THE GENETIC VARIABILITY OF CASSAVA: ORIGIN, EVALUATION AND UTILIZATION

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Several review articles (Byrne, 1984; Hahn et al., 1979; Jennings and Hersey, 1985; Roca, 1983) and a recent book (Sylvestre and Arraudeau, 1983) have been devoted to the improvement and agronomy of cassava. In this paper we therefore emphasize the theme of the genetic variability of cassava, particularly in Africa.

After a brief review of the structure of the genus Manihot and of the domestication of cassava and its dispersion in the world, we analyse:

- the origin of the genetic diversity of cassava;
- the variability of the cultivars;
- the possible applications of biotechnology to the improvement of cassava.

THE ORIGIN AND CULTIVATION OF CASSAVA

The complex of Manihot species

The genus Manihot Mill. belongs to the family Euphorbiaceae, and comprises more than a hundred species with the same chromosome number (2n=36 chromosomes). Many researchers currently refer to the taxonomic classification of Rogers and Appan (1973). By multivariate analysis of the botanical characters of specimens in a herbarium, those authors classified 98 species in 19 sections, including just one cultivated species, M. esculenta Crantz.

Many wild species are sun-loving perennials, with a bushy habit and sporadic distribution: they are found in tropical savanna regions or semi-arid regions. Certain taxa readily occupy habitats disturbed by man and behave like adventitious plants of recent formation. In contrast, shrubby species of Manihot originated in the New World, with a range extending from the southern United States (Arizona) to northern Argentina. The species is divided

into more or less discontinuous geographical groups containing many wild species. Five primary zones of diversity area distinguishable (Figure 1):

1. Central America;

2. the central Brazilian plateau;

3. northeastern Brazil;

4. southwestern Brazil and Paraguay;

5. Colombia and Venezuela.

Fig. 1. Primary zones of variability of species of the genus Manihot (IBPGR, 1983).

The diversification of the genus Manihot results from several processes acting at different times. These include:

- bioclimatic changes, in particular the flora and fauna refuge zones at the end of the least period of glaciation (Vuilleumier, 1971);

- human migrations, with their trail of food-crop plants, in the pre-Colombian era (Nassar, 1978);

- genetic exchange within and between species, an active, continuous phenomenon.

The study of genetic resources and the evolutionary organization of the genus Manihot in the Americas is worth special attention. Regions of high priority were surveyed in the 1980s: Brazil (Nassar, 1980), Colombia, Venezuela, Peru, and Mexico (1982-1983). This collecting should be continued to learn more about the situation of the natural populations in relation to the ecology, and to limit the effects of genetic erosion (IBPGR proposals, 1983).

The domestication and dispersal of cassava

M. esculenta is a cultigen domesticated in the Americas and is unknown in the wild state. The ancestors of cultivated cassava were tuberous root plants eaten by man during their migrations in the Americas. Several ethnobotanical and archaeological studies suggest that cassava was utilized in northwestern South-America 2000 to 4000 years B.C. Recently, Ugent et al. (1986) described cassava fossils from the Casma valley in Peru, carbon-dated from 1500-1800 B.C. Furthermore, cassava seems to have been domesticated several times in different regions of the Americas (non central origin).

The domestication characteristics of cassava are as follows (Jennings, 1976):

- Large tuberous roots for starch storage, with a marked (Brazil, Venezuela, Colombia) or mild (Peru) bitterness.

