CASSAVA AND BIOTECHNOLOGY

PRODUCTION CONSTRAINTS AND POTENTIAL SOLUTIONS

carried out at the request of the Netherlands Directorate
General for International Cooperation
(Ministry of Foreign Affairs)

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EXECUTIVE SUMMARY

In order to analyse the main constraints in cassava production and processing and to assess potential solutions by biotechnological techniques a study was carried out by the Department of Tropical Crop Science at the Wageningen Agricultural University (The Netherlands) on request of the Netherlands Directorate General of International Cooperation (DGIS). A review of institutions currently involved in the field of cassava research and biotechnology is incorporated in this study (see annex 3).

First (chapter 2) an examination is made of the main constraints in cassava production and processing. These constraints can be divided into four groups: (1) propagation and varietal aspects, (2) cultivation, (3) yield and quality, and (4) post-harvest technology. For each group the main areas for improvement are identified (summarized in table 2, p.20).
Chapter 3 presents some general information on biotechnology (summarized in table 3, p.22) and its possibilities and limitations in cassava research (summarized in table 4, p.26). The main limitation for the application of several protoplast and recombinant techniques to cassava is to obtain a proper regeneration from callus.
The following chapter (4) deals with the technical and socio-economic impact assessment of biotechnological applications for local research and infrastructure and associated risks are considered.
In chapter 5 the main areas for improvement are considered in the light of conventional research methodology and potential biotechnological contributions. Present possibilities and limitations of biotechnological strategies are formulated, followed by an impact assessment of biotechnology. Key sections of chapter 5 are summarized below:

1) Propagation and varietal aspects:

Planting material: Several important problems are associated with conventional vegetative propagation of cassava, such as low multiplication rates and low storage ability of the cuttings. Improved conventional propagation techniques are available. Propagation by plant tissue culture (micropropagation) may be an appropriate biotechnological approach, although it requires a relatively high initial capital input. Micropropagation can be used in association with conventional techniques. Propagation by seeds may overcome most propagation bottlenecks, but is not applicable yet for practical purposes. Micropropagation as well as propagation by seeds may increase the dependence of farmers on seed corporations or local institutes.

Varietal diversity and plant breeding techniques: The conservation of wild Manihot species and existing genetic accessions and improved plant breeding techniques will be extremely valuable for future development of new varieties.

Research has focused on improvement of germplasm storage, on improvement of flowering capacity, on production of hybrids and on improved hybridization techniques. In these areas biotechnology offersmuch scope. Plant tissue storage under conditions of slow growth has already become a standard method. Biotechnological improvement of plant breeding techniques seems also feasible. The flowering capacity of cassava varieties is in general low. This problem can be overcome by the induction of flowering in vitro. Furthermore, hybrid production is difficult in cassava, as a result of severe inbreeding depression. The application of anther culture for the production of inbred lines (necessary for hybrid production) avoids this barrier. Hybridization of Manihot spp., conventional crossing techniques can be used in most cases when hybridization between wild species is difficult, protoplast fusion may then be used. In general, improved germplasm storage and breeding techniques are extremely valuable for the assessment of genetic diversity in cassava and the development of new varieties respectively.

Plant morphology and physiology: Cassava is often grown in mixtures with other crops such as maize, beans or bananas. Cassava leaves are an important source of protein and vitamins in many local diets.

The improvement of morphological traits has to be achieved by means of conventional breeding methods. Improvement of the plant architecture (important for intercropping practices) and increased leaf production will certainly be of benefit to many local farmers. Biotechnology offers no possibility as yet.

Improvement of plant physiological factors, such as biomass partitioning, the cyanogenesis of cassava roots and improved photosynthetic capacity of cassava leaves, may substantially increase the popularity of cassava as a food crop. Selection on efficiency of storage root production and an early start of starch accumulation can be used in conventional breeding programmes to improve the biomass partitioning in cassava. Interesting research on the photosynthetic capacity of cassava leaves is carried out at CIAT. So far biotechnology provides only long term perspectives.

2) Cultivation: Cassava has the ability to grow and yield well under drought conditions and to tolerate low fertility soils one of its most attractive features for the farmer. Better control of weed, disease and pests will have a large impact on the cultivation practices of local farmers.

Environmental adaptation: Conventional research on drought tolerance has focused on high yield under drought conditions, early maturing varieties, and the improvement of the water use efficiency. However, more fundamental knowledge of these traits in cassava is needed. Biotechnology offers few perspectives as yet. Drought tolerant cassava varieties may readily be adopted by farmers in drought-prone areas. Erosion risks should be accounted for.

Research on soil fertility may focus on improvement of the efficiency of nutrient use, on mycorrhizal-fungi associations for P-uptake (which is in many regions a limiting soil fertility factor) and on associations with N-fixing bacteria. In general conventional research offers enough scope for improvement. Biotechnology provides some perspectives, as improved screening techniques for nutrient

efficiency. Attention must be paid to soil exhaustion problems when cassava varieties with improved nutrient efficiency are introduced in marginal areas.

Weeds: The young cassava crop is highly susceptible to weed competition, moreover weeding is very labour intensive. Improved weed control methods should be very beneficial. Conventional weed control methods, such as mechanical weed control or intercropping with leguminous crops, remain most suitable. Biotechnological improvement of weed control in cassava would include the development of herbicide resistant varieties. This involves considerable risks namely herbicide pollution of the environment.

Diseases and pests: Diseases and pests seriously reduce cassava yields in many parts of the world. The most important diseases are the African Mosaic Disease, the Common Mosaic Disease and Cassava Bacterial Blight, whereas the Cassava Mealybug and Cassava Green and Red Spidermites are the major pests. Improvement of varietal resistance, introduction of new biological and cultural control methods and the establishment of improved screening methods to produce disease-free planting material are possible areas of interest. Conventional breeding programmes resulted in parental lines with relatively high resistance to African Mosaic disease and Cassava Bacterial Blight. The absence of local breeding programmes which could adapt these varieties to different ecological conditions, however, is still a problem. Resistant cassava varieties may be obtained by recombinant DNA technology (cross protein resistance). Improved screening methods, such as the application of DNA probes as routine test are available. At present biotechnological contributions, especially in the development of disease resistant parental lines, deserve no priority in comparison with conventional research efforts. The risks associated with the introduction of the transformed varieties and disease break-through also have to be assessed.

Conventional research for pest control has focused on biological control, which seems especially successful for the cassava mealybug. The biological control of spidermites is also promising. Furthermore, conventional breeding programmes produce insect tolerant varieties. At present biotechnological improvement deserves no priority, compared with these low-cost pest control methods.

3) Yield and Quality:

Yield capacity: Cassava has a high yield potential, especially under poor conditions. In general, however, root yields on farmer's fields are much lower than experimental yields, because yield is depressed by socio-economic factors such as costs, infrastructure and labour. Conventional breeding programmes offer opportunities to improve the yield capacity of cassava, especially if carried out under specific regional farming conditions. Because of the polygenic character of yield, biotechnological improvements of this trait require more fundamental knowledge. Biotechnology may offer some perspectives through the production of higher yielding hybrid varieties.

Root quality: Research in this area focuses on reduction of the toxicity of cassava to humans and on improvement of the starch quality and protein content of cassava roots. Conventional breeding offers scope to reduce the toxicity of cassava varieties. Biotechnology may provide non-toxic varieties through anther culture.

non-toxic varieties might be more susceptible to pest and disease pressure.
Much more fundamental knowledge is required for conventional as well as biotechnological improvement of the starch quality of cassava roots.
Cassava roots contain only very low protein levels, which are further reduced by processing. Both attributes are serious shortcomings for the use of cassava as a food crop. The protein content of cassava roots may be improved by means of recombinant DNA technology. Most of the protein will, however, be lost during processing. Unless processing methods are developed which ensure low protein loss, biotechnological improvement of the protein content of cassava roots seems inappropriate.
Little has been done on locally important root qualities such as taste and colour.

4) Post-harvest technologies: Post-harvest technology includes the storage of fresh cassava and cassava processing.
Storage of cassava roots: Conventional methods may offer enough scope for an adequate storage of dried cassava.
The perishability of fresh cassava poses is a draw back for the distribution of fresh cassava to local markets. Prolongation of the storage period of fresh cassava is an important area of improvement. More fundamental knowledge is required for biotechnological improvements of the storage of fresh cassava.
Processing: Processing of cassava roots is necessary to control the deterioration of cassava food products, to eliminate toxicity and to make cassava food products more palatable. Improved or new processing technology will have large impact on the quality of cassava products and product diversification. The main areas of improvement are considered the introduction of fungal fermentation techniques (to improve the protein content of cassava products), the improvement of processing techniques to reduce the toxicity of cassava products and the introduction of baking processes. Fermentation techniques with moulds may reduce the toxicity to acceptable levels within a few days. The protein content of cassava products increases during processing by fungal fermentation with Rhizopus oryzae. Biotechnological improvement of processing provides good opportunities.

The perspectives for conventional and biotechnological research and the impact assessment of biotechnological applications are summarized in table 7 and 8 (p.57 and 58) respectively.

Chapter 6 presents an assessment of priorities based on the following technical and socio-economic criteria: (1) the necessary time-frame for specific biotechnological improvements, (2) the relative contribution of biotechnology compared to conventional research programmes. For the assessment of the socio-economic impact the following criteria are selected: (3) the impact of biotechnological improvements on the situation of small-scale farmers, (4) the capital input and research capacity required and (5) the potential risks associated with biotechnological applications (see table 10, p.61).

(1) **Areas of improvement with high priority for biotechnological improvement according to most criteria**: selection and identification of mycorrhizal-fungi and N-

fixing bacteria (serological techniques), improvement of germplasm storage, flowering capacity, and micropropagation (plant tissue culture), improvement of screening techniques for diseases (cDNA probes) and processing methods, which reduce the toxicity of cassava roots, enhance the protein content of cassava products (use of fungi) or include alcohol production.

(2) **Areas of improvement with high priority for biotechnological improvement according to some criteria, but with low priority on others**: selection of drought tolerant cassava varieties (with improved water use efficiency) and varieties with high nutrient efficiency (in vitro selection), hybrid production and interspecific crossings, utilization of true seed and improved varietal resistance to virus diseases and to cassava hornworm and red and green spidermites, and improved storage methods of fresh cassava. In general biotechnology is recommended to overcome these constraints, but only after careful assessment of potential outcomes and impacts.

(3) **Areas of improvement with low priority for biotechnological improvement**: selection for early maturity (drought tolerance), improvement of leaf production, plant architecture and morphological characteristics, improvement of photosynthetic capacity and efficiency of storage root production, herbicide resistance, improvement of varietal resistance to Cassava Bacterial Blight and cassava mealybug, improvement of yielding capacity and several root quality factors. In general more fundamental research is needed to reveal the polygenic characterization of these traits or to implement new, advanced techniques. Sometimes conventional research offers more scope here than biotechnology.

Finally (chapter 7) the conclusions of the study are presented, followed by a short discussion. The main conclusions are:

1) The main limitation for the application of several protoplast and recombinant techniques to cassava is obtaining regeneration from callus. Research should be focused on establishing a proper regeneration protocol.

2) Priority areas (where biotechnological research should be initiated or further developed) are improvement of associations with mycorrhizal-fungi and N-fixing bacteria, improvement of plant breeding techniques, improvement of screening methods for diseases (for breeding programmes and quarantine purposes) and the improvement of processing methods. For these areas, improvements are technically feasible and of considerable socio-economic importance.

1. INTRODUCTION

1.1 General

Cassava (Manihot esculenta Crantz) is the fourth most important staple crop in the tropics (FAO, 1988). It is grown almost exclusively in the hotter lowland tropics, where it accounts for approximately 10 per cent of the total caloric value of staple crops (Cock, 1985). Cassava is grown mainly as a subsistence food crop, but a considerable part of the world production is used as animal feed and for industrial uses. Increasingly the crop is grown as a cash crop by many farmers. In Asia and South America, for example, up to 45 per cent of the total cassava production is used as animal feed or exported (IFPRI, 1987).

Especially in Africa cassava is a staple crop of immense importance. It is grown as a food crop in 39 African countries, and all countries in which cassava is the principle staple food are situated in that continent. Cassava accounts for about one third of the total staples produced in sub-Saharan Africa (Hahn et al., 1987). In the past cassava has been readily adopted by farmers and integrated into the traditional farming systems, owing to a number of favourable characteristics (Uriyo, 1982):

(1) Cassava is well adapted to marginal soils (low fertility, high acidity) on which most other crops fail.
(2) Cassava has the ability to tolerate environmental stress (drought) or pest and disease attacks and to recover readily.
(3) Cassava gives relatively high yields, compared to other staple crops, and is an excellent source of carbohydrate.
(4) Harvesting of cassava roots can take place from 6 to 36 months after planting, thus providing the farmer with a permanent source of food.

These favourable characteristics result, among other things, in high stability of production. The fluctuation of cassava production, measured according to the yearly coefficients of yield variation from 1966 to 1986 (cassava: 4.3 % ; maize: 36.2 %), is the lowest among the major food crops in the world (Hahn et al.,1987).

Major constraints, however, still remain in cassava production and processing. Diseases such as the African Mosaic Disease and pests like the cassava mealybug or the green spidermite reduce the cassava yields enormously in many areas. Furthermore, the food quality of the cassava root is affected negatively by the presence of cyanide (HCN) and by low protein content. Cassava is often grown on marginal soils, but its tolerance to low soil fertility and to drought conditions probably still can be improved. Finally, the rapid deterioration of cassava roots after harvesting is a problem to the fresh cassava trade. In Asia, where processing techniques are relatively sophisticated, crop management and

proper cultivation practices need improvement. In South America, on the other hand, processing and marketing can be improved (CIAT, 1988a). In Africa, pests and diseases are the main limiting factors in cassava production (IITA, 1988).

1.2 Objectives

This study was carried out to gain an insight into the main aspects of cassava production and processing and the options for biotechnological improvement. Furthermore the impact of these potential biotechnological applications on the situation of the main cassava producers was analyzed. The study focuses on the following questions:

1) What are the main areas for improvement in cassava production and processing?
2) What biotechnological applications are feasible in those areas?
3) Which biotechnological applications deserve priority in cassava research?

Attention should, however, not only be paid to the technical feasibility of such biotechnological applications, but also to the social and economic impact of such techniques in rural areas. The relevance of advanced techniques to small subsistence farmers, responsible for most of the cassava production in the world, must be considered. Their needs and strategies should determine the rate and scale for the application of biotechnology.

The aim of this paper is not to present a detailed analysis of possible socio-economic changes resulting from biotechnological applications. The economic status and future of cassava in different regions of the world are discussed in a workshop paper of the International Food Policy Research Institute (IFPRI,1987). Furthermore, no attention will be paid to general information on the botany, origin and historical background of cassava. These aspects are dealt with in a standard work on cassava: 'Cassava; New Potential for a Neglected Crop' by Cock (1985).

1.3 Framework and scope

This study has been prepared by the Department of Tropical Crop Science at the Wageningen Agricultural University (The Netherlands), on request of the Netherlands Directorate General of International Cooperation (DGIS) . It will provide background information for a conference on the role of biotechnology in international cooperation, to be held in the autumn of 1989 in The Netherlands. The study has been carried out by ir. J. Panman and ir. A.J. Scheepens, under the supervision of dr. G.H. de Bruijn and dr. L.O. Fresco.

In chapter 2 the main aspects of cassava production and processing are described. For each aspect areas of improvement are mentioned. Chapter 3 presents a survey of the most important aspects of biotechnology used in crop improvement today, a resume of the conditions for the application of biotechnology in cassava research. Chapter 4 presents an outline of the methods used for the impact assessment of biotechnology. In chapter 5 the three preceding chapters are correlated. Biotechnological strategies are formulated for the main areas of relevance and compared to conventional research options. A short analysis of the impact on the situation of local farmers, of the research and infrastructural requirements, and of the associated risks is made for each biotechnological improvement proposed. Chapter 6 contains the priority assessment and chapter 7 contains conclusions and discussion of this report.

1.4 Methodology

This study is a literature review. Results of interviews with some experts in the field of biotechnology are also incorporated in this study (list of interviewed experts is presented in annex 1). Also major results and discussions of two recently international meetings on the possibilities to incorporate biotechnology into cassava research programmes are incorporated in this study. In August 1988, a conference was held at the International Institute of Tropical Agriculture (IITA) at Ibadan, Nigeria. Much interest was shown in the possibilities to incorporate biotechnology into national research programmes. In September 1988, scientists from the international agricultural research centres and several universities established the Advanced Cassava Research Network (ACRN) at the Centro Internacional de Agricultura Tropical (CIAT). These meetings resulted in a research agenda, identifying key constraints in the cassava production and processing which possibly could be resolved by means of biotechnology. The major results and discussions of both meetings are incorporated in this study.

2. CASSAVA; MAIN ASPECTS IN CASSAVA PRODUCTION AND PROCESSING

2.1 Introduction

Cassava is grown under a wide variety of ecological conditions and forms an element of many different cropping systems. To properly select the main aspects of cassava production and processing is complex. Research institutes may well pay attention to aspects of cassava cultivation other than those of small farmers in the field. Furthermore, the main aspects of cassava production and processing will differ by ecological zone. Nevertheless, this chapter attempts to review aspects relevant to the cassava production and processing and indicates possible areas of improvement. These include propagation and varietal aspects, cultivation, yield and quality, and post-harvest technologies.

2.2 Propagation and varietal aspects

2.2.1 Planting material

The propagation of cassava is generally carried out with stem cuttings, of different length and of various age (Toro and Atlee, 1980). Fresh stakes from mature plants are ideal, but are not always available. Several important problems, associated with the vegetative propagation of cassava, are summarized below:

(1) It is very difficult to select disease-free planting material without sophisticated techniques. Especially virus accumulation can cause severe yield losses.
(2) The nutritional status of the original cuttings partly determines the vigour and yield of its progeny. Special attention must always be given to careful selection of parent material.
(3) Conventional propagation methods involve stem cuttings from the woody parts of the stem. This results in low multiplication rates, only 3- to 30-fold per year, rates which are much lower than those of most sexually propagated crops (Cock, 1985). Simple techniques for rapid propagation have been developed.
(4) Special problems arise when planting material has to be stored for several months. Farmers then have to rely on preserved cuttings, which often show poor sprouting and reduced plant vigour (Toro and Atlee, 1980; Sales-Andrade and Leihner, 1980).

Identified main areas for improvement :

1. Introduction of propagation techniques to overcome the problems of long storage periods and low multiplication rates
2. Development of true seed propagation

2.2.2 Varietal diversity

The foundation of any breeding programme is a broad germplasm base. High genetic variability for important traits as disease and pest resistance, tolerance to abiotic stresses and biochemical traits as low cyanogenic glucoside content and high protein content in the breeding gene pool is extremely valuable for future development of new varieties (Chavez et al., 1988). For this reason the conservation of wild Manihot species is important. Considerable genetic erosion of wild species currently takes place naturally (Nassar, 1986).

