM 6438-14 are particularly adapted to savanna conditions, while clone CM 6740-7 adapts better to conditions that are not as extreme such as found at "La Libertad" Experiment Station (Villavicencio), and under the conditions of the Piedemonte, a hilly region lying between the Eastern Cordillera of the Andes and the Eastern Plains. We point out that, except for CM 6438-14, these experimental clones are among those materials selected to participate as parents in crosses to be carried out during year 2000.

During 2001, CORPOICA released the clone CM 6438-14, with a name that honors the memory of farmer Juan Vergara, who constantly promoted the cassava crop in the Orinoquia and shared his experience and progressive vision with the team in charge of this crop's genetic improvement. CM 6438-14 (Figure 18-12) has high levels of resistance to bacterial

Table 18-3. Average of the most relevant variables of clones evaluated in three regional trials for the Orinoquian Region (Restrepo, CORPOICA–La Libertad, and Matazul, all in the Department of Meta). Order is based on the selection index across the three environments.

Clone	Fresh-root yield (t/ha)	Dry matter content (%)	Dry matter yield (t/ha)	Plant type (1–5)	Harvest index (0–1)
SM 1363-11	24.44	36.61	8.90	3.00	0.51
SM 1152-13	23.89	35.34	8.42	4.00	0.54
SM 1794-18	22.19	36.14	8.09	3.33	0.50
CM 6438-14	20.59	35.90	7.49	3.67	0.52
SM 1821-7	23.27	33.88	7.98	3.00	0.53
SM 1143-18	21.88	32.18	7.11	4.00	0.59
SM 1854-23	22.10	32.28	7.22	3.67	0.58
M Bra 502	21.54	33.91	7.30	3.33	0.49
CM 6921- 3	18.49	34.84	6.54	4.33	0.48
CM 6740-7	18.73	34.19	6.42	3.33	0.55
Brasilera	18.80	33.90	6.49	3.67	0.52
CM 4574-7	19.38	34.03	6.58	3.00	0.53
SM 1483-1	22.93	32.07	7.42	2.67	0.48
SM 2219-11	21.64	31.48	6.84	3.00	0.53
CM 6975-14	19.14	34.80	6.70	2.00	0.47
SM 1241-12	18.89	31.08	5.82	3.67	0.58
CM 523- 7	14.54	34.03	5.09	4.00	0.54
SM 1862-25	17.07	33.31	5.56	3.33	0.51
SM 1697-1	20.32	31.09	6.46	3.33	0.48
CM 7052- 3	19.66	30.52	6.08	3.00	0.52
SM 1812-69	18.02	30.72	5.71	3.33	0.56
SM 1694-2	14.41	34.21	4.92	4.00	0.44
SM 1565-15	17.25	33.09	5.82	2.67	0.47
CM 2177- 2	16.27	32.30	5.28	4.00	0.45
SM 1674-1	14.91	32.39	5.02	3.67	0.53
SM 1859-26	19.10	30.14	5.81	2.33	0.54
CM 7073- 7	14.10	33.45	4.74	3.00	0.47
CM 5306- 8	14.85	32.44	4.76	3.33	0.42
SM 2068-3	17.01	30.65	5.27	2.00	0.45
SM 1881-17	13.86	28.91	4.11	3.33	0.42
Minimum	13.86	28.91	4.11	2.00	0.42
Maximum	24.44	36.61	8.90	4.33	0.59
Average	18.98	33.00	6.33	3.30	0.51
SD	3.10	1.91	1.19	0.58	0.04

blight and superelongation, and can thus present large volumes of young foliage at harvest.

Clone CM 6740-7 or 'Reina' (Figure 18-13) demonstrates its extraordinary potential, both for fresh roots and dry matter. In fact, being unquestionably superior, this material replaces the last cultivar released in the region (CM 523-7 or 'Catumare'). Furthermore, 'Brasilera' had been used until now for the production of pre-cooked cassava croquettes. However, CM 6740-7 has the advantage of a higher dry matter content than 'Brasilera' and a higher yield potential. 'Reina' will serve not only as animal feed but also for the food-processing industry.

Inter-Andean valleys. This ecosystem shares many characteristics with the Eastern Plains. Some of their sites present acid soils and share the same typical diseases (bacterial blight and superelongation). Unsurprisingly, therefore, clone CM 6740-7 is also an outstanding performer this region. For this type of environment, high dry matter yield is a significant

Figure 18-12. The new cultivar for the Orinoquian Region (CM 6438-14). Its name honors the memory of local farmer Juan Vergara Carulla.