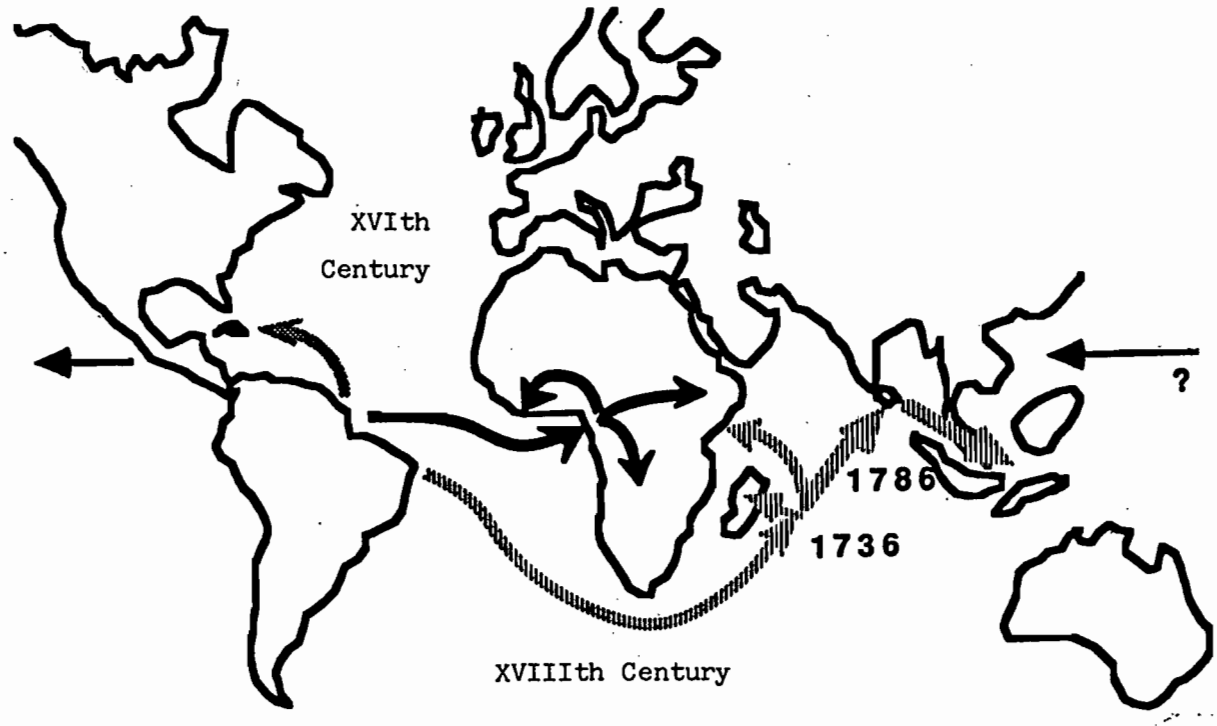
- An erect habit with little branching; this trait is related to flowering, as domestication has resulted in fewer flowers.

- Ready multiplication by cuttings, a character favoured by the reserves stored in the cauline axes; furthermore, sexual reproduction is no longer considered (sterile plants).

The dispersal of cassava in the Americas has favoured an increase in its genetic diversity there. Like other plants originating in the New World, its introduction into Africa and Asia is historically recent, dating from after the discovery of America by Christopher columbus.

Figure 2 summarizes the principal routes of intercontinental dispersion:

Fig. 2. Cassava dispersion.



In Africa, two main introductions are known, one in the Gulf of Guinea, in Central Africa, in the second half of the 16th century, and the other in the region of Madagascar and the eastern coast of Africa, during the 18th century. On the basis of ethnobotanical information, Kent (1969) situated the arrival of cassava in Madagascar with the migrations of populations from Eastern Africa in the 16th century. Cassava spread on the African continent in the second half of the 19th century and especially in the 20th century. The recent extension of its cultivation has been encouraged by the building up of standing food reserves (as protection against famines), and the plant's tolerance to drought and locusts. Jones's (1959) comparative study of the Congo, Guinea, and Eastern Africa clearly establishes the place of cassava in Africa.

In Asia, cassava was probably introduced directly from Mexico to the Philippines, and indirectly from the Mascarene Islands via Ceylon (1786), India (1794), and Southeast Asia.

In addition, several shrubby wild species of the genus Manihot which produce latex were introduced into Africa. The principal one was M. glaziovii from Brazil. Cross's survey in 1876 was widely publicized by Kew Gardens. At the beginning of this century, M. glaziovii was grown on about 50,000 hectares in Eastern Africa and it was utilized to a more limited extent in Western Africa (sometimes as a shade tree). The species M. dichotoma and M. piauhyensis were considered unsatisfactory for latex production.