Several research institutes are closely collaborating in the collection of as many wild Manihot species as possible.

Identified main areas for improvement :

1. Improvement of storage techniques for germplasm banks
2. Assessment of genetic diversity of wild Manihot species as well as of existing genetic accessions

2.2.3 Plant breeding techniques

Cassava is an allotetraploid ($2n = 36, x = 9$; Bai, 1987). Important features of species with such a polygenic basis are their wide adaptability and the slow release of their variation (Jennings and Hershey, 1984). Partly as a result of this, cassava breeding has always been a slow process (Cock, 1985). Plant tissue techniques have considerably improved the rate of progress in breeding programmes (Uriyo, 1982).

Identified main areas for improvement :

1. Shortening of breeding programmes by tissue culture techniques
2. Improved hybridization techniques between different species

2.2.4 Plant morphology and physiology

Plant morphology: Cassava is often grown in mixtures with other crops, including yams, maize, cocoyams, beans, bananas and various vegetables. Intercropping produces maximum benefits when interplant competition for light, water and nutrients is

minimized. Differences in plant architecture partly determine the suitability of cassava varieties for intercropping (Ezumah, 1981). The selection of plant types with a range of plant architectures suitable for the predominant cropping systems in all the major cassava producing zones of Africa, is an important research goal of IITA (IITA, 1988).
Cassava leaves are an important source of protein , vitamins and minerals. In many African and Asian countries cassava leaves are a popular vegetable dish. Leaf harvesting may reduce root yields, but this depends on harvest frequency and variety (Ezumah, 1981). Leaf production ability may thus be an important selection criterion in breeding programmes.
C4-crops have the Kranz anatomy as a morphological characteristic. The Kranz anatomy consists of two specialized layers of cells, the outer mesophyll cells which concentrate CO2 into C4-organic acids and the inner bundle sheath cells which assimilate the CO2 in sugars. Wild species of the family of Euphorbiaceae do have the Kranz anatomy, but cassava lacks the structure. Genetic improvement of cassava in this direction may be possible (CIAT, 1988a).

Identified main areas for improvement :

1. Selection of cassava genotypes well adapted to intercropping systems
2. Emphasis in breeding programmes on the importance of cassava leaves as supplementary food source
3. Incorporation of C4-crop characteristics

Plant physiology: Several plant physiological factors determine the root storage and total dry matter production of cassava (De Bruijn, 1982; Veltkamp, 1986) : (1) the initiation of starch accumulation, the efficiency of storage root production and the resulting harvest index, (2) the leaf area index and the leaf photosynthetic rate and (3) the sink potential of the roots.
Stress conditions, such as drought, water or nutrient stress, do affect the efficiency of storage root production. Stress results in an increase of biomass distribution to the roots (Cock, 1984).
The ideal plant type for cassava would have a leaf area index of 3 and maintain that index for the whole growth period. Maximum root growth occurs at a leaf area index that is much lower than that required for maximum total dry matter production (Cock, 1982).
The leaf photosynthetic rate is strongly correlated to the C4- crop characteristics of cassava. In cassava the PEP-carboxylase system (a C4-crop characteristic which improves the photosynthetic efficiency) exists, varying in activity between different varieties (CIAT, 1988).
Sink capacity varies between different cassava varieties (Veltkamp, 1986). It is determined by the number and size of storage roots (De Bruijn, 1982), but probably also by hormonal influences (Gifford and Evans, 1981).

An important feature of the cassava plant physiology is the cyanogenic glucoside content of the crop. Although much is known on the biosynthesis of cyanogenic glucosides and their enzymatic degradation, little is known about the biochemical and physiological role

of these glucosides in different cassava plant tissues (Hughes, 1988). The problems of cyanide toxicity are discussed in chapter 2.4.2 (Root quality).

Identified main areas for improvement :

1. Establishment of a more efficient storage root production, linked to an early initiation of starch accumulation and a good sink capacity
2. Improvement of the photosynthetic capacity of cassava leaves
3. Fundamental research on the importance of cyanogenic glucosides in the physiological functions of cassava plants.

2.3 Cultivation

2.3.1 Environmental adaptation

Drought tolerance: Cassava can be grown in areas where the annual rainfall is less than 1000 mm per year, for example the northeastern part of Brazil or in East Africa, but is seldom cultivated where rainfall is below 700 mm per year (Cock, 1985).
Cassava is highly tolerant to drought conditions. Dry periods up to 6 months impose few problems to cassava cultivation. In Thailand, the two major cassava growing regions are predominantly dry for 6 consecutive months, receiving less than 50 mm rainfall per month. Here the major cassava variety, Rayong 1, is very well suited to the dry conditions (Sinthuprama and Tiraporn, 1987; CIAT, 1988a).
During dry spells cassava reduces its leaf area by reducing leaf size, delays new leaf formation, and branching, and closes the stomata of the remaining leaves (Connor et al., 1981,a,b,c; Cock, 1984). The photosynthetic capacity of the remaining leaves recovers remarkably quickly when water is again available. In more vigorous cassava varieties a water stress period may even increase yield (Connor et al., 1981a).
Very long dry periods, however, depress the yield of cassava. The crop may survive dry periods of 10 to 13 months within a total growth period of 18 months (Silvestre and Arraudeau, 1983), but then yields drop to less than one third of the average yield.

Research at CIAT has shown that specific characteristics , which improve a crop's capability to withstand drought conditions, do occur in cassava (CIAT, 1988; see Plant morphology and physiology, 2.2.4).

Identified main areas for improvement :

1. Selection of early maturing varieties, to intensify the land utilization in dry regions and to reduce the chances of crop failure
2. Study of morphological characteristics which may improve the WUE (Water Use Efficiency)
3. Improved cultural practices such as land preparation and planting time

Soil fertility: Cassava grows relatively well on infertile soils and is tolerant to soil acidity and high levels of aluminium (Cock, 1985). The crop is grown on a wide variety of soils, mainly highly weathered and leached soils (Howeler, 1985). Important soil fertility problems encountered in these soils include phosphorus(P) deficiency, potassium(K) deficiency, soil acidity, and aluminum(Al) and manganese(Mn) toxicity (Baker, 1976; Benites and Valverde, 1982).

Cassava is often thought to exhaust the soil. Many trials have indeed shown, that cassava extracts significant amounts of N, P and K from the soil (Howeler, 1985). Per unit of dry matter, however, the extraction of the major nutrients is not higher than for most other crops. The plant has an efficient nutrient uptake and is able to grow on depleted soils. It is often grown as the last crop in a rotation before the soil is returned to fallow (Whyte, 1987).

In soils, commonly found in tropical America, P-deficiency is the major limiting fertility factor (Gomez and Howeler, 1980). In Africa and Asia K-deficiency, and sometimes N-deficiency, is the limiting factor (Silvestre and Arraudeau, 1983; Howeler, 1985).

Identified main areas for improvement :

1. Improved efficiency of nutrient use by cassava
2. Improvement of the P-uptake by inoculation of cassava roots with mycorrhizal fungi
3. Identification of associations with N-fixing (binding the nitrogen in the air) bacteria

2.3.2 Weeds

Newly planted cassava covers the ground only slowly. The young cassava crop is therefore highly vulnerable to weed competition. Weeding is the most labour-intensive aspect of cassava production (Cock, 1982).

Identified main area for improvement:

1. Introduction of improved cultural practices for weed control

2.3.3 Diseases and pests

Cassava can be grown throughout the year. In this way, it is particularly vulnerable to diseases and pests. Moreover, cassava is often grown by subsistence farmers with low profit margins. Therefore the continual use of fungi- and pesticides is financially prohibitive and environmentally unsound (Bellotti et al., 1987). Feasible alternative control methods are host plant resistance, biological control and cultural practices.

The most important diseases and pests are those present throughout the crop's growth cycle, such as African Mosaic Disease (AMD), Common Mosaic Disease (CMD), Cassava Bacterial Blight (CBB), and Red and Green spidermites. In South and Middle America,

where cassava originates, all major disease and pest problems are present, except AMD. Pest and disease problems can be controlled relatively well in that continent, because of the presence of natural predators and an extensive genetic diversity (Bellotti et al., 1987). Recently, however the cassava hornworm and CMD became more prevalent (CIAT, 1988b). In contrast, the cassava growing regions in Africa and Asia, where cassava was introduced, lack this genetic diversity and widespread outbreaks of pests and diseases have resulted or may result in severe crop losses.In Africa especially AMD and Red and Green spidermites seriously reduce cassava yields. CBB and the cassava mealybug are rather well controlled nowadays by respectively resistant varieties and biological control. In Asia the disease and pest incidence is lower, although CBB and AMD may occur in India (Cock, 1985; Bellotti et al., 1987; Hahn and Theberge, 1987; Silvestre and Arraudeau, 1983; CIAT, 1988b; IITA, 1988c).

In table 1 the major diseases and pests are listed, along with economic importance, distribution and suggested control methods.

Table 1: Major diseases and pests of cassava (a detailed description of cassava diseases and pests is found in annex 2).

Disease or pest	Losses	Distribution	Control methods
Diseases:			
Cassava Bacterial Blight	to 100%	widespread	clean planting material, cultural practices, varietal resistance
Afric Mosaic virus	to 90%	Africa, India	clean planting material, varietal resistance
Cassava Anthracnose Disease	to 30%	Latin America	varietal resistance
Common Mosaic Disease	to 60%	Latin America	clean planting material
Pests:			
Cassava green and red spidermites	to 90%	widespread	biological control, varietal resistance
Mealybug	to 90%	Africa	biological control varietal resistance
Cassava Hornworm	to 60%	Latin America	biological control

Sources: Sylvestre and Arraudeau (1983); Cock (1985); Bellotti et al. (1987); Hahn and Theberge (1987)

In the research programmes of CIAT and IITA attention is focused on the major diseases and pests. In particular, at IITA the cassava mealybug, the green spidermite and AMD, and at CIAT viral diseases and the cassava hornworm are mentioned as important constraints. Both institutes indicate that problems with nematodes will become increasingly important, especially on intensively used farm land (CIAT, 1988b).

Enormous potential exists in the fields of varietal resistance, biological control and

improved screening methods to control the major diseases and pests effectively.

Identified main areas for improvement :

1. Improvement of varietal resistance to mealybug, green spidermite, AMD and CBB
2. Introduction of biological control methods for the major pests
3. Improvement of screening methods for viral and bacterial diseases
4. Extension of fundamental research on resistance mechanisms

2.4 Yield and Quality

2.4.1 Yielding capacity

Computer calculations, using ideal plant types and favourable environmental conditions, suggest that the potential annual yield of cassava is 90 tons of fresh roots per hectare (Cock, 1985). Experimental yields easily reach 50 to 60 tons of fresh roots per hectare; these yields have also been reported from farmers cultivating cassava as the first crop on newly cleared land (Lian, 1987). In general, root yields on farmers' fields are much lower. The world average annual yield is around 9.5 tons of fresh roots per hectare, while the highest average national yield, in India and Honduras, is around 17.5 tons of fresh roots per hectare (FAO, 1988).

Breeding of high yielding cassava varieties has an important place in nearly all national and international cassava research programmes (CIAT, 1987). Low yields under practical conditions on farmers' fields are however not often the result of low yield potential of the cultivated varieties, but due to weed, pest and disease pressure or poor soil and climatic conditions, from intercropping, and from prices of the product which hampers higher inputs of the crop.

Identified main area for improvement :

1. Development of varieties with high yield potential for specific regional and farming conditions

2.4.2 Root quality

Much attention has always been paid to the breeding of high yielding cassava varieties. Root quality should also be considered as an important selection criterion, as this is the final factor in acceptability to farmers and consumers. Farmers often prefer a lower yielding variety of good quality to a higher yielding variety of poor quality (Mahungu, 1987; Ibe and Ezedinma, 1981).

The root quality characteristics that are considered most important are low cyanogenic

glucoside content, high dry matter percentage and high protein content (IITA, 1988c). Other criteria like colour, flavour, storage ability and cooking time are also important, but vary according to product and regional preferences.

Cyanogenic glucoside content : The fresh roots of cassava contain the cyanogenic glucosides linamarin and lotaustralin, in varying amounts. Cassava can be toxic to humans because of the presence of these glucosides. When the roots are damaged by peeling or grating, enzymes such as linamarase are liberated, which hydrolyze the cyanogenic glucosides during which cyanide (HCN) is formed. Cyanide is highly toxic. High level of intake may cause acute poisoning, whereas long-term ingestion of low levels of cyanide may cause goiter, cretinism, tropical ataxic neuropathy and tropical diabetes (Cock, 1982). Detoxification of cyanide takes place by the formation of thiocyanide. Sulphur containing amino acids are essential for thiocyanide formation. Tropical ataxic neuropathy is associated with low levels of sulfur amino acids in the blood, whereas thiocyanide inhibits iodine transport resulting in goiter and cretinism (Rosling, 1987). Although processing methods can reduce the total cyanogenic glucoside content to levels acceptable for human consumption, there always remain glucoside fractions which can cause chronic toxicity (Bourdoux et al., 1983; Bruinsma et al., 1983, Essers, 1988).
Cassava varieties are often described by taste as "bitter" (high glucoside content) or "sweet" (low glucoside content), but the dividing line is not always well defined. The glucoside content does not often correlate with the farmers' criteria to discriminate between sweet and bitter varieties (Bourdoux et al., 1983). Bitter varieties are often preferred for reasons of taste of the processed products or a better storage ability (Fresco, 1986; Essers, pers. comm.).
Environmental conditions influence the cyanogenic glucoside content of the cassava roots (De Bruijn, 1971). Low soil fertility and drought, but also a high nitrogen fertilization, increase the glucoside content (Cock, 1985). Highest glucoside concentrations are found in the root peel, lowest concentrations in the parenchyma or edible part of the roots, while intermediate levels are found in the leaves (Oke, 1983; Akinrele, 1986). Cultural methods may also influence the glucoside content of the roots. Lorenzi et al. (1978) found that pruning before harvesting caused significant reduction in root cyanide levels. It is sometimes suggested that a high glucoside content is associated with high yield (Cock, 1974).
Part of the variation in cyanogenic glucoside content is genetically controlled (CIAT, 1988b). Low glucosides or glucoside free varieties should eliminate toxicity problems. A point of consideration, however, is that chronic cyanide toxicity only occurs in specific regions in Africa, where cassava consumption is high and processing of fresh roots is not properly carried out, and where the consumption of iodine and additional protein is extremely low (Rosling, 1987; Essers, 1988).

Dry matter content : Cassava roots contain 30 to 40 per cent dry matter, which is higher than for most other roots and tubers possess (Cock, 1985). High dry matter content is an important characteristic in evaluating the quality of roots for preparing gari or farinha, cassava products resulting from grating and fermentation (see 2.7.2, processing). Significant correlation exists between the dry matter percentage and the garification rates (IITA, 1980; Ibe and Ezedinma, 1981). In fact dry matter and starch content of the roots

are strongly linked (Mahungu, 1987). Starch forms about 90 per cent of the dry matter of the roots (Cock, 1985). Little is known about specific starch characteristics and the quality of starch under different stress conditions (CIAT, 1988c).

Protein content : The crude protein content of cassava roots is 2 to 3 per cent on dry matter basis which is low compared to other starchy root crops. It is deficient of sulfur containing amino acids (Lancaster, 1982). It is often suggested that if cassava roots had a higher protein content, protein malnutrition and cyanide toxicity problems would occur less frequently (IITA, 1988b; Rosling, 1987). The consumption of protein rich leaves may partly solve malnutrition problems.

Other nutritional qualities : Fresh roots (and leaves) are very rich in vitamin C (Lancaster, 1982). Cassava is very low in vitamin A, but yellow roots with improved beta-carotene levels are selected at IITA (IITA, 1988a). Cassava also provides minerals, especially calcium (Lancaster, 1982).

Identified main areas for improvement :

1. Development of cassava varieties with low levels of cyanogenic glucosides
2. Development of screening methods for the cyanogenic glucoside content of cassava products, which are easily applicable under field conditions
3. Development of cassava varieties with higher starch content and better starch quality
4. Development of cassava varieties with high protein contents
5. Enhancement of research on locally important root qualities

2.5 Post-harvest technology

2.5.1 Storage of cassava roots

Storage of fresh cassava: About one third of the current cassava production is destined for fresh consumption, which is more common in rural areas than in urban areas (FAO, 1980). The perishability of fresh cassava (normally deterioration starts after 3 to 7 days) complicates large problems to the distribution of fresh cassava to urban markets.
Deterioration of fresh cassava roots takes place in 2 phases. Primary deterioration is characterized by vascular discoloration, the accumulation of phenolic compounds and increased activity of oxidases. The deteriorating roots are subsequently infested by specific varieties of bacteria and fungi (CIAT, 1988b).
The most reliable storage technique for fresh cassava roots is to provide moisture to the roots by keeping them wrapped in moist, absorbant material in the ground or in containers. These techniques are widely used around the world (Chinsman and Fiagan, 1987).

Identified main area for improvement :

1. Prolongation of the storage period of fresh cassava roots by inhibition of physiological deterioration processes

Storage of dried cassava: Approximately 38 insects, mainly Coleoptera, are reported to occur on dried cassava chips or products. Many are polyphagous; only those able to reproduce on dried cassava are important. Most damage is reported from Asia and Africa, and on imported dried cassava in Europe (CIAT, 1985).

Identified main area for improvement :

1. Improved control methods of storage pests

2.5.2 Processing

The purpose of processing is to check the deterioration of cassava food products, to eliminate cyanide toxicity and to make the food products more palatable. Processing techniques involve several operations, the most important being peeling, soaking (which eliminates toxic substances through the release of hydrolyzing enzymes), drying and cooking (which eliminates hydrocyanic acid). The main products obtained from these various processing operations are cooked fresh cassava, cassava meal (obtained by sun-drying and milling; gaplek in Indonesia and kokonte in Ghana), gari and farinha (obtained by different fermentation processes), cassava chips and pellets (animal feed), and cassava starch (for industrial purposes and alcohol production). The main processing methods are summarized by Chinsman and Fiagan (1987).

Conventional cassava processing is very labour-intensive. This labour requirement may equal or exceed that of all pre-harvest production activities (IITA, 1988c). Conventional processing is carried out by women.

Important characteristics of the processed products are the protein and vitamin content, cyanogenic glucoside content and the suitability for human or animal food, industrial applications and alcohol production.