Figure 18-13. Clone 6740-7 or 'CORPOICA–Reina' was recently released for the Colombian Orinoquia but has excellent adaptation to other regions of the country.

criterion for selection (clone SM 1219-9 has shown excellent potential). Other materials are also being looked at for good culinary quality (landraces M Per 183 and M Bra 383 not only have high yield potential, but also excellent culinary quality and characteristics for the food-processing industry).

Table 18-4 presents the yields of the best clones in the RT held at CIAT–Palmira and harvested in May 2000. On average, the evaluated clones yielded more than 7 t/ha of dry matter. The best 10 clones had dry matter yields of almost 10 tons (9.77 t/ha), whereas the five checks (including variety Catumare or CM 523-7) had average dry matter yields of 6.35 t/ha.

Table 18-5 presents the results of four consecutive harvests carried out on varieties adapted to this ecosystem, in the Municipality of Jamundí, south of the City of Cali. The first harvest was carried out 7 months after stakes were planted. Even at that time, the crop's general performance was satisfactory overall (average of 11.2 t/ha of dry matter).

The consecutive harvests helped identify clones with high-yielding potential in early development. Being a perennial plant, cassava can be harvested at any time without reference to reasons of physiology or senescence to determine optimal time (except for dry matter content, which is lower when the plant renews growth after an adverse condition such as drought).

Many farmers find it strategic to have early and late varieties, so that root production is more or less continuous. During these harvests, data on the production of young foliage were also taken. On average, this site presented small variations over time (fluctuating around 8 t/ha), but, depending on variety, foliage production ranged between 6 and 14 t/ha when roots were harvested. This information is also useful for determining optimal harvest times for each variety, taking into account the production of both roots and foliage.

Variety dissemination and release

When a material demonstrates genetic superiority across numerous environments and over several years, it will be profiled as a candidate for official release by institutions accredited for this purpose in the country. In Colombia, first the Colombian Institute of Agriculture (ICA), and later CORPOICA, traditionally fulfilled this important role. Hence, close collaboration exists between CIAT and these institutions.

Two modalities exist for the final evaluation of materials and to confirm their genetic advantages. The traditional scheme involves planting trials with replications over several years at different sites to confirm the new varieties' superiority. In these cases, checks are always planted that adequately represent the best clones available to farmers at that time. A new variety must be superior to the checks in one or more characteristics and must demonstrate sufficient stability across variable environmental conditions. Hence, new varieties can only be officially released after having been evaluated for several years and in different environments, thereby determining its stability and tolerance or resistance to different production constraints, whether of biotic or abiotic origin.

Table 18-4. Results of the 10 best of 48 varieties evaluated in the regional trial conducted at CIAT–Palmira, Colombia.

Clone	Yield (t/ha)		Dry matter (%)	Evaluation foliage (1–5)	Harvest index	Selection index
	Fresh roots	Dry matter				
10 best genotypes						
CM 8370-11	31.89	13.07	41.00	2.00	0.63	13.60
SM 1855-15	23.67	10.00	42.25	2.00	0.65	9.70
SM 1602-13	32.19	12.10	37.60	2.67	0.61	9.01
SM 1636-24	27.93	10.95	39.20	3.00	0.59	6.38
SM 1741-1	19.30	8.01	41.50	2.00	0.60	5.84
SM 2141-1	19.59	8.62	44.00	2.33	0.56	5.71
SM 1557-17	21.52	8.70	40.45	2.33	0.61	5.33
SM 1871-33	23.56	9.54	40.50	3.00	0.58	4.42
CM 3306-4	18.07	7.94	43.95	3.00	0.62	3.95
CM 8370-10	20.81	8.72	41.90	2.67	0.54	3.95
Average	23.85	9.77	41.24	2.50	0.60	6.79
SD	5.14	1.76	1.98	0.42	0.03	3.09
Checks						
CM 523-7	22.41	9.36	41.75	3.00	0.63	5.27
M Bra 12	18.19	6.66	36.65	3.00	0.58	-1.35
M Per 183	19.78	6.78	34.30	3.00	0.60	-1.49
M Col 1505	14.19	5.45	38.45	3.00	0.53	-3.22
M Col 1468	9.89	3.48	35.20	4.00	0.51	-9.75
Average	16.89	6.35	37.27	3.20	0.57	-2.11
SD	4.92	2.14	2.96	0.45	0.05	5.36
All 48 genotypes						
Average	18.17	7.03	38.50	2.91	0.56	0
SD	4.93	2.10	3.10	0.41	0.07	5.17

The second way to identify and validate the genetic superiority of materials is through *participatory research*. With this methodology, segregating materials are delivered to farmers who will then conduct the final selection of materials according to their own selection criteria. This system has the great advantage that, once a variety is selected by a farmer (or group of farmers), it would then not need promoting, as it will usually be immediately adopted by the farmers. It also has the advantage of being more specific to certain more uniform environments (e.g., for a given village district or municipality) than the traditional improvement scheme, which targets broader environments (e.g., the Caribbean or Orinoquian Region).