These migrations to Africa and Asia resulted in a founder effect, with less diversity than existed in the Americas. Since the 5th century, an intense diversification has taken place locally.

ORIGIN OF THE VARIABILITY OF CASSAVA

The genetic variation of cassava is related to the following factors:

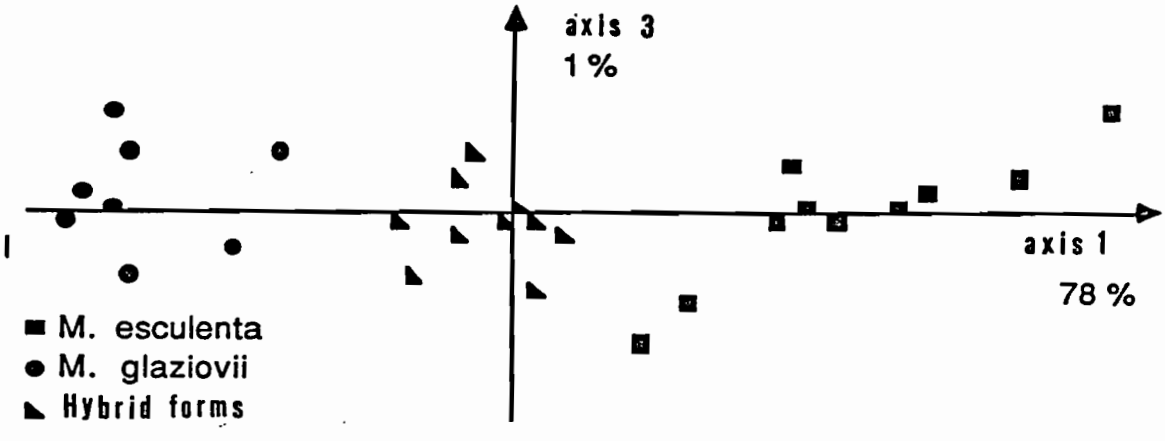
Active introgression processes

Wild Manihot species are often isolated from one another, but they seem to hybridize easily when they happen to come into contact. Nassar (1978) demonstrated this clearly in Brazil: M. reptans and M. alutacea crossed naturally in disturbed zones, with gradual introgression into M. reptans.

Likewise, in the regions where cassava originated, the cultivated forms can also intercross with the local wild species and give rise to various hybrid "swarms", which evolve towards adventitious forms ("weeds" in Harlan's (1971) sense of the word), colonizing the regions influenced by man.

Such genetic exchanges also exist in Africa between local cultivars and introduced wild species. In recent studies in the Ivory Coast, Lefèvre (1987) demonstrated the existence of spontaneous interspecific hybrids of M. esculenta x M. glaziovii. Their morphological characters (shape and size of the fruits, seeds, leaves and tuberization) suggest the presence of a very diversified population of hybrids (Fig. 3).

Fig. 3. Introgression between cultivated cassavas and M. glaziovii.



A mode of allogamous reproduction developed by a plant with a polyploid structure

The traditional agriculture based on cassava (a mosaic of cultivars) favours genetic recombination between cultivars with heterozygous structure. The descendants resulting from self-pollination and natural cross-pollination are very polymorphic and the effect of inbreeding is marked.

Magoon et al. (1969) lent strength to the hypothesis of a polyploid origin of the species M. esculenta. More precisely, its segmental allotetraploid nature is in accord with the following facts:

- bivalent pairing and disomic heredity (with several cases of duplicated genes),

- a caryotype with 3 nucleolar chromosomes and one partial duplication of chromosomes affecting 6 of the 9 chromosomes of the haploid set.

The origin of this allotetraploidy remains an enigma, as all the wild species of Manihot studied so far have 36 chromosomes.