Protein and vitamin contents : The protein level of fresh cassava is low and is further depressed by peeling (the peel or root cortex does contain most of the root proteins) and soaking (Cock, 1985). Fresh cassava roots contain significant quantities of vitamin C, thiamine, riboflavin and niacin (Lancaster, 1982). Processing (cooking or fermentation) reduces the vitamin content considerably; only vitamin C is adequately supplied by daily consumption of processed cassava (Rosling, 1987). The vitamin content of fresh cassava leaves is also reduced by boiling.

Cyanogenic glucosides : Different processing techniques result in varying levels of detoxification of cassava roots (Bruinsma et al., 1983; Oke, 1983). In general processing

techniques that involve only sun drying or boiling do not adequately detoxify the cassava roots. Sufficient detoxification relies on an adequate fermentation. The fermentation time, for example the number of days that fresh roots are soaked in water, is especially important. The best combination of techniques will be sequential soaking, sun drying and cooking (Oke, 1983). Roasting of fermented cassava roots with palm oil (garification) reduces the glucoside content even more. Mashing and subsequent boiling is an adequate method to detoxify cassava leaves.

Suitability for animal feed : Cassava chips and pellets (cassava products obtained by sun drying) are used as animal feed on a large scale (e.g. EEC-imports from Thailand). Low vitamin content and high cyanide content of the chips may reduce their suitability for animal feed.

Suitability for industrial applications : Cassava starch is used in the food industry, for paper making, as a lubricant in oil wells, in the textile industry and as substrate for the production of dextrins, which are used in glues (Silvestre and Arraudeau, 1983). Especially starch demand by the textile industry and by the sweetener producing industry (fructose syrup), and the use of cassava for alcohol production seems to be promising. Cassava is frequently mentioned as a potential biomass crop for alcohol production. In Brazil this industry is widely distributed. A major question concerning the alcohol production from cassava is the energy balance. The distillation of alcohol from the fermentation liquor needs considerable energy, which decreases the net energy ratios of alcohol production (Cock, 1982; Phillips, 1978). Alcohol production from cassava should, moreover, not impair the role of cassava as a cheap human food source.

Identified main areas for improvement :

1. Improved processing techniques to reduce the content of cyanogenic glucosides in cassava products
2. Introduction of fungal fermentation procedures to improve the protein content of cassava products
3. Enhancement of research on processing technologies with reduced loss of vitamins and proteins
4. Improvement of the net energy ratio of alcohol production from cassava.
5. Better use of cassava flour in food processing, including improved baking methods

2.6 Summary table

In table 2 the main aspects of cassava production and processing (propagation and varietal aspects, cultivation, root and quality and post harvest technologies) and identified areas of improvement are presented.

Table 2 : Main aspects in cassava production and processing and identified areas of improvement

Main aspects	Areas of improvement
Propagation and varietal aspects:	
Planting material	- Development of techniques with high multiplication rates and long storage ability - Development of true seed
Varietal diversity	- Improvement of germplasm storage techniques - Assessment of wild and cultivated Manihot germplasm
Breeding techniques	- Improvement of breeding techniques
Plant morphology	- Selection of cassava varieties well adapted to - Improvement of leaf production - Introduction of C4-crop morphological traits
Plant physiology	- Establishment of a more efficient biomass partitioning - Improvement of photosynthetic capacity - Research on cyanogenic glucoside production
Cultivation:	
Drought tolerance	- Selection of early maturing varieties - Improvement of the water use efficiency - Improvement of cultural practices
Soil fertility	- Improvement of nutrient use efficiency - Inoculation with mycorrhizal fungi for P-uptake - Identification of associated N-fixing bacteria
Weeds	- Improvement of cultural practices
Diseases and pests	- Improvement of varietal resistance - Introduction of biological control methods - Improvement of screening methods for bacterial and viral diseases
Yield and quality:	
Yielding capacity	- Improvement of yield potential
Root quality	- Reduction of cyanogenic glucoside content - Improvement of screening methods for cyanide content - Improvement of starch content and quality - Improvement of protein content - Improvement of locally important characteristics
Post-harvest technologies:	
Storage of cassava roots	- Inhibition of deterioration processes
Processing	- Reduction of cyanide content in processed products - Introduction of fungal fermentation to improve protein content - Reduction of vitamin and protein losses - Improvement of alcohol production - Improvement of baking processes

3. BIOTECHNOLOGY; GENERAL INFORMATION

3.1 Introduction

The applications of molecular biology have increased so dramatically that a new term, 'biotechnology', has been introduced. Biotechnology is defined by the Netherlands' Directorate General for International Cooperation (DGIS) as the integrated use of molecular genetics, biochemistry, microbiology and process technology to supply goods and services, employing micro-organisms, parts of micro-organisms, or cells and tissues of higher organisms (DGIS, 1989).

Biotechnology is likely to have an impact on medical care, the pharmaceutical industry, production of chemicals, the food industry, agriculture and waste processing. This chapter presents a survey of the most important technology in crop improvement nowadays, a review of the main conditions for the application of biotechnology, a time-frame for the realization of biotechnological applications.

3.2 Biotechnologies in agriculture

In the following sections short descriptions of the most important applications of biotechnology in agriculture are given. This biotechnology with their applications are presented in table 3. Additional information on these subjects can be found in (1) Primrose (1987) and Mantell et al.(1985) for the subjects plant tissue culture, protoplast fusion and recombinant DNA techniques, (2) Jones and Torrance (1986) for diagnostic techniques and (3) Walker and Gingold(1988) for enzyme technology. The main biotechnological techniques are explained in a glossary.

Table 3: Biotechnology in agriculture in general

Technique	Application
Plant tissue culture:	
Propagation	Micropropagation and germplasm conservation
Plant breeding	Embryo culture (rescue of embryos from interspecific crosses) and anther culture (production of homozygous, haploid plants from microspores for e.g. production of hybrids)
Advanced techniques	Recombinant DNA techniques and protoplast techniques (see below)
Protoplast techniques:	
Protoplast isolation	Somaclonal variation
Protoplast fusion	Hybridization between different species and production of new genotypes
Recombinant DNA technology:	Transfer of specific genes (transgenic plants) and RFLPs (identification of specific genes)
Screening techniques:	
Serological techniques and recombinant DNA	Screening germplasm collections, selection of disease-free planting material, selection for resistance in plant breeding
Fermentation and enzyme-technology:	Production of microbial biomass, conversion of substrate in more valuable compounds.

3.2.1 Plant tissue culture

Plant tissue culture is the process whereby small pieces of living tissue (explants) are isolated from an organism and grown aseptically for indefinite periods on a nutrient medium. The usual explants are buds, root tips, nodal segments or germinating seeds. These are placed on suitable culture media (including plant hormones) where they grow into a callus.

Regeneration from callus is an important feature for plant tissue culture. Callus can be induced to adventitious regeneration and somatic embryogenesis. Adventitious regeneration implies the induction of root and shoot formation on callus tissue by changing the hormone content of the medium. Somatic embryogenesis implies the induction of embryogenesis on calluses. Such cells are embryo-like but differ from normal embryos in being produced from somatic cells and not from the fusion of two germ cells. Those embryoids can develop into fully functional plants without the need to induce root and shoot formation on artificial media.

Plant tissue culture can be used for propagation purposes, plant breeding purposes and contribute to more advanced techniques.

Propagation purposes : (1) micropropagation of plant material to rapidly build up a number of mother plants as an intermediary step prior to stake propagation, or for direct transplantation to the field, (2) the production of disease-free plant material by using plant tissue culture in combination with thermotherapy and (3) germplasm exchange and germplasm conservation, especially for species for which seed storage is not applicable, like plants with low fertility (propagated vegetatively) or with low rates of germination.

Plant breeding purposes: (1) embryo culture to rescue embryos obtained from interspecific crossings and (2) anther culture to obtain plants from microspores.

Part of more advanced techniques: Plant tissue culture as part of protoplast (see 3.2.2) and recombinant DNA techniques (see 3.2.3).

3.2.2 Protoplast techniques

A protoplast is a plant cell from which the cell wall has been removed. Protoplasts can be produced from suspension cultures, callus tissue or intact tissues, e.g. leaves, by mechanical disruption or preferably by treatment with enzymes. After enzyme treatment protoplast suspensions are collected by centrifugation, washed in a medium without enzyme and separated from intact cells and debris by flotation on a cushion of sucrose. When placed on nutrient medium protoplasts will synthesize new cell walls and then initiate cell division.
Protoplasts can be induced to fuse by means of specific chemicals or electrofusion. After fusion the nuclear and cytoplasmic genomes reassort and recombine resulting in a wide array of gene recombinations not attainable through conventional breeding. The biggest drawback is that the scientist has little control over which gene combinations are retained which are eliminated.
The result of protoplast fusion can be a hybrid, which contains some chromosomes from both of the parents, or a cybrid, which contains the nucleus from one parent and the protoplasm from the other parent.

3.2.3 Recombinant DNA techniques

Recombinant DNA technology allows the transfer and the replication of genes in host organisms. Two approaches to transformation exist:

Biological approach: One of the frequently used techniques for the transmission of a new gene to a plant cell is transmission by means of a vector. The vector must be active in transferring the DNA into the plant in such a way that the DNA is maintained through cell division and plant propagation and that it will be expressed as a genetic factor. It is important that the vector will not induce disease symptoms and that it will have a broad host range.
Organisms which do have potential as a vector are caulimoviruses, geminiviruses, Ti-plasmids of Agrobacterium tumefaciens and Ri-plasmids of Agrobacterium rhizogenes.

The most frequently used vector is Agrobacterium tumefaciens, a bacterial organism which can cause crown gall in a wide variety of plant species. When the bacterium infects plant cells a portion of its DNA known as T-DNA, is transferred to the plant genome.

Physical approach: Physical methods for introducing DNA in the host plant are:
(1) Micro injection; it is possible to introduce DNA in the host plant by micro injection of cells, protoplasts or zygotes under the microscope (Zhou et al., 1988).
(2) Particle bombardment of cells with wolfram or gold particles including the gene fragments (Klein, 1987). (3) electroporation (Fromm, 1986) (4) Osmotic shock and (5) transformation with chemical fusogens (Lindsay et al., 1987).

When the regeneration from cells is a problem, it can be advantageous to transfer a gene into pollen. The transferred pollen can then be used to fertilize ovules. Regeneration from fertilized ovules is often easier than regeneration from protoplasts (Hess, 1988).

Conditions for recombinant technology

A number of areas will have to be developed before gene transfer provides a range of new phenotypes to plant breeders (Gerlach, 1985). Firstly, ways to isolate specific genes from plants and other eukaryotes must be established. One of the most promising techniques for gene identification and isolation is the use of Restriction Fragment Length Polymorphism (RFLP) (Landry and Michelmore, 1987; Lindhout and van der Mark, 1988). Stability of the new genetic information and transmission of the new gene to subsequent generations must then be achieved. Finally, expression of the cloned genes in the correct cells at the correct time must be ensured.

3.2.4 Diagnostic techniques

Diagnostic techniques for the screening of viral or bacterial diseases can be divided into conventional techniques such as detection with indicator plants and field observation, and biotechnological techniques such as serological and cDNA detection.

Historically virus detection was based on symptom occurrence and plants that developed symptoms were taken from fields. However, many viruses show no visual symptoms. The use of indicator plants for virus detection and diagnosis is both cumbersome and expensive, requiring greenhouse space, time and energy (Martin, 1985).

Serological techniques: Serology is one of the most useful tools available for identification and detection of viruses and phytopathogenic bacteria. Rapid serological tests such as immunofluorescence are well adapted for diagnosis. The most useful serological technique for a rapid screening of viruses and bacteria on a large scale is the enzyme-linked immunosorbent assay (ELISA). Other screening techniques that make use of immunological reactions are immuno fluorescence (IF) and immuno electron microscopy (Martin, 1985; Thottappilly & Rossel, 1988).
In the past all serological procedures have been limited by the non-specificity of antisera.

Polyclonal antisera contain a multiplicity of antibodies which can cross react with more than one virus (Barnett, 1986) or bacterial species (De Boer, 1987). It is now possible to produce monoclonal antibodies. Monoclonal anibodies are highly specific antibodies derived from a single clone of an antibody-producing cell and react with one site of the antigen. The advantage of monoclonal antisera is their high level of specificity and uniformity.
Diagnostic techniques should be sensitive, reliable and also rapid and easy to use and preferably capable of detecting specific virus or bacterial strains.

cDNA detection techniques: It is possible to produce a complementary cDNA strain, a probe of the cRNA of the pathogen (e.g. viruses or bacteria) (Watson, 1983; Forster et al., 1985). By varying the length of the probe the specificity of the detection technique can be changed. The probes can be labelled with radioactivity or chemicals (Forster et al., 1985). When in the test sample the pathogen is present, the probe will hybridize and can be detected by autoradiography or with conjugates that will give a positive reaction to biotin. Serological methods are in general less sensitive than cDNA techniques. Detection techniques using cDNA probes will in the future have important applications in disease diagnosis.

3.2.5 Fermentation and enzyme technology

Fermentation and other bioconversions have been used for centuries in the production of wine, beer, fermented milk and vegetables. In fermentation complete organisms are used. Another strategy is to isolate enzymes from organisms. Single-step reactions frequently utilize isolated enzymes as catalytic units.

Micro-organisms are used for the production of microbial biomass on raw plant substrate, for conversion processes, and for the production of commercially important metabolites (Stanberg, 1988). It is possible to produce micro-organisms with a high protein content known as single cell proteins. Microbial cells can also be used to catalyze (enzymatic) the conversion of substrates (raw plant material) into more valuable compounds.

An important field of enzyme technology is starch processing. Starch can be used as a source for ethanol, but also for more valuable products as special polymers, new low-calori sweeteners (such as aspartate) and biologically derived flavours (Best, 1988)

3.3 Biotechnology in cassava research

3.3.1 Limitations to biotechnological applications.

A review of the applications of biotechnology to the main areas of improvement in cassava production and processing raises the following issues:

1) A prerequisite for the application of several protoplast and recombinant techniques to cassava is to obtain a proper regeneration from callus tissue or transformed explants.
2) The introduction of new genes in cassava varieties by protoplast fusion or transformation with vectors requires standard protocols,which may meet other difficulties.

The application of diagnostic techniques as well as fermentation and enzyme technologies involve meet no great difficulties.
A time-frame for the realization of each biotechnological application must be determined.
The main biotechnological techniques in cassava research are presented in table 4, together with their application or development strategy.

Table 4: Biotechnological techniques in cassava with their (potential) application

Technique	(Potential) application
Plant tissue culture:	
Meristem or shoot tip culture	Now currently applied
Regeneration from non-meristematic explants	Embryogenesis from cotyledons, shoot tips and immature leaves is applied at present
Regeneration from callus	Not possible until now
Protoplast fusion:	Not applicable until regeneration from callus is established
Recombinant DNA technology:	Not applicable until regeneration from callus is established

Regeneration

Regeneration from callus: Callus has been induced from stems, petioles, leaves and even root sections of cassava. In general stem sections seem best suited to callus induction. Regeneration from callus has proved difficult and success has only been reported with stem callus tissues by Tilquin (1979) and with callus derived from protoplast cultures by Shahin and Shepard (1980). Both experiments could only achieve some irregular regeneration with specific genotypes. Unfortunately these results have never been reproduced (Ng, 1988; Stamp & Henshaw, 1987; Szabados et al; 1987; Robertson, pers. comm.). Only root formation can readily be obtained in callus cultures.

Regeneration from non-meristematic explants: It is now possible under appropriate conditions to induce somatic embryogenesis from seed-derived cotyledons (Stamp & Henshaw, 1987), shoot tips (Szabados et al., 1987) and immature leaves (Szabados et al., 1987). Somatic embryos and whole plants have been regenerated. The balance between embryogenic tissue and friable callus (non-embryogenic) on explants is of great importance for achieving high embryogenic responses (Roca et al., 1985). Zsabados et al. (1987) have induced somatic embryogenesis from shoot tips and immature leaves of

in vitro shoot cultures of 15 cassava genotypes.
Anatomical and morphological studies have demonstrated a similar development of embryos in mature seed and clonal leaf explants of cassava (Stamp, 1987). Embryogenesis obtained from genetically segregating tissues, such as the seed cotyledons, may help identify the requirements for somatic embryogenesis in callus of clonal origin in cassava.

Regeneration from meristem or shoot tip culture: The regeneration of shoots and plantlets from meristem segments is relatively easy. The use of plant tissue culture for micropropagation of disease free material, germplasm conservation and germplasm exchange are well developed (see 5.2 and 5.3). The explants mostly used for this purpose are stem segments with at least one node (Roca et al., 1985).

Protoplast fusion and transformation protocols

Protoplast culture: Protoplasts from mesophyll cells have been isolated successfully from several cassava varieties and induced to regenerate cell walls and form callus. The cells formed colonies at a frequency of 50%-60% (Mabanza & Jonard, 1983). However, shoot formation was only occasionally observed (Shahin & Shepard, 1980). The strategy for further research is to apply the protocols used with explants for obtaining somatic embryogenesis from single cells.

Protoplast fusion: The main limitation to protoplast fusion is the absence of proper regeneration protocols. Furthermore, the regenerated plants may not always be fertile and may not produce viable seed.

Transformation with Agrobacterium strains: Since cassava can be naturally parasited by wild Agrobacterium sp., it may be expected that transformation with engineered Agrobacterium strains is possible. The main limitation for application of transformation with Agrobacterium strains to cassava is the absence of regeneration protocols. The transformation of single cells is preferred, because transformed cells can be more easily selected than transformed tissues. Calderon-Urrea (1988) describes a method for the infection of somatic embryos, leaf and stem fragments of cassava. Transformed cell lines were obtained from the different explants. It was not possible for him to isolate transformed somatic embryos on medium containing antibiotic. Furthermore, work on regeneration protocols for transformed explants of cassava is carried out at the Free University, Amsterdam (Sakina, pers. comm.) and at CIAT (CIAT, 1988b).

3.3.2 The time-frame for biotechnological applications

The time-frame for biotechnological applications in cassava improvement programmes depends on the state of the art for a specific biotechnology. The time-frame for biotechnological applications depends also on background knowledge of aspects such as genetic, biochemical and physiological regulation of specific traits.

(1) *Short-term applications* (requiring 1 to 3 years of research on world-wide scale): Biotechnological improvements are readily applicable. Biotechnological applications in this category are plant tissue culture, embryo culture, storage techniques, immunological and processing techniques.
(2) *Medium-term applications* (requiring 5 to 10 years research on world-wide scale): Strategic research is needed but the use of biotechnology appears to be feasible. Biotechnological applications in this category are anther culture, protoplast fusion and recombinant DNA techniques for identified genes.
3) *Long-term applications* (requiring at least 10 years of research on world-wide scale): Basic research is needed to identify the future biotechnological approach. Biotechnological applications in this category are recombinant DNA techniques for unidentified genes.

It should be stressed that this time-frame for biotechnological improvement is rather arbitrary. Especially medium-term applications such as protoplast fusion and recombinant DNA techniques will only be realized within these terms if regeneration protocols are developed.