Whatever the improvement system used, the stages described in this chapter are always included. First, parents with desired attributes for exploitation should be selected. The parents are then crossed among themselves to produce a large number of segregating progenies. Each seed resulting from pollination constitutes a new genetic entity, which means that crossing produces great genetic variability. The more costly and slower activity is to select genetically superior materials from the wide variability generated by the crosses. Current technological developments enable selection to be more efficient and effective in terms of the use of resources at hand. Ultimately, of the thousands of crosses made every year, only some clones will be identified by CIAT as being superior. Of these, only some will be released as varieties by CORPOICA.

Biotechnology

The second half of the 20th century has been witness to a dizzying development of technology in the area of what is now known as "biotechnology". Perhaps the most significant characteristic of biology is that the genetic code is universal. This means that the information codified in a bacterium, for example, can

Table 18-5. Results of four successive harvests of crops of 15 elite clones at 7, 8, 9, and 10 months old. Data are from local plots in the Municipality of Jamundí (Valle del Cauca, Colombia).

Clone	Age of crop in months												Average
	7			8			9			10			
	Fresh-root yield	Dry matter content		Fresh-root yield	Dry matter content		Fresh-root yield	Dry matter content		Fresh-root yield	Dry matter content		
	(t/ha)	(%)	(t/ha)	(t/ha)	(%)	(t/ha)	(t/ha)	(%)	(t/ha)	(t/ha)	(%)	(t/ha)	
CM 7951-5	40.5	36.5	14.8	41.1	34.8	14.3	57.3	36.3	20.8	63.0	39.8	25.0	18.73
SM 1741-1	45.1	36.7	16.5	35.3	31.2	10.8	38.3	37.0	14.2	44.4	38.8	17.2	14.68
SM 1460-1	38.1	34.1	13.0	32.8	34.5	11.3	38.1	35.0	13.3	46.5	35.2	16.4	13.50
SM 1557-17	37.0	34.8	12.9	39.8	33.0	13.1	36.6	35.4	13.0	41.5	34.6	14.3	13.33
SM 909-25	33.8	34.7	11.7	34.8	30.9	10.5	42.5	35.9	15.2	39.4	37.8	14.9	13.08
M Bra 383	33.9	28.5	9.7	38.4	34.9	13.4	34.5	36.8	12.7	41.3	38.7	16.0	12.95
SM 1219-9	34.5	34.0	11.7	44.4	32.3	14.3	33.5	32.4	10.8	39.8	36.3	14.4	12.80
SM 1543-16	32.3	34.8	11.2	26.8	33.2	9.0	36.0	34.9	12.6	49.3	35.8	17.6	12.60
CM 7514-7	29.1	39.1	11.4	29.6	38.5	11.4	28.4	40.7	11.5	36.3	41.2	14.9	12.30
CM 3306-4	35.1	37.4	13.1	29.8	36.6	10.9	30.5	38.0	11.6	34.8	39.1	13.6	12.30
M Per 183	33.9	28.5	9.7	38.4	34.9	13.4	36.0	30.1	10.8	50.8	30.1	15.2	12.28
CM 6740-7	23.9	32.6	7.8	37.5	33.4	12.5	34.0	34.4	11.7	29.1	36.3	10.6	10.65
CM 523-7	29.6	34.4	10.2	33.0	34.0	11.2	36.1	35.7	12.9	23.8	34.8	8.2	10.63
CM 849-1	19.9	33.9	6.7	25.1	32.5	8.3	21.3	33.8	7.2	22.0	35.5	7.7	7.48
SM 653-14	23.9	32.6	7.8	33.9	16.9	5.8	22.5	35.0	7.8	19.8	36.4	7.3	7.18
Average	32.2	34.8	11.2	33.6	33.4	11.2	35.0	35.4	12.4	38.8	36.7	14.2	12.30
Minimum	19.9	28.5	6.7	25.1	16.9	5.8	21.3	30.1	7.2	19.8	30.1	7.3	7.18
Maximum	45.1	39.1	16.5	44.4	38.5	14.3	57.3	40.7	20.8	63.0	41.2	25.0	18.73

be interpreted by most living organisms of the planet, because all use the same code. This has major implications for agriculture.