Vegetative reproduction

Thanks to this mode of multiplication, every valuable new individual can be used to make a strain (a clone). This ancestral practice makes it possible to exploit the variability of natural reseeding depending on the selective pressures, which vary with time and over space. For example, plants from the seedbed which are free of virus are kept to renew the stock of cuttings when the cultivars in the field are very diseased. This process is eminently favourable to constant diversification and adjustment to new parasitic pressures (as in the recent example of bacteriosis in Central Africa).

Somatic mutations

Few authors refer to this subject (Leon, 1976; Martin, 1976), in the absence of experimental proof.

In conclusion, the genetic pool of the genus Manihot evolved in its area of origin according to a pattern of disruptive selection, with increase of the diversity of the cultivated pool (human selection) and of the wild pool (adventitious forms). The result is a taxonomically complex situation linked to the evolution of wild and cultivated forms in the area where cassava originated, but also in Africa.

With reference to these evolutionary patterns, it is entirely appropriate to improve the stock by exploiting the possibilities of introgression with wild species and recombination between cultivars. This option makes it of prime importance to evaluate, and circulate the genetic resources of the genus Manihot for their use in selection. This is not a new undertaking and has already led to recognized successes in Africa from virus-resistant interspecific hybrids (Jennings, 1976). The agronomic characters

of the wild species of Manihot answer many agronomic needs (Nassar, 1986):

- resistance to viral diseases and to bacteriosis (M. glaziovii);
- a high protein content (M. oligantha);
- tolerance to drought, cold, and hydromorphic soils;
- reduction of the hydrocyanic glucoside content (M. gracilis, M. oligantha);
- prolificness (M. oligantha, M. tripartitia, M. zehntneri, M. anomala).

DESCRIPTION OF THE DIVERSITY OF CULTIVARS

It is customary to distinguish the cultivars of cassava according to:

- their content of hydrocyanic glucoside (sweet or bitter cassava);
- the color of the root flesh (yellow flesh is often more bitter than white flesh);
- the length of the growth cycle (early, sweet cultivars, or bitter ones with a 1- to 2-year cycle).

This diversity of cultivars is often reflected in their vernacular names. Research centers that do work in cassava selection have built up collections of local or introduced cultivars (such as the IBPGR repository, 1980). The largest are held in Brazil (by Embrapa), Colombia (by CIAT, which has 2600 strains), India, Indonesia, and Nigeria.

There are two main objectives for the collections:

- to identify cultivars on the basis of morphological and physiological characters;
- to utilize them in selection as a faunction of their agronomic characteristics and their behaviour relative to the biotic and abiotic environment.

These descriptions are taken from the list of characteristics recommended by IBPGR (1983). Well-documented descriptive studies have been made from collections in:

- Madagascar (Cours, 1951),
- Ghana (Doku, 1966),
- Venezuela (Montaldo, 1982),

- Mexico (Galindo, 1982),

- Brazil (Costa, 1983),

- The Ivory Coast (Zoundjihekpon, 1986).

All these descriptive studies of diversity are very useful in the improvement of cassava. However, they are of limited value for describing the genomes of the cultivars, because:

- a great many characteristics are noted;

- the expression of morphological and physiological characters is very dependent on the environment, local strains of parasites, and the state of health of the collection;

- the genetic determination of characters is not known, or is not quantitative in nature.

For all these reasons, significant progress in the description of genetic variability has been made with the use of biochemical markers.

The first such methodology, put into use in the 1970s, was based on electrophoresis to determine enzyme polymorphism. Enzyme markers have the double advantage that they have simple genetic determination and selectively neutral behaviour. Its recent application to cassava is due to Zoundjihekpon (1986), CIAT (1985), and Lefèvre (1987). The latter author at present uses 10 enzyme systems revealed on starch gel: 12 loci and 28 alleles have been identified. This technique makes it possible to demonstrate:

- a simple diploid type of heredity,

- the existence of duplicated genes (phosphogluconate dehydrogenase),

- cases of fixed heterozygosity (phosphoglucoisomerase) and of interactions between loci (malate dehydrogenase).