4. SOCIO-ECONOMIC IMPACT ASSESSMENT

4.1 Introduction

Apart from the technical impact, the socio-economic impact of biotechnological improvements on cassava production and processing should also be assessed. In this chapter the main social and economic criteria for impact assessment are discussed, namely the potentials for adoption of biotechnological improvements by local farmers, the requirements for research and infrastructural capacity when biotechnology is used in cassava research, and the potential risks associated with biotechnology. It should be emphasized that these criteria are equally important for the assessment of the socio-economic impact.

4.2 Adoption by local farmers

Biotechnological improvements or their products will only be accepted by local farmers if those improvements solve the major constraints in their cassava production and processing. Farmers have a wide range of criteria, depending on differences in agro-ecology and farm size. It is important to assess the main criteria for different categories of farming systems.

The degree of access to capital-intensive (external) inputs can be used as a factor distinguishing between the different farming systems. This results in two basic types of farming systems: subsistence or market oriented. It is hardly possible to clearly distinguish farming systems in this way. Rather there is a continuous gradation of farming systems limited to ample access to capital-intensive inputs.

Appropriate biotechnological research for subsistence farming systems should focus on biotechnological improvements at no capital-intensive input cost, with special attention to cassava cultivation under marginal conditions. Subsistence and semi-subsistence farming systems, which can be found throughout the tropics, fall within this category. Small-scale farming systems, which supply the animal feed and starch industries, may also fall within this category. The cassava production by small-scale, poor farmers in Northern Thailand for the animal feed export to the European Community is an example of this type of farming system. Farmers will try to achieve high yield stability, often under marginal conditions, and to be independent of capital-intensive inputs.

Appropriate biotechnological research for market oriented farming systems also includes research on biotechnological improvements, which require relatively high input costs. Small-scale farmers on fertile soils with sufficient access to inputs and stable markets for

their outputs, and large-scale commercial farming systems are examples of this category. Farmers will opt for yield optimalization and high net incomes.

4.3 Local research and infrastructure.

For a successful establishment of a biotechnology industry in developing countries, capital inputs, trained personnel and a relatively well-functioning infrastructure are necessary (Kenney and Buttel, 1985; Dias et al., 1987). Development of new biotechnological applications specifically requires high training inputs; lack of trained personnel is often the principle obstacle.

Different biotechnologies, as summarized in chapter 3.2 , should be distinguished to assess the necessity of certain capital, training or infrastructural requirements for their development in a specific situation. In table 5 a typology of biotechnologies is given, characterized by the factors research/training requirements and capital input/infrastructural capacity.

Table 5: Typology of biotechnologies (for explanation of terms see glossary)

	Capital input/Infrastructural capacity		
Research/ Training Requirements	Low	Medium	High
Medium	Plant tissue culture for propagation and screening	Protoplast fusion Fermentation technology	Enzyme-technology Cryopreservation
High	Plant tissue culture for breeding purposes	Diagnostic techniques (serological)	Recombinant DNA techniques

In general biotechnological research will support other research programmes, mostly conventional plant breeding programmes. Sufficiently trained personnel for those research programmes must already be available, before biotechnology is introduced. Research and training capacity should therefore be at least at a medium level in a certain region or country before considering biotechnological research.

Propagation by means of plant tissue cultures, screening methods for nutrient and water stress, protoplast fusion and fermentation technologies all require low to medium levels of capital input and infrastructural capacity. Development of biotechnological applications at a more fundamental level, such as recombinant DNA techniques and certain diagnostic techniques, require a high level of capital input and infrastructural capacity.

This typology (see table 5) indicates research and infrastructural requirements for the development of new biotechnological applications. It should be realized that the

implementation of already existing biotechnology may require less research and infrastructural facilities
In that case recombinant DNA techniques, for example, will require only medium research and infrastructural facilities. In chapter 5 only research and infrastructural requirements for the development of new biotechnological applications are indicated.

4.4 Associated risks

Many environmental risks may be associated with the introduction of biotechnological improvements in agriculture. UNEP (United Nations Environment Programme; Fowler et al., 1988) has pointed out some of these risks :

1. Invasion of new, transformed organisms may bring about large ecological changes.
2. Introduction of genetically engineered crops may lead to genetic erosion.
3. Transformed crops with introduced disease and pest resistance may be more susceptible to pest and disease break-through.
4. Introduction of improved crops in marginal areas may lead to soil fertility or erosion problems.

Most of these risks are also associated with the introduction of improved varieties by conventional breeding programmes. Some risks, however, are especially associated with the introduction of biotechnological improvements. Recombinant DNA techniques, resulting in transformed organisms, may impose serious risks to the natural environment. Van Vloten-Doting (1989) stated that the risks of cultivating genetically engineered plants do not fundamentally differ from the risks encountered by the introduction and breeding of exotic species. It is possible that the introduced information is transmitted to related wild species of a crop, extending the risk to acquired properties of these wild plants. To reduce these risks, careful examination of transgenic plants in the field during several cropping cycles has to take place. Other biotechnologies, such as protoplast fusion, inflict no more environmental risks than conventional breeding programmes (Van Vloten-Doting, 1989).
In this study the following risks are considered in relation to the introduction of biotechnological improvements: (1) genetic erosion, (2) ecological risks associated with the introduction of new genomes and (3) environmental risks associated with the introduction of improved varieties in marginal areas.

5. ANALYSIS; CONVENTIONAL AND BIOTECHNOLOGICAL RESEARCH STRATEGIES IN CASSAVA PRODUCTION AND PROCESSING

5.1 Introduction

This chapter builds onto the three preceding chapters. Biotechnological strategies are formulated for the main areas of improvement in cassava production and processing, and compared to conventional research options. A short analysis of the impact on the situation of local farmers, of the research and infrastuctural requirements and of the associated risks is made for each biotechnological application. When the adoption by local farmers or associated risks are not relevant to a specific biotechnological application, they are not mentioned in the impact assessment.

5.2 Propagation

Conventional research approach

Propagation techniques: Conventional multiplication is very slow. Two improved techniques have been adopted. One utilizes sprouts grown on 2-node stakes (Cock et al., 1976), the other utilizes single leaf-bud cuttings obtained directly from the mother plants (Roca et al., 1980). The former can potentially yield up to 36.000, and the latter up to 3000 stakes per year per mother plant respectively.

True seed: Propagation by true seeds is difficult. For most cassava cultivars the fertility is low and the seeds have a low germination rate which appears to be due to dormancy (Jennings & Hershey, 1984). The seeds are also very heterozygous, caused by the allo-tetraploidy of cassava. Improvement of the fertility and removal of the dormancy could be achieved by a positive selection on these traits in a plant breeding programme.

Potential for biotechnological improvements

Micropropagation: Micropropagation allows a rapid multiplication of cassava varieties. The simplest cassava micropropagation technique is plant tissue culture of stem segments with at least one node, obtained from in vitro plantlets. Three to five plantlets can be obtained per culture per month per meristem (CIAT, 1988a). For the basic protocol for meristem and shoot tip culture reference is made to CIAT (Roca et al., 1985). Micropro-pagation is also used for the production of disease-free planting material (Roca, 1985; see 5.10; Diseases).

Propagation by true seeds: The production of the true seeds for cassava propagation depends on the establishment of homozygous cassava varieties through anther culture or apomixis (see 5.4; Plant breeding techniques)

Micropropagation can be adopted within a short time. The production of true seeds may be realized on a medium term. The time-frame for the production of true seeds depends mainly on the introduction of a good anther culture protocol.

Research on improvement of propagation techniques is conducted at several centres, the most important being CIAT and IITA.

Impact assessment

Adoption by local farmers: Propagation by true seeds as well as micropropagation may increase the dependence of farmers on seed corporations or local institutes.

Local research and extension capacity: For the application of micropropagation a simple plant tissue laboratory and a modest infrastructural and research capacity will be adequate. For the production of true seeds a medium-level infrastructure and research capacity is sufficient.

Conclusion

Improved conventional propagation techniques are available. Micropropagation may be an appropriate biotechnological approach. It requires a relatively high capital input, but micropropagation can be used in association with conventional techniques. True seed production may overcome many propagation constraints, but is not yet possible.

5.3 Germplasm storage

Conventional research approach

Cassava germplasm collections are conventionally maintained in the field. New germplasm plantings often use freshly cut stakes from old fields. Besides the high costs, field maintenance often exposes the valuable germplasms to insect attack, disease infection, and soil and climatic problems.

Seeds can also be used to maintain cassava germplasm. Seeds stored at 5°C and 60% relative humidity have maintained their viability for several years (Hahn et al., 1973). IITA (1979) has reported that seeds under these conditions can be stored for up to seven years with no loss of germination ability. Although cassava seedlings are free of most diseases, adapted genotypes can not be preserved due to their high genetic segregation. Another difficulty of seed storage is the low germination rate of cassava seed which appears to be due to dormancy (Jennings & Hershey, 1984)

The increasing need to accelerate genetic conservation techniques for several clonally propagated crops has been widely recognized. In 1982 the International Board for Plant Genetic Resources (IBPGR) established an international committee to assess scientific research and advise on in vitro storage for crops in general. Withers and Williams (1985) summarized successful attempts with several crops. Wild germplasm of Manihot spp, with useful traits, which should be preserved, is listed in table 6.

Table 6: Sources of useful genes in some wild Manihot species.

Species	Useful trait
M. alutacea	Resistant to mites
M. angustiloba	High starch content
M. anisoplylla	High starch content
M. attenuata	Tolerant to low temperatures
M. carthaginensis	Tolerant to dry conditions and high PEP carboxylase activity
M. chlorostica	Tolerant to saline soils
M. dichotoma	Resistant to African Mosaic Disease and drought tolerant
M. esculenta subsp. melanobasis	High protein content
M. filamentosa	High potential as fodder crop
M. glaziovii	Resistant to African Mosaic Disease
M. gracili	Dwarf type
M. grahami	Tolerant to low seasonal temperatures
M. guarantica	Tolerant to low seasonal temperatures
M. irwinii	Tolerant to aluminium toxicity
M. longipetiolati	Dwarf type
M. neusana	Resistant to mealybug
M. orbicularis	Tolerant to aluminium toxicity
M. peltata	Tolerant to acid soils
M. pohlii	Resistant to mealybug
M. pringlei	Low cyanide content
M. pseudoglaziovii	Resistant to bacterial blight and high PEP-carboxylase activity
M. reptans	Resistant to bacterial blight
M. sagittato-partita	Tolerant to acid soils
M. tripartita	Tolerant to aluminium toxicity
M. tristis	High protein and starch content

Source: Chavez et al. (1988).

Potential for biotechnological improvements

Biotechnological improvement of germplasm storage may take place by storage under conditions which slow down growth or by storage in liquid nitrogen (cryopreservation).

Storage under conditions which slow down growth: Plant tissue culture provides possibilities for storage of germplasm. The storage temperature, illumination and variations in the composition of the medium (osmotic level, nutrient limitation, growth hormones, and for example the addition of activated charcoal to the medium) influence the rate of growth and viability of single node cultures (Roca et al., 1983). For a basic protocol for preservation under slow growing conditions reference is made to CIAT (CIAT, 1988a).

More than 2200 cassava varieties from CIAT's germplasm collections have been transformed into plant tissue cultures for slow growth storage (CIAT, 1988a). Two other large accessions are at IITA in Nigeria (2000 varieties) and at Central Tuber Crops

Research Institute (CTCRI) in India (1800 varieties) (Jennings & Hershey, 1984). Several of the national institutions have developed plant tissue culture facilities for the maintenance and micropropagation of cassava germplasm through cooperative efforts with CIAT.

Cryopreservation: A promising future possibility for vegetative conservation is the storage of meristem tips in liquid nitrogen. Procedures for cassava are still at the experimental level, but successful regeneration of cryopreserved clones has recently been accomplished (Kartha et al., 1982). Bajaj (1983) has obtained complete cassava plants and callus tissues from excised meristems which were freeze-preserved in liquid nitrogen (-196°C) for three years. From the cryopreserved plantlets 29.3 % survived and developed into shoots and plantlets. These explants have been successfully established in the soil and grown to maturity. The major problem has been the low rate of recoverability.

Genetic stability can cause concern in both techniques. Preliminary tests have not shown noticeable changes in plant characteristics after storage under conditions which slow down growth and cryopreservation (CIAT, 1988a).

Impact assessment

Adoption by local farmers: Though farmers have no influence on germplasm storage at institutes the importance of germplasm maintenance of their local varieties should be realised.

Local research and extension capacity: Maintaining a germplasm bank with plant tissue culture under conditions of slow growth requires little in this way of infrastructure, a medium level of research capability is needed. Cryopreservation will require a well developed infrastructure and research at medium level.

Associated risks: Phytosanitary and quarantine aspects of germplasm exchange in vitro require standardized procedures to prevent the spread of pests and diseases (Wither and Williams, 1985).

Conclusion

Biotechnology offers large scope for the improvement of germplasm storage. Plant tissue storage of germplasm under conditions of slow growth has already become a standard method. Cryopreservation may be applicable before long.

5.4 Plant breeding techniques

Conventional research approach

Plant breeding techniques such as stimulation of flowering and hybridization, also with wild Manihot spp, are important for the improvement of cassava.

Flower induction: A serious constraint for plant breeders is the inability of many cassava genotypes to flower. This should be solved by selection of varieties, which flower well.

Hybridization: Hybrids may increase the yields of cassava. Factors important for the development of hybrid seed are:

(1) Production of homozygous inbred lines. With successive inbreeding, inbreeding depression occurs. Alternating inbreeding or apomixis, which occasionally occurs in cassava, can be used (IITA, 1988a).

(2) Flowering ability of the inbred lines

(3) Introduction of male sterility in the cassava parent lines. Several mechanisms of male sterility present in cassava have been described (Byrne, 1984).

Both CIAT and IITA place strong emphasis on the value of hybrid progenies, with appropriate adaptation and resistance, for national programmes (Hershey, 1985).

Hybridization techniques: In most cases interspecific crossing between different species of Manihot presents no problems, although in some interspecific crosses the embryo will abort. Crosses of cassava with some arboreal Manihot species (for example M. brachyandra, M. leptophylla, M. glaziovii, M. pseudoglaziovii) failed, fruit drop being 100% three weeks after pollination (Nassar, 1980).

Potential for biotechnological improvements

Flower induction: Floral induction may occur on meristem-derived cassava plantlets from tissue culture (Tang et al., 1983). Five genotypes out of 13 consistently responded to culture conditions giving rise to male or female flowers. Male flowers contained anthers in which meiosis occurred. Apparently normal seeds were formed.

Genetic characterization: The efficiency of selection in plant breeding programmes can be improved if tight linkages are detected between mapped RFLP markers and loci governing important traits. Since the detection of RFLPs is very time consuming, only long term results can be expected.

Hybridization: Hybrids can be bred by means of anther culture. Using anther culture inbreeding depression can be avoided. Other objectives of anther culture may be the production of true seed production and acyanogenic cassava lines (see 5.2 and 5.13). Anther cultures of cassava genotypes results in 100% calluses (CIAT, 1982a). The same results have been obtained at IITA (Wenzel et al., 1985). Differentiation of embryo-like structures recently occurred on anther-derived callus of a specific genotype in a particular medium (CIAT, 1988a). This finding suggests that regeneration from single microspore-derived calluses may be possible in the near future.

Hybridization techniques: Many species can be crossed by conventional means, but the use of protoplast fusion can extend the gene pool (see 3.2.2; protoplast techniques).

Because of the transfer of unknown genes, protoplast fusion is useful for only those traits which can be selected at cellular level.
Crosses between distantly related species of plants have a slight chance to yield an agriculturally beneficial hybrid. Interspecific crosses often result in abortion of the embryo. Embryos can be rescued by means of plant tissue culture. One of the most commonly used methods is to excise the undifferentiated embryo and culture it on a specified medium. With this technique hybrid embryos from cereals and legumes have been rescued. The youngest cassava embryos which could be rescued with embryo culture were 25 days old (Roca et al., 1985). Biggs et al. (1986) described a method which involves the excision of embryogenic plantlets from mature cassava seeds. This method aims to break seed dormancy and to increase the recovery of plants from cassava. Anther culture and embryo culture may be realized within an intermediate time span. The application of protoplast fusion in this area may be realized in the long term.

Research on the application of anther and embryo culture in cassava breeding programmes is mainly carried out at CIAT.

Impact assessment

Local research and infrastructure: Once regeneration becomes a routine technique applications such as anther culture, embryo culture and protoplast fusion are relatively simple. They require a medium level of infrastructural and research capacity.

Conclusion

Biotechnological improvement of plant breeding techniques is possible. Flowering of cassava can be improved by the induction of flowering in vitro. The production of hybrids may be improved by the introduction of anther culture (male sterility), thus avoiding inbreeding depressions. Protoplast fusion and embryo culture may facilitate crossings between cassava and other Manihot spp. However, most of the Manihot spp. can also be crossed using conventional techniques.

5.5 Plant morphology

Conventional research approach

As main areas of improvement, leaf production ability, plant architecture (considering intercropping) and the incorporation of morphological characteristics of C_4-crops are selected.

Leaf production: Breeding efforts to select cassava varieties for both foliage and root production have focused on high yielding varieties. Root production of such varieties is less affected by leaf harvests (Dahniya, 1981).

Plant architecture: An important objective of many breeding programmes is the development of cassava varieties with a good plant architecture for intercropping (Sinthuprama et al., 1988; Nayar et al., 1988). For intercropping erect, non-branchy and vigorously growing cassava varieties are often selected (Jayawardena and De Silva, 1988). Branching is mostly caused by flower initiation, which is partly daylength sensitive (De Bruijn, 1977). The ideal architecture varies according to the cropping system. Conventional breeding programmes offer possibilities to select for better intercropping ability of cassava varieties.

Kranz anatomy: Interspecific barriers (the Kranz anatomy is present in some species of the family of the Euphorbiaceae) will probably impede the incorporation of the Kranz anatomy in cassava by means of conventional breeding methods.

Potential for biotechnological improvements

Biotechnological improvement in this area is long term undertaking. More knowledge about the genetic control of morphological traits has to be obtained. Most of these traits are determined by a number of genes. This renders achievements with recombinant DNA techniques in the near future improbable.
Biotechnological research in this area has not been carried out so far.

Impact assessment

Adoption by local farmers: Improvement of leaf production and plant architecture for intercropping are especially important to small-scale farmers.

Local research and extension capacity: Basic research on the genetic regulation of morphological traits requires a well developed infrastructure and highly qualified personnel.

Conclusion

The improvement of morphological traits has to be achieved by means of conventional breeding methods. Biotechnology offers no perspectives as yet. The Kranz anatomy could perhaps be introduced in cassava by interspecific crossings or protoplast fusion, but this is a long-term perspective.