First, if a gene of economic interest exists in any living organism, this gene can be ultimately identified, multiplied, and transferred to a crop where it can be expressed in the same way as it did in its natural environment. This is the case of genes for resistance to insects or herbicides. Second, the technologies developed for one crop can serve for another crop. Hence, cassava has benefited enormously from all the knowledge generated mainly for cereals (e.g., rice, wheat, and maize) and legumes (e.g., soybeans and beans).

The tools developed for biotechnology may be grouped into three large categories, each of which has specific uses: tissue culture, molecular markers, and genetic transformation. This is a very dynamic field of research and a detailed description of protocols and updated results goes beyond the purpose of this publication.

Changes in the Implementation of the Cassava Genetic Improvement Scheme

From the viewpoint of quantitative genetics, the cassava improvement scheme described earlier in this chapter is essentially based on the selection of numerous segregating clones derived from a cross between two progenitors that were selected for a diversity of reasons and purposes. This selection is based on phenotypic characteristics, the variance (σ^2_P) of which can be separated as follows:

$$\sigma^2_P = \sigma^2_A + \sigma^2_D + \sigma^2_{Ep} + \sigma^2_E$$

where,

σ^2_A is the variance due to additive genetic effects,
σ^2_D is the variance due to the effects of genetic dominance,
σ^2_{Ep} is the variance due to epistatic genetic effects
σ^2_E is the variance due to environmental effects (experimental error), as well as all the components of the genotype-by-environment interaction.

Only the additive fraction of the variability observed (on which selection of genotypes is based) can be taken advantage of by the present system of recurrent selection. Both σ^2_D and σ^2_E introduce a "distortion" because, even though they influence the performance of a determined genotype, their effects cannot be transmitted to later generations, unlike genes that have additive effects.

Non-additive components of genetic variance (σ^2_D and σ^2_{Ep}) for the main characteristics of cassava have been demonstrated to be highly significant (Cach et al. 2005, 2006; Calle et al. 2005; Jaramillo et al. 2005; Pérez et al. 2005a, 2005b). Hence, whatever method increases the proportion of additive effects in the selection process will greatly increase its efficiency. Another equally valid alternative is to implement an improvement method that can also take advantage of the effects of dominance and epistasis. Some alternatives for improving cassava that may be implemented in the near future are presented below.

Improvement schemes that include self-fertilizing stages offer some advantages that have been reported in the literature. With successive self-fertilizations, different loci in the genome are obliged to progressively reach the stage of homozygosis. This is prejudicial for individual performance (particularly for cross-pollinated crops such as cassava or maize), because vigor and productivity will gradually diminish. However, this process has the advantage of eliminating deleterious or undesirable genes from the population that remain "hidden" because of the generalized heterozygosity of these types of crops in their natural state. The totality of effects of these undesirable genes is known as the "genetic load", which is estimated to be prominent for cassava. However, as progress is made in the degree of inbreeding of segregating populations, the proportion of total phenotypic variance that is additive variance increases (Hallauer and Miranda 1988).

Table 18-6 illustrates the effects of successive self-fertilizations in the distribution of genetic variance. The obvious result is that, with total homozygosis, σ^2_D is eliminated as a component of phenotypic variance. Another obvious result is that the additive effects present in the F1 generation (full-sib family) now has double the influence than in the original situation. From the genetic viewpoint, a homozygotic line is stable (on using it as progenitor, the genetic segregation mentioned previously does not occur), in contrast to what happens with hybrid F1, which, even if it could reproduce vegetatively, its use as a progenitor is affected because the genetic effects of dominance cannot be transmitted to later generations.

The industry of maize hybrids is based precisely on the design of the progenitors, which, on combining, specifically produce a material of excellent performance

Table 18-6. Distribution of genetic variance between and within families when increasing inbreeding through successive self-pollinations.

Family	Proportion of homozygosis	Between families		Within families	
		σ^2_A	σ^2_D	σ^2_A	σ^2_D
Full siblings	0	1/2	1/4	1/2	3/4
F_2	50.00	1	1/4	1/2	1/2
F_3	75.00	3/2	3/16	1/4	1/4
F_4	87.50	7/4	7/64	1/8	1/8
F_∞	100.00	2	0	0	0

in the field. The process of self-fertilization of cassava thus offers two very attractive advantages: (1) it contributes to the automatic reduction of the genetic load in populations that have been improved in part, and (2) it permits the design of parents for producing more competitive hybrids. Current improvement concentrates on producing and identifying good hybrids from selected progenitors. In the future, such emphasis will produce individuals especially designed to be optimal progenitors and thus generate outstanding hybrids. The great advantage is that this process guarantees a more sustained genetic progress, which is quick, at least from the theoretical viewpoint.