The study of a collection of 168 Ivory Coast cultivars illustrates this approach to the genetic diversity of a collection:

- the 12 loci so far known identify 78 different electrophoretic genotypes;

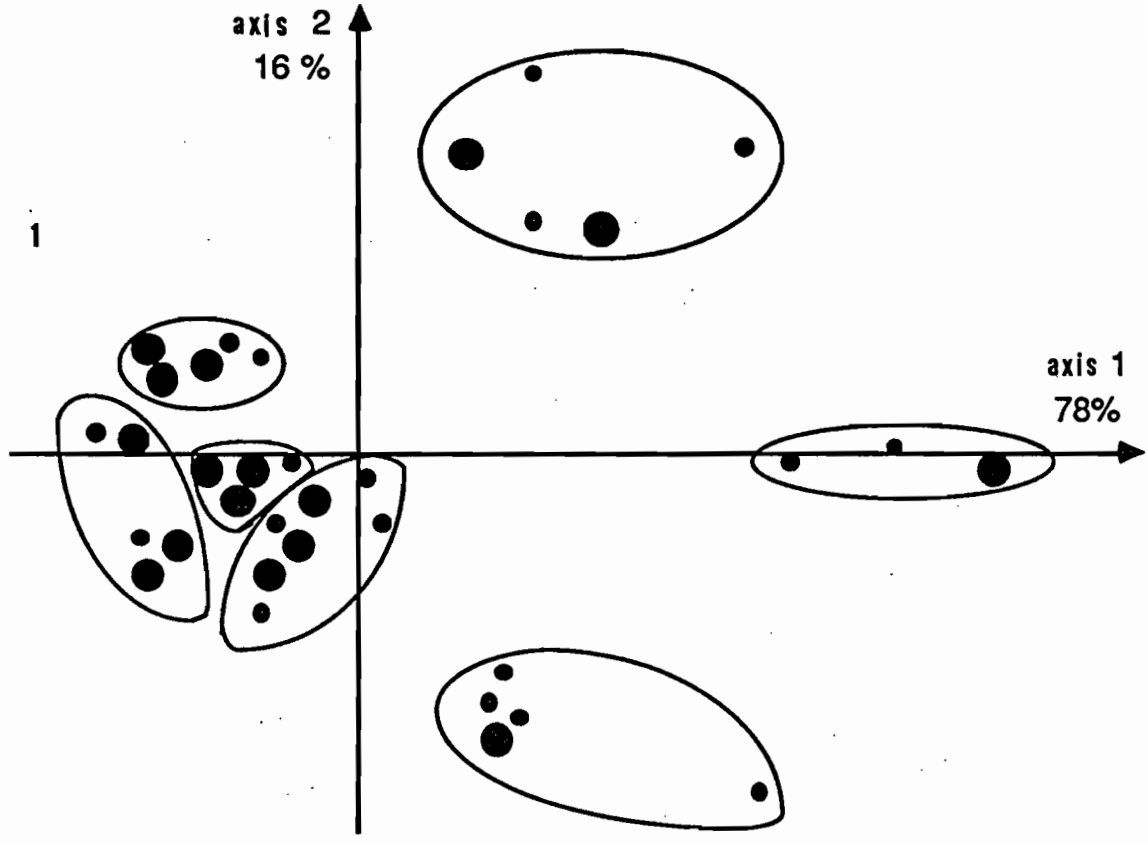
- 19 alleles have a frequency higher than 30%;

- the various possible allelic combinations can exist, many of them in the heterozygotic state.