5.6 Plant physiology

Conventional research approach

Efficient root storage: Research should be focused on the efficiency of storage root production and the selection for an early start of starch accumulation (Veltkamp, 1986). These criteria, however are still not used in cassava breeding programmes. The harvest index is seen as a more practical tool in selecting for high yield potential since the

selection on efficiency of storage root production requires more data (Lian, 1987).

Photosynthetic capacity of cassava leaves: Large differences in photosynthetic rate between cassava varieties occur, but these are not correlated with the crop's growth rate in the field (CIAT, 1988). It was found, that the PEP-carboxylase system differs in activity between cassava varieties.

Potential for biotechnological improvements

Efficient root storage: Little is known about the basis of genetic control of the initial start of starch accumulation and the efficiency of storage root production. Biotechnological improvements are not yet possible. The CIAT conference (CIAT, 1988b) indicated that the sink strength in cassava has to be studied. Through biochemical means the genotypic capacity of biomass partitioning to roots can be identified. Such measurements are used for other crops like potato. Biotechnological improvement in this field may be realized in the long term.

Photosynthetic capacity of cassava leaves: More basic information is needed to understand the physiological and biochemical processes that are related to the photosynthetic capacity of cassava leaves. Antibody techniques may be used to identify certain key enzymes of the PEP-carboxylase system in cassava varieties (Black, 1988b) and to select varieties with a large photosynthetic capacity. Biotechnological improvement will be realized on the long term.

At present research activities on cassava physiology are carried out at CIAT and at the University of Georgia (dr. C.C. Black).

Impact assessment

Local research and infrastructure: Fundamental research on plant physiological characteristics of cassava requires a highly developed infrastructure and highly qualified personnel.

Environmental aspects: The introduction of cassava varieties with high yields on marginal soils may accelerate the exhaustion of these soils.

Conclusion

Selection on the storage root production and on an early initial efficiency of starch accumulation can be used in conventional breeding programmes to improve the biomass partitioning in cassava. Biotechnological improvement of biomass partitioning as well as the photosynthetic capacity of cassava leaves requires much more fundamental research.

5.7 Drought tolerance

Conventional research approach

Improvement of the drought tolerance of cassava has focused on high yield under drought conditions, early maturing varieties and the improvement of morphological characteristics.

High yield under drought conditions: Selection of cassava varieties with improved drought tolerance is conventionally carried out by selection for high yield and good yield stability under naturally dry conditions. Varieties, which have a leaf area index above the optimum under well watered conditions, show good yield stability and high yield under drought conditions (Cock, 1985b). However the gain in yield is not consistent in different seasons and locations, making this approach inefficient and often even ineffective (Blum, 1983; Ludlow and Muchow, 1988).

Early maturing varieties: Early maturing varieties with reasonable yields are urgently needed (IITA, 1988b; Jayawardena and De Silva, 1988; Sinthuprama et al., 1988). Conventional breeding for early maturity is done by selection for high yield at early harvest (Sinthuprama and Tiraporn, 1987). Early yields show large differences. In Thailand experiments with 6 to 8 months' cropping periods were carried out, resulting in acceptable dry matter yields of some varieties (12 per cent yield loss compared to 12 to 14 months' harvests; Sinthuprama et al., 1988).

Morphological characteristics: Trials at CIAT suggest, that morphological traits such as leaf formation and leaf life and stomata incidence vary among different cassava varieties (CIAT, 1985; CIAT, 1988a).

Potential for biotechnological improvements

Complex crop characteristics expressed at plant level, such as high yield under drought conditions, early maturity and morphological traits as leaf movement or stomata incidence, do not as yet offer much possibility for biotechnological improvement.
Screening of varieties through plant tissue techniques may be used to improve the drought tolerance of cassava, but only if desirable traits are expressed at cellular level (as for example osmotic adjustment).
Advanced research on drought tolerance is only carried out at CIAT. This research focuses on the photosynthetic capacity of cassava under drought stress (CIAT, 1988). Considerable interest in research on the photosynthetic capacity is shown too at the University of Georgia, Athens, USA (Dr. C.C. Black).
Biotechnological improvement of the drought tolerance of cassava may be realized in the long term.

Impact assessment

Adoption by local farmers : Cassava varieties, which offer a good yield stability under drought conditions or which are early maturing, will readily be adopted by farmers in drought-prone areas.

Local research and infrastructure : Fundamental research on the genetic characterization of drought tolerance in cassava will need a high level of research and infrastructural capacity.

Associated risks : The introduction of drought tolerant cassava varieties may lead to an increase of cassava cultivation in drought-prone areas, often on soils sensitive to erosion. Hence special attention should be paid to anti-erosion control.

Conclusion

More fundamental knowledge is needed on the biochemical regulation and the genetic characterization of drought tolerance in cassava, for conventional as well as for biotechnological improvement. At present biotechnology for the improvement of drought tolerance of cassava for traits which are expressed at cellular level some promise.

5.8 Soil fertility

Conventional research approach

As main areas of improvement the efficiency of nutrient use, VAM-fungi associations and associations with N-fixing bacteria are selected.

Efficiency of nutrient use : It is possible to improve the efficiency of nutrient use in crops. Research on tomatoes and Phaseolus beans (Gabelman and Gerloff, 1982) and maize (Ramirez et al., 1987) revealed the existence of intraspecific variation in efficiency of N, P and K use.
Screening of cassava germplasm accessions and breeding lines for low P tolerance is carried out at CIAT (CIAT, 1988a). Varieties are screened in the field. Few research results on nutrient use efficiency of N and K are available.

VAM-fungi associations : Vesicular-arbuscular mycorrhizal (VAM)-fungi, associated with higher plants, improve their P-uptake. The external hyphae of the fungus enlarge the uptake capacity of the plant roots, especially for nutrients of low mobility in the soil solution such as P, Zn (Zinc) and Cu (Copper) (Howeler et al., 1987; Rajapakse and Miller, 1987). Important tropical crops, as cowpea, soybean, tea, coffee and cassava, have mycorrhizal associations (Bowen, 1980).
Cassava is highly mycotrophic and its response to P-application depends entirely on the mycorrhizal association (Howeler, 1983). Genetic variability in response to root colonization by VAM-fungi exists in cowpea (Rajapakse and Miller, 1987), but has not

been examined in cassava. Large differences in effectiveness of P-absorption exist between different VAM-fungi strains. Often local fungi populations, on acid or degraded soils, are small and ineffective. Inoculation of cassava roots with improved strains (for example Glomus manihotis and Entrophosphora colombiana) may result in yield increases of 20 to 25 per cent on these soils (Howeler et al., 1987). VAM-fungi strains are collected and evaluated at CIAT, at present the mycorrhizal collection comprises 1200 isolates (Howeler et al., 1987).

Associations with N-fixing bacteria : Associations between N-fixing bacteria and higher plants may take place in root nodules, as is the case with leguminous crops, or in a loose symbiosis, with free-living bacteria. Free-living, N-fixing bacteria, such as Azotobacter, Pseudomonas and Azospirillum, have been found on many non-leguminous plants, but mainly on monocotyledons like grasses and grains (Doebereiner and Pedrosa, 1987). Free living, N-fixing bacteria, associated with cassava, have not been identified.

Potential for biotechnological improvements

Efficiency of nutrient use : Biotechnological improvement of the nutrient use efficiency of cassava may be effected through improved screening methods for efficient varieties, for example by cell and tissue culture on nutrient deficient media.
Especially K-utilization of crops offers possibilities for biotechnological improvement (Gerloff, 1987). Differences in K-efficiency may appear on cellular level, as has beenfound with research on tomato (Figdore et al., 1987). Nitrogen efficiency differences may also be observed by means of in vitro techniques, as N-response is often linked to nitrate reductase activity at cellular level (Davey and O'Hara, 1984).
Efficiency of nutrient use is not a research priority at CIAT and IITA. Biotechnological improvement in this field may require an intermediate time span.

VAM-fungi associations : Biotechnological improvement in this field may first take place by isolation and identification of effective fungi strains as well as by their conservation. Up till now the identification and conservation are still carried out in bio-assays in greenhouses, using highly mycotrophic test plants like kudzu or cassava (Howeler et al., 1987). Effective fungi strains can be identified by means of cDNA diagnostic techniques (which are also proposed for the identification of effective Rhizobium strains for peanut; Bunders et al., 1989). Special attention should be given to strains effective on marginal soils.
Identification and evaluation of effective VAM-fungi strains is carried out at CIAT and IITA and is a research priority (CIAT, 1988b; IITA, 1988b). The application of cDNA techniques and of biotechnology for conservation of the fungi conservation may be realized on a medium term.

Associations with N-fixing bacteria : Biotechnological improvements may focus on the establishment of associations between Rhizobia bacteria and cassava, and the improvement of natural associations between cassava and free living, N-fixing bacteria (Mantell et al., 1985).

Symbiotic associations between Rhizobia bacteria and cassava are still impossible. The genetic background still needs to be revealed Most bacterial determinants have been identified, but only a few of the many plant genes involved (Dale Noel, 1984; Bisseling et al., 1986). Little is known about the presence of free living, N-fixing bacteria.
At present no research is being carried out on these subjects. Biotechnological improvement in this area may have a long term time-frame. Only the identification of effective free living N-fixers may be realized in the short term.

Impact assessment

Adoption by local farmers : Farmers, with limited access to fertilizers, will benefit from biotechnological improvement in this field; they will readily adopt innovations.

Local research/infrastructure : The identification, production and evaluation of effective VAM-fungi strains and N-fixing bacteria will require intermediate research and infrastructural capacity. Fundamental research on associations with Rhizobia as well as on the efficiency of nutrient use will need highly qualified personnel and high capital inputs.

Associated risks : Cassava varieties with effective VAM-fungi strains and N-fixing bacteria, or with an improved nutrient efficiency, will probably be cultivated on marginal soils. This may cause serious soil erosion and soil exhaustion problems.

Conclusion

Biotechnology provides some improvements such as improved screening techniques for VAM-fungi strains or screening methods at cellular level for nutrient efficiency. In general, however, conventional research offers enough scope for improvement of soil fertility related cassava characteristics. More fundamental research is needed for both conventional and biotechnological improvements.

5.9 Weeds

Conventional research approach

Weed may be controlled by mechanical means, by herbicides or by early closure of the cassava canopy through suitable varieties (Toro and Atlee, 1985). Intercropping with other crops such as leguminous crops may also control the weed growth.
The selection of early closing, vigorously growing varieties may be important for the control of the weeds, but mechanical weeding will still be needed and the correlation to yield characteristics should be emphasized.

Potential for biotechnological improvements

Herbicide resistant cassava varieties may be produced. A proposed strategy is the

isolation of mutant (insensitive) enzymes from bacteria grown in the presence of the herbicide. The gene encoding this enzyme is inserted in the genome of the cassava plant. Transgenic Petunia spp. proved to be resistant to normal agricultural concentrations of herbicides (Kishore, 1987). This strategy could probably be applicated to cassava on a medium term.
No research on herbicide resistance in cassava has been carried out.

Impact assessment

Adoption by local farmers: Small scale farmers with limited access to inputs, rarely use herbicides. Herbicide resistant varieties may not be adopted.

Associated risks: The introduction of herbicide resistance may be transferred to related wild species. The application of chemicals such as herbicides may also pollute the environment.

Conclusion

Resistance to herbicides in cassava may only be realized in the long term. This biotechnological application involves considerable environmental risks. Conventional weed control remains the most suitable method.

5.10 Diseases

Conventional research approach

Conventional research approach has concentrated on the development of resistant varieties, on cultural, biological and chemical control methods and on screening methods.

Varietal resistance : Breeding programmes at IITA and CIAT have developed varieties resistant to AMD, CBB, CAD, Cercospora leaf spots and Superelongation disease (Bellotti et al., 1987). Tolerance levels differ, but some varieties are practically immune to AMD and CBB, e.g. IITA-varieties TMS 30572 and 30211 (Hahn and Theberge, 1987). In general, varietal resistance offers good possibilities to control diseases. However, resistant varieties developed at institutes such as IITA and CIAT need to be used in local breeding programmes for adaptation to local farming systems.
Furthermore, durable resistance has to be established. The continuous resistance of IITA-varieties to AMD and CBB for nearly 15 years and its apparent polygenic nature suggests a durable resistance. Little is known about other diseases (Hahn and Theberge, 1987; Lozano and Bellotti, 1986).

Biological control : Biological disease control has only been recorded for CBB. CBB disease pressure may be reduced by foliar applications of Pseudomonas fluorescens and P. putida strains (Lozano, 1986). Yields of susceptible varieties increased on average 2.7 times in CIAT trials, when Pseudomonas spp. were applied 4 times a month during the

growing season (Hernandez et al., 1986).

Cultural control : Many cultural control methods may be used, depending on local farming systems, type of disease and farmers' ability (Lozano et al., 1985). The use of disease-free planting material is the most important cultural control method. For virus diseases it is, apart from varietal resistance, the only way to reduce disease pressure. Crop rotation or fallowing may also be successful, for example to overcome heavy CBB-infestations (Lozano, 1986). Delaying planting time till the end of the rainy season (with high incidence of CBB, Superelongation, CAD and Cercospora leaf spot) reduces disease pressure (Cock, 1985a).

Chemical control : Chemical control may be effective for fungal diseases. AMD or CBB can not be controlled in this way.

Screening methods : Effective screening methods are extremely important for the selection of disease-free planting material. Field screening for varietal resistance under high disease pressure is generally used as screening method for resistance to CMD, AMD, CBB and CAD (Hahn and Theberge, 1987). Experience in India and Kenya has shown, that careful roguing can result in a drastic reduction of the incidence of AMD (Cock, 1985).
Apparently healthy material may still contain latent bacterial or viral diseases (Robertson, 1987). Secondary selection of healthy sprouts from sprouting stakes has been used in Colombia and Malaysia to produce CBB-free planting material (Cock, 1985a). A combination of heat treatment of cassava plants followed by meristem culture has proven to be effective in eliminating AMD from planting material at IITA (Ng, 1988). This method is now used to distribute AMD-free planting material throughout Africa.

In general, most diseases can effectively be controlled by an integrated use of resistant varieties, biological control and cultural methods. Shortcomings in conventional technology are the adaptation of resistant varieties to local farming systems and adequate screening techniques for planting material.

Potentials for biotechnological improvements

Varietal resistance : Biotechnological improvements are possible whereas virus resistance ('cross protein resistance' and 'anti-sense DNA resistance') and bacterial/fungal resistance ('anti-bacterial proteins') are concerned.
Tobacco and tomato plants, resistant to Tobacco Mosaic Virus, were obtained by the transfer of the TMV-coat protein encoding gene to the plant genome. Beachy et al. (1987) isolated this gene and a transcriptional promotor, transferred these into a strain of Agrobacterium tumefaciens and transformed tobacco and tomato leaf cells. This kind of viral resistance is known as 'coat protein resistance' or 'cross-protection'. Research on many different crops (e.g. potato and alfalfa) has indicated that coat protein resistance can be useful in the control of many different types of plant viruses (Powell et al., 1986; Beachy et al., 1988). Transgenic cassava , expressing the coat proteins of CMD and AMD, may also show resistance to those virus diseases. However, it is not clear whether a long

duration crop as cassava (in which a gradual build-up of virus strains occurs) will show the same kind of resistance as short duration crops.
The in cassava introduction of genes encoding potent anti-microbial proteins derived from insects may significantly augment the level of the crop's resistance to bacterial and fungal diseases. Although none of these genes have been transferred to plant cells yet, this approach offers much scope (Jaynes, 1988). Jaynes and Dodds (1987) have already identified and isolated a large number of broad spectrum anti-microbial proteins from pupa of the giant silk moth (Hyalephora cecropia).
Research on recombinant DNA techniques to improve the disease resistance of cassava is carried out at the Washington University at St. Louis, USA (Dr. R. Beachy), by Monsanto Company at St. Louis, USA and at ORSTOM at Paris, France (Dr. C. Fauquet). Scientists at the CIAT conference in September 1988 estimated, that successful development of coat protein resistance in cassava will be on a medium-term task. The genetic characterization of anti-microbial proteins and the utilization of antisense DNA require more fundamental research. Biotechnological improvement in these fields may be long-term ventures.

Screening methods : Immunological techniques and cDNA techniques can be used.
Immunological techniques (e.g. ELISA) can be efficient and reliable methods for screening viruses and some bacterial diseases. Most often antisera of polyclonal origin are used, but this polyclonal origin poses some difficulties. Thoevenel et al. (1984) found that polyclonal antisera against AMD were insensitive in ELISA tests since as virus levels were very low in cassava leaves. Monoclonal antibodies may solve these problems and may lead to a better control of virus and bacterial diseases (Mantell et al., 1985).
DNA hybridization techniques for screening purposes have been developed. These techniques are very specific and sensitive, and allow the detection of viroids which lack a protein coat and therefore are undetectable by immunological methods. Robinson et al. (1984) showed that in spot hybridization tests DNA-probes of AMD reacted with extracts of plants, which were healthy according to immunological tests. One of the main constraints which retard the application of this technique is the need for radio-active probes for detection. Non radio-active probes should be developed, e.g. chemically labelled probes (Forster et al., 1985; Robinson, 1988). With the introduction of non radio-active probes, DNA hybridization techniques can easily be applied as a routine test in any small laboratory.
Advanced research in this field is carried out at CIAT, IITA and the U.S. Department of Agriculture in Beltsville, USA. Biotechnological improvement of screening methods may be realized in the short-term.

Impact assessment

Adoption by local farmers : The development of disease resistant varieties and of improved screening methods will benefit both small and large farmers. Small farmers will be able to use improved varieties at a larger scale, as improved screening methods should lead to rapid distribution of disease-free material.

Local research and infrastructure : Fundamental research on the identification of resistance genes, as well as on the development of improved screening methods, requires high grade research and capital inputs. When standard procedures for transformation or for screening are available, less advanced laboratories and medium-level research and training facilities are sufficient.

Associated risks : The risks, related to the introduction of transformed cassava plants in the natural environment, have not yet been analyzed. The risk of vertical resistance (e.g. cross protein resistance), which can easily be broken, has to be examined. Multilevel resistances should have priority.

Conclusion

In much recent literature bacterial and virus diseases are still seen as the most serious biotic constraint to the cassava cultivation. Conventional breeding programmes have resulted in cassava parental lines with relatively high resistance to AMD and CBB. The absence of appropriate local research to adapt those parental lines to different ecological conditions is primarily responsible for the remaining disease problems in cassava. The absence of effective screening methods is a problem for adequate disease control. Biotechnological improvement may, however, result in cassava varieties with a multilevel resistance to various diseases. Conventional breeding programmes can only produce such varieties, at a very slow pace. Biotechnology is absolutely necessary in the screening of diseases, and can be realized within a short time.