Now, some problems exist that explain why, so far, these ideas have not been implemented, principally:

a. The genetic load in cassava is so large that reaching high degrees of homozygosis is difficult with plants that can survive. Although this is currently a limitation, it also justifies the urgent need to begin cleaning out the genetic load from the crop as soon as possible. We point out that tolerance of inbreeding can be increased in cross-pollinated crops, as was shown irrefutably for maize. No scientific reason exists to assume that the same cannot be achieved for cassava (Contreras R et al. 2009).

b. Because of the cassava plant's peculiar method of reproduction, self-fertilization can be greatly delayed. To achieve a high degree of homozygosis, at least 4 or 5 successive self-fertilizations are needed. In cassava, this requires about 10 years. However, a procedure exists that is widely used for other crops whereby totally homozygotic materials, known as *doubled haploids* can be immediately obtained (Griffing 1975). This procedure normally uses gametophytic tissue, which is subjected to tissue culture that is initially haploid and becomes doubled haploid through the automatic or induced duplication of the number of chromosomes (CIAT 2009).

For the reasons given above, changes are planned for the way the cassava genetic improvement project at CIAT will be carried out in the future. Below, we briefly describe the scheme that may be implemented over the next few years. We emphasize that this is only at a preliminary phase of definition and many changes will surely be introduced, depending on how the crop responds at different stages.

Development of homozygous progenitors

Important efforts are underway to develop a protocol for the production of doubled haploids through approaches such as microspore, anther, or ovule culture or through wide crosses with *Ricinus communis*.

Taking advantage of general combining ability

By definition, to eliminate deleterious genes in each segregating population, its characteristics as progenitor are improved, as the deleterious genes can no longer be transmitted to later generations. Genetic designs exist for improving, in a systematic and efficient way, genes with additive effects, those that, in an integral way, define the general combining ability of each individual or population.

Defining heterotic groups and taking advantage of specific combining ability

Once the genetic load has been successfully reduced in the populations, improvement can start by focusing on producing progenitors that mutually complement each other from the genetic viewpoint. This implies the start of producing materials that, when crossed with each other, will produce exceptional hybrids. This is precisely what occurs when parents of commercial maize hybrids are crossed; the parents have been designed and gradually improved to produce, each time, more productive hybrids with a more stable performance. This process inevitably derives from the definition of heterotic groups, that is, groups that characteristically demonstrate good hybrid vigor when crossed with each other.

For cassava, because the presence of heterotic groups has not yet been determined, a reasonable way to begin would be to define the genetic distances between the lines produced during the last stage described above. Once the heterotic groups are defined, we can then begin to effect a selection directed towards taking advantage of not only the additive effects (σ^2_A), but also dominance (σ^2_D) and epistasis (σ^2_{Ep}). These latter effects are also known as *specific combining ability*, and the improvement method that can exploit them efficiently is known as *reciprocal recurrent selection* (Hallauer and Miranda 1988).

Major emphases on qualitative aspects of the cassava root for different types of consumption

Recently, notable advances have been made to generate cassava with distinct qualitative characteristics. For the starch industry, mutations have been identified, whereby the starch does not contain amylose ("*waxy*" starch) or it has small granules and higher than normal contents of amylose (Ceballos et al. 2007b, 2008). Typical characteristics of normal cassava starch have also been studied in detail (Sánchez et al. 2009).

In the ethanol industry, the existence of "sugary" cassava has been known for a long time. This cassava accumulates sugar polymers simpler than starch. In 2004, Carvalho and co-workers carried out a detailed description of this type of material, which was also identified recently by the cassava improvement project at CIAT. The costs of converting this type of root to ethanol would be considerably lower, but how much energy per hectare this type of cassava could produce is still not clear.

In terms of nutritional quality, CIAT first characterized (Chávez et al. 2005) and then triplicated the original levels of carotenoids in roots through a system of rapid recycling of materials (CIAT 2009). In 2008, materials with more than 18 μg of total carotenoids per gram of fresh root had already been obtained. As well as nutritional advantages, high carotenoid contents offer tolerance of postharvest physiological deterioration (Sánchez et al. 2005; CIAT 2008). Variability indices exist for protein contents in roots, even though this type of research requires more precise quantification methods (Ceballos et al. 2006).

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