By multivariate analysis (Fig. 4), it has been shown that the local cassavas of the Ivory Coast form several groups defined by particular combinations of common alleles. This structuration closely reflects their genetic relatedness, the genetic distance between groups, and the origin of intermediate groups. The improved varieties introduced in this country present the same

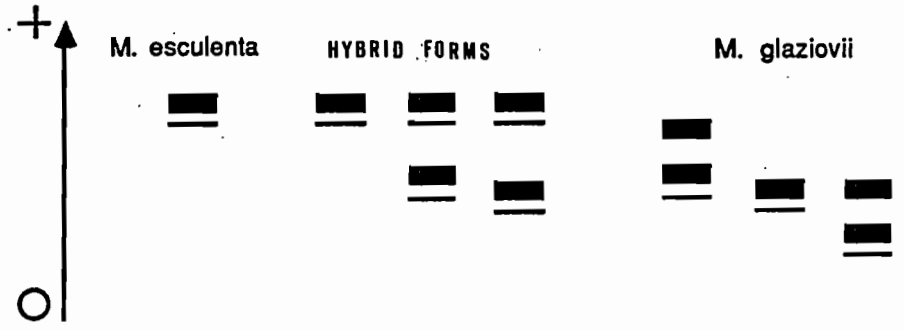
structuration, with several previously unreported allelic combinations.

Fig. 4. Structure of enzymatic diversity of 168 Ivory Coast cultivars (AFC with 10 enzymatic alleles).



The enzymatic markers also make it possible to follow the phenomena of interspecific hybridization and introgression (Fig. 5., leucine aminopeptidase).

Fig. 5. Enzymatic markers of the introgression between cultivated cassavas and M. glaziovii, (LAP zymegrams).



In the case of the spontaneous hybrids M. esculenta x M. glaziovii, one allele from each of the parent species is found at each locus. An exception, one of the hybrids, is enzymatically no different from M. esculenta and reflects a reversion to the type of the cultivated parent. Conversely, older traces of introgression can be identified in some cultivars (rare, common, or fixed alleles in M. glaziovii).

The technique of enzyme electrophoresis seems to be the main effective biochemical approach. Other chemotaxonomic markers can also be used (such as phenol polymorphism). But, particularly since the beginning of the 1980s, workers have turned to direct marking of nuclear, chloroplast, or mitochondrial genomes.

With the use of restriction enzymes and molecular probes, it is possible to locate a specific fragment of DNA and to study its intraspecific or interspecific polymorphism. For example, it is possible to distinguish the chloroplast genomes of the parents, and to obtain the origin of the cytoplasm of sexual hybrids, introgressions, and hybrids (somatic hybrids). The analysis of restriction fragments of mitochondrial DNA is a convenient tool for distinguishing the fertile or sterile male cytoplasms. The detection of a nuclear DNA fragment by hybridization with a specific probe makes it possible to reveal a polymorphism, called restriction-fragment-length polymorphism (RFLP). This can be exploited to:

- characterize a cultivar (biochemical identity card),

- mark a character of agronomic interest (if the appropriate molecular probe is available), or

- establish a detailed genetic map of a cultivated plant species.

In the case of cassava, such molecular studies on DNAs are indispensable if one wishes eventually to attempt genetic manipulations.

BIOTECHNOLOGIES APPLICABLE TO THE IMPROVEMENT OF CASSAVA

Sanitation

In this cultivated vegetatively reproducing species, the risks inherent in diseases caused by systemic agents transmitted through cuttings are well understood on the basis of studies by Martin and Morel. The new techniques of plant multiplication in vitro have made it possible to regain normal state of health for many vegetatively propagated species. The effectiveness of in vitro culture of meristems combined with heat treatment was established for various viral diseases of cassava in the 1970s (Kartha, 1975; CIAT, 1980; IITA). The important problem is to have reliable indexing methods available for each viral strain; decisive progress has been made in this field, associated with molecular biology (serological tests, monoclonal antibodies). The gradual degeneration of heavily virus-infected cassava significantly affects their growth, their vigour, and their production (estimated losses of 40 to 70%) and makes it difficult to assess their agronomic value. There are several hundred healthy clones at CIAT (1982). Furthermore, this technique of micropropagation of cassava makes possible a faster vegetative multiplication of healthy strains to set stocks of cuttings.