5.11 Pests

Conventional research approach

Conventional research approach focuses on varietal resistance, biological control and cultural control methods.

Varietal resistance : Screening programmes at IITA, CIAT and EMBRAPA (Empresa Brasileira de Pesquisa Agropecuaria, Brazil) identified mite-resistant cultivars. Resistance mechanisms may differ; one is associated with leaf pubescence (Byrne et al., 1982). However, most of the germplasm accessions identified as resistant are agronomically inferior types (Bellotti et al., 1987). Mealybug-resistant varieties are also found (Hahn, 1983). Very little is known about varietal resistance to cassava hornworm and nematodes (CIAT, 1988b).

Biological control : In Africa cassava mealybug seems nowadays to be effectively controlled by the parasitic wasp Epidinocarsis lopezi. A large biological control programme has been established in Africa, which is very successful (Neuenschwander et al., 1987; IITA, 1988a). Important achievements were the establishment of the wasp in different ecological environments and the rapid distribution of the wasp. A point to consider is whether the wasp will survive if mealybug populations have been reduced

considerably.
Field experiments at CIAT showed, that predatory mites from the family Phytoseiidae can control green spider mite populations. Suitable species have been collected, sent to Africa (IITA) and released into farmers' fields in several African countries (CIAT, 1988a). The results have not yet been evaluated. Effective biological control of the cassava hornworm has been achieved using a virus, Baculovirus erinnyis (Schmitt, 1988). The virus can easily be obtained from dead larvae by mashing them in water and freeze them consequently. Biological control methods for nematodes are being developed, including fungi and bacteria (IITA, 1988b).

Cultural control methods : Many cultural practices can reduce pest problems. These include the distribution of pest-free planting material, the planting of several varieties in a field and crop rotation (Bellotti et al., 1985).

In general it seems possible that the cassava mealybug as well as the cassava hornworm can be controlled by conventional control methods, in particular biological control. Control of the cassava green spider mite is still a problem.

Potential for biotechnological improvements

Biotechnological improvement is only foreseen in varietal resistance.

Varietal resistance : Possibilities exist to create transgenic cassava plants, which are resistant to insects through the expression of insecticidal proteins. At present Bacillus thuringiensis (Peferoen, 1988) is preferred as source of such proteins.
Bacillus thuringiensis produces many different kinds of insecticidal proteins, of which 3 major pathotypes are described : strains toxic to Lepidoptera, Diptera and Coleoptera. Scientists of Plant Genetic Systems NV at Gent (Belgium) have, in collaboration with Advanced Genetic Sciences Inc. in California (USA), isolated a protein-encoding gene from B.t., toxic to the tobacco bud- and hornworms. Transformation experiments resulted in tomato and potato plants resistant to both bud- and hornworm. The cassava hornworm, which is a major pest in Latin America, can be controlled by spraying with B.t. spores. It seems very likely, that certain B.t.-proteins are toxic to the cassava hornworm, thus offering scope for the transformation of cassava plants. B.t.-proteins toxic to mites or mealybugs have not yet been identified (CIAT, 1988b).
Another source of insect resistance is chitinase, an enzyme which is often produced by insect predators. Chitinase attacks the skeleton and gut of the insect, leaving it prey to bacterial infections. Chitinase encoding genes have already been identified and isolated (Jaynes and Dodds, 1987).
Research on novel mechanisms of insect resistance in crops in general is carried out by Plant Genetic Systems in Belgium (M. Peferoen), the Louisiana State University at Baton Rouge, USA (Dr. J.M. Jaynes) and the Washington State University at Pullman, USA (C.A. Ryan). Interest has been shown in applying this research to cassava improvement as well. The transformation of cassava to express B.t.-toxins may on a medium-term undertaking.

Impact assessment

Local research and infrastructure : Fundamental research is needed to identify B.t.-proteins toxic to CGM and CM. Co-operation with the private sector may be possible. Therefore it is difficult to assess research and infrastructural requirements.

Associated risks : The risk is great that this kind of vertical resistance is rapidly broken down.

Conclusion

Conventional research has focused on biological control, especially for the cassava mealybug. Biological control of the mealybug seems to be effective in Africa. The biological control of spidermites is promising, as predatory mites have been identified at CIAT (CIAT, 1988). With this low-cost control method raisis the question whether a high-technology approach is needed. Furthermore breeding programmes produce insect-tolerant varieties (Bellotti et al., 1987). At this moment there is not much reason to give priority to biotechnology in research on pest control.

5.12 Yielding capacity

Conventional research approach

Environmental factors such as temperature, rainfall, solar radiation, soil conditions and cultural practices strongly influence the physiological processes of a cassava plant and, ultimately, its yield (Cock, 1985b). Because of the importance of adaptation of cassava varieties to specific environments, selection for yield should be carried out in those environments (where they will ultimately be cultivated).
For the selection of high yielding cassava varieties the harvest index , the efficiency of storage root production (ESRP) and the initial start of starch accumulation are important indices (Hershey, 1985).
Little information on the genetic control of various physiological parameters is available. Unfavourable conditions may be bypassed by the selection for short duration or earliness.

Potential for biotechnological improvements

Improving the root yield under stress conditions by means of biotechnology at prsent to be unlikely, owing to the polygenic character of this trait.
Very little is known about the genetic regulation of traits that determine the yield, such as C4 characteristics and biomass partitioning.
Application of protoplast fusion or the induction of somaclonal variation can broaden the genetic variability. The results obtained with somaclonal variation depend on a good selection system in an early phase and on the scale of the experiment. Tissue culture could offer possibilities to select some traits in an early phase,the number of tuberous roots for example.

Impact assessment

Adoption by local farmers: Cassava varieties, which offer an acceptable yield, a good yield stability and acceptable secondary qualities will be easily adopted in all farming systems.

Associated risks: The introduction of varieties with high yield capacity under stress conditions may accelerate soil erosion through increased cultivation in marginal areas.

Conclusion

There is much diversity in cassava varieties due to the allo-tetraploid character of the plant. This suggests that with conventional breeding programmes progress can still be achieved, especially for polygenic traits as yield. The introduction of hybrid varieties can improve the yield. Homozygous lines could be obtained with anther culture, after development of successful regeneration protocols.

5.13 Root quality

Conventional research approach

Cassava varieties with low levels of cyanogenic glucosides: The development of cassava varieties with low cyanide levels or acyanogenic varieties is important to reduce the incidence of cyanide toxicity (IITA, 1988). Part of the variation in cyanogenic glucoside content in the cassava roots is genetically controlled (Mahungu, 1987). This genetic variation is also observed in crops like clover (Trifolium sp.), rubber (Hevea brasiliensis) and sorghum (Sorghum bicolor) (Hughes, 1988).
Categories, based on HCN content of the fresh roots, have been distinquished by Bolhuis (1954): innocuous varieties, moderately poisonous varieties and dangerously toxic varieties. About 65 per cent of the cassava varieties developed at IITA can be considered innocuous (IITA, 1981). Low cyanogenic glucoside content is a routine objective in cassava breeding programmes (IITA, 1988c).
Several wild, related species have been found with low cyanide content in the roots. Nassar (1986) described a wild species in Central Brazil, M. oligantha ssp. nesteli, with a low HCN level. Chavez et al. (1988) indicated another species, M. pringlei, containing low HCN levels in the roots. However, no acyanogenic species or varieties have been found. It is supposed, therefore, that the cyanogenic glucoside content of cassava is regulated by a recessive gene complex (Hahn, 1983).
However, even cassava varieties with low cyanide content will need adequate processing (Bourdoux et al., 1983; Rosling, 1987). Little is known about the effect of low cyanogenic glucosides level on crop protection against insects and herbivores as well as on the physiology of cassava (Ayanru and Sharma, 1984).

Screening methods of the cyanogenic glucoside content: A major constraint in the quantitative screening of large numbers of cassava varieties or processed products for

low HCN content was the lack of an effective analytical technique. Sodium picrate tests and enzymatic assays are used nowadays (Mahungu, 1987). An automated enzymatic assay, developed by Rao and Hahn (1984), can handle 300 analyses a day.

Starch content and quality : A high dry matter content is an important criterion for the cassava processing industries as well as for local consumers (Cock, 1985). Starch content of the roots is strongly associated with the dry matter content (Mahungu, 1987). Moderate to high heritability for dry matter content is reported by IITA (1980). However, low genetic improvement is observed in breeding programmes, mainly caused by strong influences of the environment and the maturity of the crop . Cassava varieties with high dry matter percentages of up to 43 per cent, have been observed in Zaire (PRONAM, 1984).
Some research has been carried out on the starch quality (Blanshard, 1988). Little is known, however, about the pre-harvest, environmental and genetic factors that affecting starch quality.

Protein content : Several wild relatives of cassava show more elevated crude protein levels in the roots (up to 7 per cent; Nassar, 1986). Interspecific hybridization of cassava with wild relatives has resulted in cassava varieties with a relatively high protein content in the roots, but when propagated the protein content of these selections fell back to typical levels (Jones, 1959). Polyploid cassava varieties contain more crude protein than diploid varieties (Jos et al., 1972).
An increase in protein content should be accompanied by an increase in essential sulfur amino acids. Crosses with related Manihot spp. may therefore not be the right approach to improve the protein content of cassava (Carneiro and Barreto de Castro, 1988). Furthermore, it is not known if and how the protein content of cassava is correlated with yield levels and fertilizer (N) requirements.
Processing techniques, however, decrease the protein content of roots to very low levels. Increasing the protein content of cassava products by breeding of high root protein containing varieties therefore does not seem to be an appropriate solution to protein malnutrition. Diet diversification (consumption of cassava leaves or beans) or enhancing the protein content of cassava products by fungal fermentation seems to be more promising (Josis et al., 1987).

Other root qualities : At IITA research is carried out on the enhancement of beta-carotene levels in cassava roots (IITA, 1988c). Other root qualities, like flavour, size, colour, and taste are included in present evaluation trials at IITA (Mahungu, 1987).

Potential for Biotechnological improvements

Cassava varieties with low levels of cyanogenic glucosides : Anther culture would allow selection of dihaploid recessive lines, which may be acyanogenic. Genetic manipulation of the synthesis of cyanogenic glucosides may be another approach to reduce cyanide levels in cassava. Fundamental knowledge on the biosynthesis of cyanogenic glucosides and their enzymatic degradation is necessary (Hughes, 1988). Studies on species as clover, which produce the same cyanogenic glucosides as cassava,

are particularly useful (Boersma et al., 1983; Hughes and Ayre, 1976). First isolation and characterization of genes producing key enzymes in the glucosides synthesis must take place. Then anti-sense genes could be produced, whose products would target and inactivate the mRNAs of the genes of the involved enzymes, so that accumulation of cyanogenic glucosides is prevented.
At present research on the genetic and biochemical regulation of the cyanogenic glucosides synthesis in cassava is carried out at the University of Newcastle upon Tyne, United Kingdom (Dr. M. Hughes) and at the Ohio State University, USA (Dr. R. Sayre). Research on the synthesis of cyanogenic glucosides in clover is carried out at the Free University, Department of Ecophysiology, Amsterdam, The Netherlands (Dr. Kakes). Biotechnological improvement in this field may require a long term approach.

Screening methods of the cyanide content : Once mRNAs, governing the production of key enzymes, have been isolated (see above), DNA probes can be developed to screen cassava varieties on their glucoside content (Hughes, 1988). DNA probes would provide a quick and efficient screening method, though not easily applicable in the field.
Biotechnological improvement of screening methods may have a long term time-frame.

Starch content and quality : Biotechnological research on potato shows that improvement of the starch quality by means of anti-sense RNA techniques is possible (Visser, 1989; Jacobsen, 1989; pers. comm.). More fundamental knowledge of the starch synthesis in cassava is necessary.
Research on the starch quality is carried out at CIAT, at the Nottingham University, United Kingdom (Dr. J.M.V. Blanshard) and at the Agricultural University of Wageningen, The Netherlands (Dr. E. Jacobsen and Dr. A. Staritsky).

Protein content : Several strategies are proposed to engineer the cassava plant to produce proteins with a balanced content of essential amino acids (Rao and Singh, 1986):

(1) The expression of nutritionally-rich storage protein genes can be selectively enhanced by manipulation of enzymes involved in the synthetic pathway of proteins. More knowledge about the existence of these genes in cassava is necessary.

(2) Modification of the existing storage protein genes to code for more of the essential amino acids. This can be done by introducing and cloning the gene for a specific protein, inserting extra codons for the deficient amino acids and then introducing the modified gene back into the cassava plant (Goldsbrough et al., 1986). Genetic characterization of specific genes is necessary.

(3) The transfer of genes encoding for nutritionally rich storage proteins from other species into cassava. Patatin is a storage protein, which is specifically located in the tubers of potato. Patatin may be a useful storage protein for cassava (Van Vloten-Doting, 1989; pers. comm.).

(4) The transfer of artificial genes encoding nutritionally rich storage proteins. Synthetic genes have already been constructed and successfully inserted into the genome of potato plants (Jaynes et al., 1986).

Very little is known about the molecular processes that determine the distribution of amino acids and proteins in the plant. It is possible that most of the enhanced protein production will be distributed to the cassava leaves instead of to the roots. Furthermore,

processing methods reduce the protein content of cassava roots considerably. Therefore, improvement of the protein content of cassava roots does not seem to be an appropriate strategy (see 5.15)
Advanced research in this field is carried out at the Louisiana State University, Baton Rouge, USA (Dr. J.M. Jaynes) and at the Washington State University, Pullman, USA (Dr. C. Ryan).
Biotechnological improvement of the protein content may be realized on a medium term, when the transfer of artificial genes or of storage proteins as patatin are concerned, but may otherwise have a long term time-frame.

Impact assessment

Adoption by local farmers : Though low cyanogenic varieties are often preferred for human health reasons, acyanogenic cassava varieties may in some cases not easily be adopted by farmers, who cultivate cassava as a food crop. Bitter varieties are still preferred in many regions, e.g. for their taste or storability.

Local research and infrastructure : Recombinant DNA techniques are suggested for the improvement of starch quality and protein content of cassava. Application of these techniques, accompanied by fundamental research, requires a high level of research and a well developed infrastructure. The application of anther culture for the production of acyanogenic varieties requires an intermediate level of research and infrastructural capacity.

Associated risks : Acyanogenic cassava varieties may be more suceptible to pest and disease.

Conclusion

Conventional breeding offers scope to reduce the cyanide content of cassava varieties. Acyanogenic varieties, however, are not found in conventional breeding programmes. Apomixis may provide a solution, but for the production of acyanogenic varieties biotechnology offers more scope.
Conventional research as well as biotechnology only offer perspectives for the improvement of starch quality, when more knowledge is available about the genetic regulation of starch quality.
Conventional research approach offers hardly any perspective to improve the protein content of cassava roots. Biotechnological improvement may be achieved in a relatively short period when certain techniques are considered. Improvement of the protein content of the cassava roots, however, does not seem to to be an appropriate strategy.

5.14 Storage of cassava roots

Conventional research approach

Storage of fresh cassava: Fresh cassava roots have in a 2-3 week shelf life if they are treated with fungicide and kept in perforated plastic bags. This method has been developed at CIAT. The cassava roots .

Storage of dried cassava: Limited research is carried out on the storage of dried cassava roots. Proper sanitary measures, such as cleaning and disinfecting warehouses prior to restocking and rapid removal of infested material, are the most effective control measures (Bellotti et al., 1985).

Potential for Biotechnological improvements

Storage of fresh cassava: Physiological deterioration is enzymatically mediated. For the application of recombinant DNA technology (e.g. inactivate specific enzymes in the synthetic pathways with antisense DNA techniques or the incorporation of a gene encoding for linamarase activity) more knowledge about the biochemical regulation is needed (CIAT, 1988b). Biotechnological improvements may be realized on a long term basis.
Current efforts on basic research of root deterioration of cassava is carried out at ODNRI, U.K. (Dr. R. Cooke) and at the University of Nottingham (Prof. J.M.V. Blanshard). Both institutes are working in cooperation with CIAT.

Impact assessment

Adoption by local farmers: The introduction of new varieties which can be preserved for longer periods are especially important to farmers who are producing fresh cassava for the local market.

Local research and extension capacity: Before biotechnology can be applied, more basic knowledge about root deterioration is essential. This requires a high level of research and infrastructural capacity.

Conclusion

Fundamental knowledge is insufficient to enable root deterioration to be tachled by biotechnology. The storage of cassava has to be improved by means of conventional methods.

5.15 Processing

Conventional research approach

Conventional research has focused on the inventarization and analysis of small-scale, locally developed processing methods as well as large-scale, industrial methods. Much information is available on starch extraction, alcohol production and chips and pellet production. However, in this study attention is paid to the reduction of cyanide content, limiting protein loss at household level, and to bread making.

Protein content : The effects of various processing methods on the protein content of cassava products were examined by Favier et al. (1971). Peeling depressed the protein content most, 57 per cent, whereas subsequent soaking and pressing reduced the protein content by another 30 per cent. Since peeling accounts for most protein loss, selection of cassava varieties with more protein in the edible part of the root and less in the peel would be valuable.

Cyanogenic glucosides : Processing methods, which involve a sufficient long fermentation phase, reduce the cyanide level of cassava products to non-toxic levels (Bourdoux et al., 1982). Problems occur, however, when fermentation is not possible because of water shortage or food shortage (no time available for sufficient fermentation).
Under food shortage conditions a quick detoxification method should be available. Essers (1986) developed a 24 hours processing method to reduce cyanide content to non-toxic levels. Cassava tubers are grated, sieved and sun-dried, using a very laborious process. The method is not yet practiced.

Bread production : It is possible to bake bread, with a good taste and texture, from cassava flour without using wheat flour (Satin, 1989). A little amount of the cassava flour is cooked, which results in a gelatinous substance with raising properties. The baking process has to be monitored cautiously, in particular the temperature.

Potential for biotechnological improvements

Protein content : The protein content of cassava products can be improved by fungal fermentation. Fungal fermentation with the use of Rhizopus oryzae results, with the addition of salts as nutritive source , results in protein enrichments of up to 11 per cent of digestible protein (Daubresse et al., 1987). Different strains of fungi have been tested (Silvestre and Arraudeau, 1983). Interest focuses on R. oryzae as this fungus is frequently isolated from fermented cassava and is not known to cause toxicity problems. Experiments at village level with non-sterile fermenters are carried out in Burundi (Josis et al., 1987).

Cyanogenic glucoside content : A fermentation technique without using water, includes the growing of moulds on fresh cassava roots (Essers, 1986). Freshly peeled cassava roots are covered with banana leaves for a couple of days. Moulds develop on the roots and partly ferment the cassava. After sun drying the cyanide level appears to be

reduced to acceptable levels (Essers, 1986). More research is needed on the possible formation of aflatoxins.

Alcohol and high fructose syrup production : The distillation of alcohol from the fermentation liquor requires large amounts of energy. Alcohol tolerant yeasts can increase the alcohol concentration of the fermentation liquor from 6 to 12 per cent, which would reduce the amount of energy necessary for alcohol distillation by 30 per cent (Cock, 1985). High-fructose syrups are produced from cassava starch by means of microbial enzymes. Factories for making fructose syrup have been established in Indonesia (Cock, 1985).

Impact assessment

Adoption by local farmers : The low technology improvements mentioned above will be easily adopted. Biotechnological improvement of alcohol production and fructose syrup may be of indirect interest to farmers producing cassava for the starch industry.

Research and infrastructure : Medium to low levels of research and infrastructure are required for these improvements.

Associated risks : The production of large amounts of waste water by starch extraction factories may create pollution problems. Attention must be paid to microbial processing of waste water.

Conclusion

Biotechnology offers large scope for improvement of cassava processing methods. Although the development of biotechnological applications may require medium levels of research and infrastructure, most applications are relatively simple techniques. Fungi technology can be used at village level. Conventional research approach in both areas also offers scope for improvements and should be used in combination with biotechnology.

5.16 Summary table

In table 7 the perspectives for conventional and biotechnological research approaches and the time-frame for each biotechnological improvement are reviewed. Perspectives for conventional and biotechnological research are indicated by + (perspectives) or - (no perspectives). The time-frame of biotechnological improvements is indicated, if perspectives are positive. A summary of the socio-economic impact assessment is presented in table 8.

In this table the expected adoption of biotechnological improvements by local farmers in general, the research and infrastructural requirements for biotechnological research in a specific area and risks associated with biotechnological applications are indicated.

Table 7: Perspectives for conventional and biotechnological research approaches for each area of improvement

	Perspectives for conventional research	**Perspectives for biotechnological improvements**	**Time-frame for biotechnological improvements**
Areas of improvement			
1) Propagation:			
micropropagation	+	+	short term
true seed propagation	-	+	medium term
2) Germplasm storage:	-	+	short term
3) Plant breeding techniques:			
Flowering capacity	-	+	short term
Hybrid production	-	+	medium term
Hybridization techniques	+	+	medium term
4) Plant morphology:			
Leaf production	+	-	-
Plant architecture	+	-	-
C4-crop morphology	-	+	long term
5) Plant physiology:			
Biomass partitioning efficiency	+	+	long term
Photosynthetic capacity	+	+	long term
6) Drought tolerance:			
- Selection of early maturing varieties	+	-	-
- Improvement of water use efficiency	+	+	long term
7) Soil fertility:			
Nutrient efficiency	+	+	medium term
N-fixing bacteria	+	+	medium term
VAM-fungi	+	+	medium term
8) Weeds:			
Herbicide resistance	-	+	long term
9) Diseases:			
AMD and CMD	+	+	medium term
CBB	+	+	long term
Screening methods	-	+	short term
10) Pests:			
Green/red spidermite	+	+	long term
Mealybug	+	+	long term
Cassava hornworm	+	+	medium term
11) Yielding capacity:	+	+	long term
12) Root quality:			
Cyanogenic glucoside content	+	+	long term
Starch quality and content	+	+	long term
Protein content	+	+	medium term
13) Storage of cassava roots:			
storage of fresh cassava	+	+	long term
storage of dried cassava	+	-	-
15) Processing:			
Protein content	+	+	short term
Cyanogenic glucoside content	+	+	short term
Bread production	+	-	-
Alcohol and syrups	-	+	short term

Table 8: Impact assessment for biotechnological application in each area of improvement

	Expected adoption by local farmers	Research and infrastructural requirements	Associated risks
Areas of improvement			
1) Propagation:			
micropropagation	+	low	-
true seed propagation	-	medium	-
2) Germplasm storage:	n.a.	low	-
3) Plant breeding techniques:			
Flowering capacity	n.a.	low	-
Hybrid production	+	medium	genetic erosion
Hybridization techniques	n.a.	medium to high	-
4) Plant morphology:			
Leaf production	+	high	-
Plant architecture	+	high	-
C4-crop morphology	-	high	-
5) Plant physiology:			
Biomass partitioning efficiency	+	high	exhaustion
Photosynthetic capacity	+	high	exhaustion
6) Drought tolerance:			
- Selection of early maturing varieties	+	high	exhaustion
- Improvement of water use efficiency	+	medium to high	soil erosion
7) Soil fertility:			
Nutrient efficiency	+	medium to high	exhaustion
N-fixing bacteria	+	medium	-
VAM-fungi	+	medium	exhaustion
8) Weeds:			
Herbicide resistance	-	medium	pollution
9) Diseases:			
AMD and CMD	+	medium	break-through
CBB	+	high	break-through
Screening methods	n.a.	medium	-
10) Pests:			
Green/red spidermite	+	medium to high	break-through
Mealybug	+	medium to high	break-through
Cassava hornworm	+	medium	break-through
11) Yielding capacity:	+	high	exhaustion
12) Root quality:			
Cyanogenic glucoside content	+	high	disease pressure
Starch quality and content	-	medium to high	-
Protein content	+	medium to high	increase N-uptake
13) Storage of cassava roots:			
storage of fresh cassava	+	high	-
storage of dried cassava	+	high	-
15) Processing:			
Protein content	+	low	-
Cyanogenic glucoside content	+	low	-
Bread production	+	low	-
Alcohol and syrups	+	medium	waste production

n.a. = not applicable

6. ASSESSMENT OF PRIORITIES

6.1 Criteria

Following the extensive analysis of biotechnological improvements in cassava production and processing, as presented in the preceding chapter, it is very important to assess those improvements which should receive priority in biotechnological research programmes. The process of setting priorities, used in this study, is rather similar to the one used in the Rockefeller Foundation's 'International Programme on Rice Biotechnology' (Toenniessen and Herdt, 1988).

The process starts with a listing of all mentioned in chapter 4 areas of improvement in cassava production and processing. Each area of improvement receives an estimate of importance, using several criteria. For the introduction of biotechnology in cassava production and processing, the technical feasibility of certain biotechnological applications, but also the socio-economic impact on local farming systems have to be analyzed. In most studies on biotechnology the socio-economic the socio-economic impact assessment is neglected wihout any reason.
In this study the following technical criteria are selected: (1) the necessary time-frame for specific biotechnological improvements, (2) the relative contribution of biotechnology compared to conventional research approach. For the assessment of the socio-economic impact the following criteria are selected: (3) the impact of biotechnological improvements on the situation of small-scale farmers, (4) the capital input and research capacity required and (5) the potential risks associated with biotechnological applications.
The introduction of biotechnology is often compared with the "green revolution"(Buttel et al., 1985; Wolf, 1987). The basis of this green revolution has been the production of improved seeds which yields considerably more than most local varieties. However, high inputs of fertilizer, pesticides and water were often required to achieve those high yields. Many small-scale farmers hardly benefitted from these impacts, as they did not have access to the necessary inputs. It should be avoided that the introduction of biotechnology will have similar effects on the position of small-scale farmers.

A system of 'weights' is associated with each criterion, expressing the relevance or necessity of a certain biotechnological improvement according to that criterion. Thus, for example, the time-frame for specific biotechnological improvements is characterized by 3 categories, namely short-term, medium-term and long-term applications. The 'weight' of each category is indicated by none, 1 or 2 asterisks (-,*,**), ranging respectively from not preferred (-) to preferred (**).

The different selected criteria, with their 'weights', are presented in the following table.

Table 9: Selected criteria for priority assessment

Weights:	-	*	**
Criterion:			
Time-frame of biotechnological improvement (1)	long-term	medium-term	short-term
Relative contribution of biotechnology (2)	low	moderate	high
Impact on small-scale farming systems (3)	negative	positeve (non-specific)	positive (specific)
Required capital input and research capacity (4)	high	medium	low
Potential associated risks (5)	many	few	none

Time-frame : Biotechnological improvements with short-term applications are preferred, whereas long-term applications have less priority. Biotechnological improvements with long-term application often include polygenic determined traits and highly advanced techniques.

Relative contribution : Biotechnological improvement of traits, that are relatively difficult to improve by means of conventional research, are given priority. In particular the introduction of foreign genetic material in the cassava plant falls within this category.

Impact on small-scale farming systems : Biotechnological improvements that have a positive effect on small-scale farming systems (subsistence) in particular, are preferred. Biotechnological improvements that have a positive impact on all farming system types, receive less priority.

Research and capital input requirements : Biotechnological improvements, which require little capital input and research capacity, receive high priority. Low technology improvements, easily applicable under field conditions, fall within this category.

Associated risks : Biotechnological improvements, which may entail many risks, are given low priority.

6.2 Assessment

The main constraints in cassava production and processing, as well as a weighing of the above mentioned criteria, are presented in table 10.

Table 10 : Comparison of areas of improvement using several criteria

Constraint:	Criteria: time-frame	relative contribution of biotechnology	impact on small-scale farming systems	required capital input and research capacity	potential associated risks	T
Propagation:						
Micropropagation	**	*	*	**	**	8
True seed	*	**	-	*	**	6
Germplasm storage:	**	**	*	**	**	9
Breeding techniques:						
Flowering capacity	**	**	*	**	**	9
Hybrid production	*	**	*	*	*	6
Interspecific crossings	*	*	*	*	**	6
Plant morphology:						
Leaf production	-	-	**	-	**	4
Plant architecture	-	-	**	-	**	4
C4-crop morphology	-	**	*	-	*	4
Plant physiology:						
Photosynthetic capacity	-	*	*	-	*	3
Efficient root storage	-	*	*	-	*	3
Drought tolerance:						
Early maturity	-	-	**	-	*	3
Water use efficiency	-	*	**	*	*	5
Soil fertility:						
VAM-fungi	*	*	**	*	**	7
N-fixing bacteria	*	*	**	*	**	7
Nutrient efficiency	*	*	*	*	*	5
Herbicide resistance:	-	**	-	*	-	3
Diseases:						
AMD and CMD	*	-	**	*	*	5
CBB	-	-	**	-	*	3
Screening techniques	**	**	*	*	**	8
Pests:						
Green/ red spidermite	-	*	**	*	*	5
Mealybug	-	-	**	*	*	4
Cassava hornworm	*	-	**	*	*	5
Yielding capacity:	-	*	*	-	*	3
Root quality:						
Cyanogenic glucoside	-	*	**	-	-	3
Starch quality	-	*	*	-	**	4
Protein quality	*	*	*	-	*	4
Storage of cassava:						
Fresh cassava	-	*	**	-	**	5
Dried cassava	-	-	**	-	**	4
Processing:						
Cyanogenic glucoside	**	*	**	**	**	9
Protein content	**	*	**	**	*	9
Alcohol and syrups	**	**	-	**	*	7

T = number of asterisks scored for all five criteria

The research topics in the table can graded in three groups of decreasing priority for biotechnology:

(1) **Areas of improvement with high priority for biotechnological improvement according to most criteria (7 to 9 asterisks)**: selection and identification of VAM-fungi and N-fixing bacteria (serological techniques), improvement of germplasm storage, flowering capacity, and micropropagation (plant tissue culture), improvement of screening techniques for diseases (cDNA probes) and processing methods, which reduce the cyanogenic glucoside content, enhance the protein content of cassava products (use of fungi) or include alcohol production. Biotechnological research focusing on these areas of improvement is given first priority.

(2) **Areas of improvement with high priority for biotechnological improvement according to some criteria, but with low priority to others (5 to 6 asterisks)**: selection of drought tolerant cassava varieties (with improved water use efficiency) and varieties with high nutrient efficiency (in vitro selection), hybrid production and interspecific crossings (anther culture and protoplast fusion), utilization of true seed (anther culture) and improved varietal resistance to viral diseases (cross protein resistance) and to cassava hornworm and red and green spidermites (B.t. resistance), and improved storage methods of fresh cassava. In general biotechnology is recommended to overcome these constraints, but only after careful assessment of potential outcomes and impacts.

(3) **Areas of improvement with low priority for biotechnological improvement (1 to 4 asterisks)**: selection for early maturity (drought tolerance), improvement of leaf production, plant architecture and C4-crop morphological characteristics, improvement of photosynthetic capacity and efficiency of storage root production, herbicide resistance, improvement of varietal resistance to CBB and cassava mealybug, improvement of yield capacity and several root quality factors. In general more fundamental research is needed to reveal the often polygenic characterization of these traits or to implement new, advanced techniques. Sometimes conventional research methods offer more scope for crop improvement.

6.3 Weaknesses

In preparation for the ACRN-conference at CIAT in September 1988, CIAT as well as IITA made a priority list of research problems in cassava production and processing (CIAT, 1988). The selected priority constraints represented areas, where conventional research held limited promise and biotechnology would be helpful in solving problems (see annex 4). In fact only one criterion, the relative contribution of biotechnology compared to conventional research approach, was used for this priority assessment. Although our approach yields similar results for some topics such as, the high priority for improvement of screening methods for virus diseases, the development of true seed and the improvement of processing methods, the use of other, supplementary criteria results in a more 'balanced' priority assessment.

It should, however, be recognized, that this kind of priority setting, thus shows several important weaknesses :

(1) The selection of criteria is very subjective. Associated risks as well as the position of small-scale farmers are considered as important criteria in this study, but may be less important in the eyes of other authors.

(2) Considering all criteria to be of equal importance is an arbitrary point of view. Different weights may result in a totally different setting of priorities.

(3) The relative importance of biotechnology compared to conventional research methods may change. When fundamental research reveals more of the crop's characteristics or when new biotechnologies evolve, this relative importance will change.

(4) Biotechnological improvements are not evaluated in relationship to one another in this priority assessment. Potential negative or positive correlations between different areas of improvement should also be incorporated.

(5) There is a lack of reliable statistics on cassava in general. Furthermore, large regional differences in cassava production and processing techniques exist. Selected areas of improvement will sometimes only reflect regional concerns (e.g. cassava hornworm is a constraint only in Latin America). These aspects all hamper a thorough and final priority assessment.

7. CONCLUSIONS AND DISCUSSION

7.1 Conclusions

The main conclusions of the study are the following:

i) With respect to current cassava production and processing the main areas for improvement are:
   1) development of propagation techniques (such as micropropagation and true seed propagation)
   2) improvement of propagation techniques for germplasm banks
   3) shortening breeding programmes and improvement of hybridization techniques
   4) improvement morphological traits such as leaf production and C4-crop characteristics
   5) improvement of physiological traits such as biomass partitioning, cyanide content of cassava roots and the photosynthetic capacity
   6) drought tolerance aspects such as high yield capacity under drought conditions, early maturing varieties and the improvement of the water efficiency
   7) improvement of soil fertility aspects such as the efficiency of nutrient use, mycorrhizal-fungi associations for P-uptake an associations with N-fixing bacteria
   8) introduction of improved cultural practices for weed control
   9) improvement of varietal resistance to mealybug, green spidermite, AMD and CBB and extension of fundamental knowledge on resistance mechanisms
   10) introduction of biological control methods for the major pests
   11) improvement of screening methods for viral and bacterial diseases
   12) development of varieties with high yield potential
   13) improvement of root quality aspects such as toxicity, starch quality and protein content of cassava roots
   14) prolongation of the storage period of fresh cassava roots by inhibition of physiological deterioration processes and improvement of control methods for storage pests
   15) improvement of processing techniques for the reduction of cyanogenic glucoside content (such as fungal fermentation) and for alcohol production, and improvement of baking techniques with cassava flour

ii) Biotechnological contributions to cassava research provide perspectives to tackle some of the constraints in cassava production and processing, such as reduction of

cyanide content, improvement of the protein content, increased varietal resistance to pest and diseases and improved screening techniques for viral and bacterial diseases.

The main limitation for the application of several protoplast and recombinant techniques to cassava is establishing regeneration from callus. Research in these areas should be focused on obtaining a proper regeneration protocol from callus. This protocol is a prerequisite for the development of true seed propagation, hybrid production, interspecific crossings and disease resistant cassava.

iii) Areas where improvements are technically feasible and of considerable socio-economic importance are given first priority. Priority areas (where biotechnological research should be initiated or further developed) are improvement of associations with VAM-fungi and N-fixing bacteria, improvement of plant breeding techniques, improvement of screening methods for diseases (for breeding programmes and quarantine purposes) and the improvement of processing methods.

7.2 Discussion

perspectives for biotechnology to cassava

Cassava's yield potential is severely reduced under farmer conditions , because of labour shortage, deficient infrastructure, unfavourable price relations and lack of inputs. While there is considerable scope for the application of biotechnology in specific fields, its overall contribution must not be exaggerated. Furthermore, improved cassava yields and increases in cassava area may enhance farmers' dependence on the crop and might have negative ecological effects. Biotechnological as well as conventional research on cassava should always be integrated in an overall agricultural and rural development programme.

priority assessment

It should be emphasized, that the time-frame for biotechnological improvement of cassava is largely determined by current state of the art in research on cassava regeneration and transformation. Up till now no definite success has been reported with regeneration of cassava plantlets from callus tissues. Recombinant DNA techniques as well as protoplast fusion techniques are therefore still not applicable in cassava improvement programmes, which reduces the possibilities for many biotechnological methods. Low-technology applications such as plant tissue culture for propagation and germplasm storage or the use of fungi in cassava processing, may however have a large impact on cassava improvement.

In most studies the technical feasibility of biotechnological improvements are identified. The assessment of the socio-economic impact is however of importance too. For the assessment of priorities criteria such as the impact of biotechnology on the situation of small-scale farmers, the capital input and research capacity and the potential risks associated with biotechnological applications should not be overlooked.

Furthermore a fair comparison between conventional and biotechnological strategies is very difficult to establish. The lack of varieties resistant to AMD, for example, is often seen as a solid ground for the introduction of cross protein resistance to AMD into cassava varieties (CIAT, 1988b). Reports of different research institutes state, however, that highly AMD-resistant varieties are available at experimental stations, but that these varieties are not adapted to local ecological conditions (IITA, 1988b). Lack of production of locally adapted and resistant varieties by local breeding programmes is therefore the main bottleneck in the development of resistance to AMD. Conventional research methods offer in this case more scope for improvement than the application of biotechnology.

As cassava is grown mainly as a subsistence food crop in small-scale farming systems, biotechnological improvements should in the first place be directed towards the needs of these small-scale farming systems. It is very difficult to assess the impact of different biotechnological improvements on the position of small-scale farmers. In fact much socio-economic research has to be carried out before adequate conclusions can be drawn. More statistical information should also be gathered on the growing importance of processed cassava products on local markets.

research capacity

Fundamental research on new biotechnological applications, for example on recombinant DNA techniques, are supposed to require high capital and research inputs as well as a good infrastructure. Plant tissue culture labs may be relatively cheap, but scaling up for production is very costly. Recombinant DNA facilities entail large investments, and creating the infrastructure for large-scale production will be considerably more expensive (Kenney et al., 1983).The question is then raised as to where this kind of research should be carried out: on central, well-equipped research institutes as CIAT and IITA, or also at national institutes and universities? Whenever fundamental research has resulted in standard protocols, the costs of such biotechnological applications decrease singificantly, making them available at any level of research. It will be clear that aneffective, international cassava research network is absolutely necessary.