Conservation of genetic resources

The in vitro culture of cassava satisfactorily resolves the difficulties of conservation and diffusion of genetic resources. Every selector knows the problems of maintaining several hundred cultivars in a collection of live plants in the field (diseases and parasites, climatic accidents, etc.). The culture of meristems in vitro can be used for this purpose in two ways:

- The preservation of microcuttings in slowed growth (at 20-22 C) makes storage possible for 4-5 years with periodic pricking out; 1500 cultivars are preserved in this form at CIAT.

- Meristems can be frozen if protected with cryoprotectors (such as DMSO or sugars) adn then stored in liquid nitrogen at -180 C; using this technique, Kartha (1982) obtained 90% survival of meristems and 10% regeneration of plants. Such long-term preservation is still very demanding to use (in technique and regeneration).

Culture of tissues and cells *in vitro*

Though the micropropagation of cassava has technically come into use, the other modes of reproduction *in vitro* have not been very successful so far:

- Culture *in vitro* of immature cassava embryos would be helpful in work on interspecific hybridization; this has not been tried.

- The regeneration of plants from tissues has been known to succeed in cassava. The first such plant was obtained by Stamp and Henshaw (1982) by somatic embryogenesis from explants of cotyledons, with large doses of auxins favouring the induction of variants. Likewise, Mabanza and Jonard (1984) found up to 95% regeneration by neoformation of buds on callus from cotyledons from seeds at maturity.

- The regeneration of plants from cells does not yet work. The protoplasts of leaf mesophyll develop into microcolonies and microcallus, but the regeneration only occasionally goes on to the formation of cauline axes (Shahin and Shepard, 1980).

- Obtaining haploid plants, which are so useful in the analysis of genomes and the fixation of characters in the homozygous state, has not yet succeeded. Cell culture stumbles against organogenesis (with the formation of callus and root axes only) (Liu and Chen, 1978; CIAT, 1982). Other possibilities for generating haploid forms remain to be surveyed: direct culture of microspores, gynogenesis, induction of haploidy by fertilization with the pollen of another species or of irradiated pollen.

Somaclonal variation and genetic engineering

The development of these new approaches to selection is blocked by the difficulty of regenerating whole cassava plants by somatic embryogenesis:

- Culture *in vitro* of various explants has demonstrated the capacity of isolated cells to regenerate whole plants, phenomena of rejuvenation during successive microcuttings, and even the loss of cellular identity so that it becomes possible to start off in new directions (variants resulting from mutation, chromosomal remodelling, genetic regulation, etc.). In cassava, this route could be exploited with pressures such as physical stress, toxins, and specific antibodies.

- The usefulness of interspecific hybridization products has been demonstrated in cassava in particular. Approaching it by somatic hybridization may open up the range of possibilities of recombination of genomes and of cytoplasms: this gives rise to more or less viable, more or less stable transgenic plants.

- Finally, plant transformation by genetic engineering has resulted in several transfers of bacterial genes and plant proteins. The transformation can be direct or by intermediary

vectors derived from plasmid T1 of Agrobacterium. There have so far been only a few projects involving the improvement of plants (TMV capsid protein, or resistance to glyphosate).

CONCLUSIONS

This general review of the genetics of cassava sheds light on the gaps and need for research in this field:

- Knowledge of the cassava genome and of the structure of the genus Manihot is very limited. It may progress rapidly with the use of various molecular markers.

- Genetic resources have a primary influence in the improvement of cassava. Prospecting for wild forms and cultivars should thus be encouraged, as should the circulation of plant material. A decisive step was the building up of tissue culture collections of healthy microplants.

- The classic methodologies of vegetative and sexual selection remain entirely applicable to cassava.

In contrast, modern methods will be applicable to cassava only after the following points have been resolved:

- knowledge of how to regenerate normal, stable transgenic plants in vitro;

- knowledge of how to identify and isolate the genes to be transferred (probes) aplicable to simple genetic characters (such as resistance);

- an understanding of how to control the expression of transferred genes;

- the availability of equipment and of personnel competent in plant biotechnology and molecular biology.

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