For the local institute of developing countries it is essential to frequently exchange personnel and to cooperate with other more advanced laboratories and to attend trainee courses

associated risks

Too little attention is given to the potential risks associated with biotechnological applications. Biotechnological improvement may bring known problems along with it, like erosion problems on drought-prone soils when improved, drought tolerant cassava varieties are introduced, but they also entail new, unknown problems as potential risks related to the introduction of transgenic plants. A continious monitoring of transgenic plants in the field should be assessed to reduce environmental risks. The need for

ecological research in this area is felt by many authors (e.g. Fowler et al., 1988; Sarink, 1989; Boon and Bunders, 1987).

Biotechnology is still hardly used in cassava research programmes. An international network on advanced techniques in cassava research has just been established. Problems with patents and proprietary information have not yet materialised, as there is still rather little interest for cassava in the industrial world. Furthermore, most of the advanced research on cassava is carried out at independent, international institutes as CIAT and IITA. These conditions offer the possibility to develop an appropriate cassava development programme, focused on the needs of farmers as well as the cassava industry, but without neglecting the needs of local, small-scale farming systems. Biotechnological applications, which are appropriate in a social and cultural context and which match with local ecological diversity, can be developed. At present a careful and balanced assessment of research priorities in biotechnological research on cassava should take place, to avoid a negative impact on small-scale farming systems.

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ABBREVIATIONS

AMD	African Mosaic Disease
CBB	Cassava Bacterial Blight
CIAT	International Centre for Tropical Agriculture
CM	Cassava Mealybug
CGM	Cassava green spidermite
CMD	Common Mosaic Disease
DAS-ELISA	'Double Antibody Sandwich'-ELISA
DGIS	Directorate-General for International Cooperation-the Netherlands 'Ministry of Foreign Affairs
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ESRP	Efficiency of Storage Root Production
FAO	Food and Agriculture Organization of the United Nations.
IBPGR	International Board for Plant Genetic Resources
IFPRI	International Food Policy Research Institute
IIRI	International Rice Research Institute
IITA	International Institute of Tropical Agriculture
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
VAM-fungi	Vesicular-Arbuscular Mycorrhizal fungi

GLOSSARY

adventitious: Adjective used to describe organs developing from positions on the plant from which they would not normally be derived, e.g. shoots from roots, leaves or callus and embryos from any cell other than a zygote.

anther culture: Tissue culture of pollen, resulting in haploid plants

antibody: Specific protein produced by the immune system of higher animals and humans as part of the immune response to the presence of a specific antigen

antigen: Substance or well defined part of a substance which is recognized and bound by a matching antibody

antisense RNA: is exactly the complement of the DNA sequence of a specific gene, it impedes the expression of that gene by hybridization.

apomixis: reproduction which has superficial appearance of ordinary sexual cycle, but actually occurs without fertilization and or/meiosis

Bacillus thuringiensis (Bt): A common soil bacterium which produces a protein toxic to insects. The bacterium or its toxin can be used as a biopesticide. It is also possible to transfer the gene encoding for the toxin to the genome of a plant. The genetically engineered plant could subsequently produce its own pesticide.

biotechnology: Development of products by exploiting biological processes or substances. Production may be carried out by using intact original or modified organisms, such as yeasts and bacteria, or by using active cell components, such as enzymes from organisms.

callus: A cluster of undifferentiated plant cells that can, in some species be induced to form the whole plant.

cell: The smallest structural unit of living organisms that is able to grow and reproduce.

cDNA complementary DNA obtained by copying mRNA transcripts into DNA

clone: A group of cells or organisms that are descended from only one ancestor

cryopreservation: Conservation of genetic material by freezing it at very low temperatures

Deoxyribonucleic acid (DNA): The molecule that carries the genetic information for almost all organisms. The DNA molecule consists of a long succession of nucleotides (see nucleotide), the sequence of whose base-components is the actual carrier of the genetic information. At times when the DNA is not being used in an organism it exists as the so-called double helix- two intertwined strands of DNA linked together through the base pairs.

embryo rescue: When cross-pollination occurs between genetically different plants, the resulting embryo may ba aborted because of parental mutual incompatibility. Such embryos may be extracted and grown on an appropriate medium

embryogenesis: A pathway of differentiation which is characterized by the formation of organized structures that resemble zygotic embryos.

embryoids: Embryo-like structures produced as a consequence of differentiation

processes such as embryogenesis and androgenesis.

enzyme: A protein catalyst that facilitates a specific biochemical reaction necessary for a function of an organism

fermentation: Processing of food or other mixtures by micro-organisms or cultured cells or by enzymes derived from them.

gene: A segment of DNA carrying, due to its base sequence, a very specific information. Some genes carry the information for the synthesis of proteins (structural genes), others carry information for regulatory functions (regulatory genes)

gene characterization: (or gene mapping) In classical genetics this means only the determination of the relative locations of genes on a chromosome. In biochemical work it is used for localizing any isolated piece of DNA on the chromosome, even the function of the DNA piece is not known.

gene transfer: A method in which an isolated piece of DNA is inserted into the genome of a host cell.

genome: The entirety of genetic material of a cell.

germplasm: Often synonymous which 'genetic material' it is the name given to seed or other material from which plants are propagated

haploid: Having a single set of unpaired chromosomes in each nucleus. Characteristic of gametes.

herbicide: Chemical used to control weeds

herbicide tolerance (HT): Ability of plants to tolerate herbicides.

heterokaryon: A cell in which two or more nuclei of unlike genetic make-up are present.

hybrid: The offspring genetically dissimilar parents (e.g., a new variety of plant or animal results from cross-breeding tow different existing varieties, a cell derived from two different cultured cell lines that have fused).

hybridization: The act or process of producing hybrids.

immunoassay: A method of making the connection between antibody and protein visible. A marker is linked to the antibodies; this may be an enzyme (enzyme immunoassay) or a radioactive substance (radioimmunoassay).

in vitro: Literally 'in glass' (Latin), meaning in a laboratory container or apparatus under laboratory conditions as opposed to 'in vivo'. Both refer to biochemical experiments and methods.

meristem: Localized region of active cell division in plants from which permanent tissue is derived.

micro-organism: Any organism that can be seen only with the aid of a microscope. Also called microbe.

monoclonal antibodies: Highly specific antibodies derived from a single clone of an antibody-producing cell, therefore exactly the same type. They specifically recognize only one site of the antigen.

mutagenesis: The induction of a inheritable physical change in the genome of the cell. The change can occur spontaneously; in the course of germ cell maturation; due to the action of a chemical or radiation; or other influences on the organism.

nitrogen fixation: A biological process which involves the binding of nitrogen in the air to form ammonia, which is needed as a nutrient since it is essential for the building of all proteins and DNA. Some plants (e.g. leguminous plants) form a symbiosis with nitrogen fixing micro-organisms and thus receive their nitrogen requirement directly

from them. (The plants themselves are not able to fix nitrogen.)

nucleotide: A DNA unit consisting of one of the four organic bases-adenine, guanine, cytosine and thymine- a sugar and a phosphate.

plasmid: An extrachromosomal, self replicating, circular segment of DNA; plasmids (and some viruses) are used as 'vectors' for cloning DNA in bacterial 'host cells'.

polygenic: Deriving from more than one gene.

probe: A radiolabelled nucleic acid molecule used to detect the presence of its complementary strand by hybridization.

promoter: Region of the DNA which is recognized by RNA polymerase in order to initiate transcription.

protein: A molecule composed of amino acids. Proteins are ubiquitous and most abundant in all organisms. They serve many crucial purposes in an organism, e.g. as enzymes to catalyse all the reactions, as structural proteins to support the organism, as transport proteins transporting nutrients to all parts of the organisms, etc.

protoplast: A plant cell from which the cell wall has been removed by mechanical or enzymatic means.

recombinant DNA (rDNA): A strand of DNA synthesized in the laboratory by spicing together selected parts of DNA strands from different organic species or by adding a selected part to an existing DNA strand.

recombinant DNA technology: The use of recombinant DNA for a specific purpose, such as the formation of a product or the study of a gene.

regeneration: The cultivation of a whole plant from a single cell.

Restriction Fragment Length Polymorphism: DNA chains are specifically cut into small fragments which can then be separated on a gel.

Rhizobia: Family of soil bacteria. They can form symbiosis with leguminous plants little nodules are developed at the roots of the plants. When in symbiosis they are capable of nitrogen fixation, which also benefits the plant.

Ri-plasmid: Root-inducing plasmid of Agrobacterium rhizogenes.

single cell protein: cells, or protein extracts, of micro-organisms grown in large quantities for use as human or animal protein supplement.

somaclonal variation: The natural occurring genetic variation that appears in plants regenerated from somatic cells grown in tissue culture: in vitro methods of propagating cells from animals or plant tissue.

somatic: Referring to the vegetative or non-sexual stages of a life cycle.

somatic embryogenesis: Induction-via hormones- of the development of an embryo out of somatic cell cluster.

Ti-plasmid: Tumour-inducing plasmid of Agrobacterium tumefaciens

tissue culture of higher plants: The culturing of plants, seeds, embryos, tissues, cells or protoplasts on artificial nutrient mediums under sterile conditions.

transgenic organism: A genetically manipulated organism containing in its genome one or more inserted genes of another species.

transcription: Copying of a gene into RNA by a DNA-dependent RNA polymerase.

translation: Copying of mRNA into protein.

ANNEX 1

List of interviewed experts:

ir S. Essers, Scientist at the department of nutrition technology
Experience: processing techniques of cassava and toxicity problems

dr. ir. J.J. Hardon, Director of the Centre for Genetic Resources
Experience: research on collection, management and use of genetic resources

prof. dr. B. de Groot, Experience: the development of biotechnology in Indonesia with cassava as model crop

prof. dr. E. Jacobsen, Professor at the department of Plant Breeding, agricultural university Wageningen
Experience: the development of new breeding procedures with the application of cell and molecular biological methods

dr. L. Vloten-Doting, Director of the research institute ITAL, Wageningen
Experience: the use of genetic manipulation of plants and micro-organisms for plant breeding and crop protection

ANNEX 2

Deatailed description of cassava diseases and pests:

Cassava diseases

Key diseases

1. Cassava Bacterial Blight (CBB) Xanthomonas campestris pv. manihotis.

Kind : Bacterial disease

Losses : Up to 100 per cent (Lozano, 1986). The disease is now of minor importance as the result of integrated control measures.

Distribution : Widespread distribution, but especially in areas with large T-fluctuations and on poor sandy soils.

Characteristics : Primary symptoms are wilting of the young germinated sprouts shortly followed by die-back. Secondary symptoms are angular leaf spotting, followed by blight, defoliation and die-back. The pathogen is spread by infected cuttings and insects.

Control methods : Disease-free planting material, varietal resistance, pruning, delay of planting time till end of the rainy season and biological control with Pseudomonas spp.(Lozano et al., 1985).

2. African Mosaic Disease (AMD)

Kind : Viral disease

Losses : Up to 90 per cent (Hahn et al., 1980). Although resistant varieties have been developed at IITA, ACMD is still important under local circumstances with susceptible varieties.

Distribution : Africa, Madagascar, India.

Characteristics : Symptoms are a mosaic pettern on the leaves and leaf distortion. The disease is disseminated by whiteflies
(Bemisia spp.) and infected cuttings (Hahn and Theberge, 1987).

Control methods : clean planting material (meristem cultures and careful roguing) and

resistant varieties.

3. Cassava Anthracnose Disease (CAD) Colletotrichum gloeosporioides pv. manihotis.

Kind : Fungal disease

Losses : Occasionally severe losses, up to 30 per cent (Muimba, 1984).

Distribution : Latin America, Africa

Characteristics : Black stems, resembling CBB, and shoot die-back. The disease severely decreases fresh quality of planting material. Humid conditions necessary for contamination.

Control methods : Varietal resistance, cultural control methods, fungicide treatment.

Occasional diseases :

1. Cassava Common Mosaic Disease (CMD)

Kind : Viral disease

Losses : Up to 60 per cent, but at low incidence (Lozano et al., 1985).

Distribution : Latin America

Characteristics : Very similar to ACMD

Control methods : Disease-free planting material.

2. Frogskin disease (FSD)

Kind : Viral disease

Losses : Up to 100 per cent (Cock, 1985)

Distribution : Very limited, Latin America

Characteristics : Reduced root size and presence of deep cortical cracks. No above-ground symptoms. Inconclusive evidence that whiteflies are involved as transmittors.

Control methods : Clean planting material.

3. Superelongation disease Sphaceloma manihoticola

Kind : Fungal disease

Losses : Up to 100 per cent, but at low incidence (Cock, 1985).

Distribution : Latin America (Lozano et al., 1987).

Characteristics : Marked elongation of the internodes and malformation of the leaves. Development during rainy season.

Control methods : Varietal resistance, fungicide treatment.

4. Cercospora leaf spots Cercospora spp.

Kind : Fungal disease

Losses : Up to 30 per cent (Terry et al., 1984)

Distribution : Widespread

Characteristics : Leaf spots, resulting in lesions and leaf fall, thus reducing yields. Development during rainy season.

Control methods : Cultural methods (to reduce humidity), fungicide treatment, resistant varieties.

Incidental diseases :

1. Phoma Phoma spp.

Kind : Fungal disease

Losses : Up to 100 per cent

Distribution : Restricted to cool, humid areas. The most important disease in the highland areas.

Characteristics : Brown leaf spots, resulting in leaf fall and shoot die-back.

Control methods : Varietal resistance , fungicide treatment.

2. Preharvest Root Rots

Most important are Phytophtora spp., Rigidoporus lignosus (white thread disease), Armillaria mellea and Botyodiplodia theobroma (Silvestre and Arraudeau, 1985).

Kind : Fungal diseases

Losses : Up to 80 per cent (Lozano et al., 1985).

Distribution : Restricted to poorly drained areas

Characteristics : Infection of roots is visualized by a white mycelium cover and results in partial or complete wiliting and soft or dry rot of the thickened roots.

Control methods : Good drainage, ridging, crop rotation

Cassava Pests

Key pests :

1. Mites: Green Spider Mite Mononychellus spp. and Red Spider Mite Tetranychus spp.

Losses : Up to 90 per cent (Bellotti et al., 1987)

Distribution : Green spider mite occurs in Africa and Latin America, whereas red spider mite is found in Asia.

Characteristics : Population growth in dry season.

Control : Biological control by means of Phytoseiidae predators, already effective on low populations, fungi (Entomohtora spp. and Amblyseius fustis) and varietal resistance associated with pubescense and antibiosis.

2. Mealybugs Phenacoccus manihoti and Phenacoccus herreni

Losses : Up to 90 per cent on susceptible varieties

Distribution : Phenacoccus manihoti and phenacoccus herreni

Losses : Up to 90 percent on susceptible varieties

Distribution : Population growth in dry season

Control : Varietal resistance (at a low level; CIAT, 1988a; IITA, 1988a), biological control with the wasp Epidinooearsis lopezi (in Africa) and other natural enemies like Encyrtidae or Coccinellids

3. Thrips Frankliniella williamsi

Losses : Up to 30 per cent (Bellotti et al., 1987).

Distribution : latin America

Control : Highly resistant varieties are available

Occasional pests

1. Nematodes

Most important are root knot nematodes, root lesion nematodes, renoform nematodes and spiral nematodes.

Losses : Not exactly known. Nematodes may cause severe yield losses on exhausted soils (IITA, 1988b)

Control : Biological control by means of fungi and bacteria.

2. Whiteflies Aleurotrachelus socialis and Bemisia tabaci

Losses : Up to 80 per cent (Bellotti et al., 1987).

Distribution : Aleurotrachelus socialis in latin America, Bemisia tabaci in Africa and Asia.

Characteristics : A. socialis feeds on cassava plant, whereas B. tabaci is important as transmitter of the Cassava Mosaic Disease.

Control : Insecticides, very difficult to control

1. Nematodes

ANNEX 3

Review of scientists and institutions currently involved in the field of cassava and biotechnology.

field of research	Scientist or institution
plant tissue culture	A.I. Robertson, University of Zimbabwe W.M. Roca, CIAT C.H. Henshaw, CIAT S.Y. Ng, IITA K.K. Kartha, PBI , Canada G. Staritsky, Department of Troical Science, Agricultural University Wageningen
protoplast techniques	W.M. Roca, CIAT R. Jonard, University, Montpellier
genetic characterization of cassava toxicity	M.A. Hughes, Univesity of Newcastle, Upon Tyne, UK R.T. Sayre, The Ohio State University, Ohio, USA dr. P. Kakes, Free University, Department of Ecophysiology, Amsterdam, The Netherlands
biochemistry of starch quality of cassava	J.M.V. Blanshard, universty of Nottingham, UK R.T. Sayre, the Ohio State university, Ohio, USA R.D. Cooke, ODNRI, London, UK
DNA recombinant technology for disease or pest resistance	R. Beachy, Washington University, St. Louis, USA C.A. Ryan, Washington State University, Pullman, USA J. Jaynes, Louisiana State University, Baton, USA M. Peferoen, Plant Genetic Systems, Gent, Belgium
Screening methods	US Department of Agriculture in Beltsville, USA D.J. Robinson J.C. Thoevenel
DNA recombinant technology for protein improvement	J. Jaynes, Louisiana State University, USA
starch quality improvement	J.M.V. Blanshard, University of Newcastle Upon Tyne, UK dr. E. Jacobsen, Department of Plant Breeding, Agricultural University, Wageningen
photosynthetic capacity	C.C. Black, University of Georgia, Athens, Georgia, USA
root deterioration	dr. R. Cooke, ODNRI, U.K.

ANNEX 4

Constraints identified by CIAT and IITA

CIAT and IITA prior to the workshop identified, individually the following research problems in cassava production, utilization considered to have relevance for advanced research approaches (CIAT, 1988b).

CIAT		IITA	
*	1. cyanide toxicity	*	1. cyanide toxicity
**	2. post-harvest root deterioration		2. shy flowering
	3. propagation related problems		3. mealy bug and green mites
	4. viral diseases	****	4. nutritional quality
	5. cassava hornworm	***	5. starch quality under stress
***	6. starch quality under stress	***	6. nematodes
	7. photosynthetic capacity under stress	**	7. post-harvest root deterioration
****	8. nutritional quality		8. mycorrhiza

* indicate coincidence between CIAT and IITA