Cassava

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Cassava is cultivated for its roots, which develop into tubers. It is part of the diet of the poorest countries of the tropics, particularly in Africa, which accounts for more than half of the world production (Fig. 1). Young cassava leaves are also consumed: they are a significant source of supplementary protein, vitamins, and minerals for the populations of Central Africa and northeastern Brazil that essentially survive on cassava tubers. The leaves are sometimes used as forage.

Cassava is cultivated most often in traditional agricultural systems, which rarely use improved cultivation practices. It is in the Asian countries, where cassava is a cash crop grown for export and industry, that improved varieties are adopted most readily by farmers. A typical case is that of Thailand, which in two decades has become among the major world producers along with Brazil, Nigeria, Zaire, and Indonesia.

Cassava is usually planted from a stem cutting of around 20 cm, but domestication has involved sexually reproduced plants using techniques that are still found in Africa and the Amazon region (Emperaire et al., 1998). Seeds are used exclusively for varietal improvement, but improved techniques are being researched for their direct use in cultivation (Iglesias et al., 1994).

Grafting wild cassava with robust vegetative growth and resistance to leaf diseases, such as *Manihot glaziovii*, on a cultivated cassava stock at the beginning of the cycle may triple the individual yield. This is the *mukibat* system described by de Bruijn and Dhamaputra (1974) and it is easy to implement on small areas.

Cassava prefers light and well-drained soils that are predominantly sandy, but it can tolerate heavier, more clayey soils, if they are broken down. Mineral fertilization is still rarely applied, but the response to various manures is well known. Nitrogen favours the development of above-ground parts, sometimes to the detriment of tuberization, while potassium fertilization considerably increases yields (Howeler, 1990). The presence of endomycorrhizae on roots improves phosphorus nutrition.

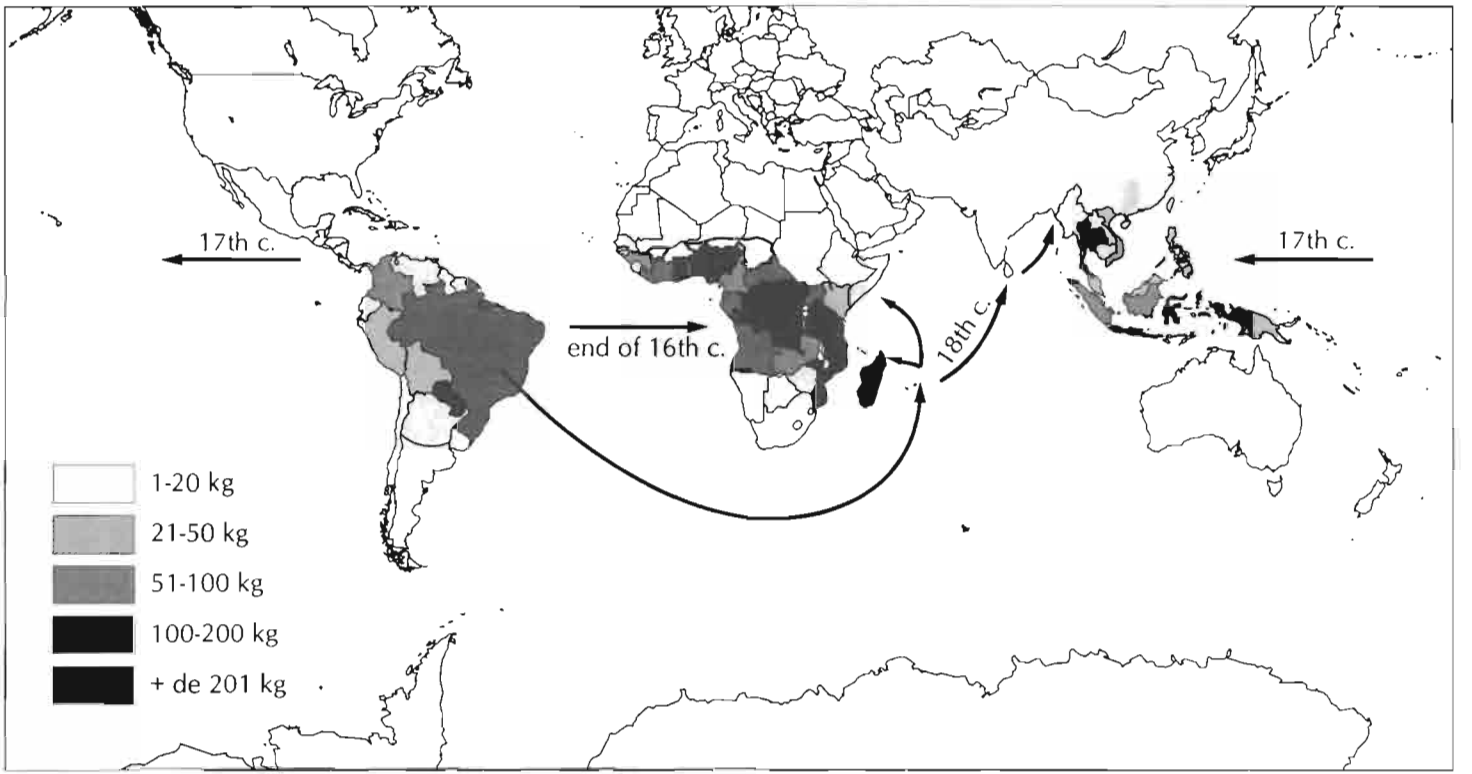


Fig. 1. Production of cassava according to FAO data (in kg per year per inhabitant, average of three years: 1995, 1996, 1997). The arrows indicate the diffusion of cassava from its centre of origin, Brazil, and also probably from Mexico (Purseglove, 1992).

The major phytosanitary constraints are: African mosaic—a geminiviral disease transmitted by the whitefly *Bemisia;* vascular bacteriosis, caused by *Xanthomonas campestris* pv. *manihotis*); and attacks of mealy bugs (*Phenacoccus manihoti*) and acarids (*Mononychellus tanajoa*). These diseases and pests are significant in Africa.

The length of the culture cycle depends on the varieties and environmental factors—temperature, length of dry season, insolation; it varies from six months for the highly precocious varieties to three years and more under unfavourable climatic conditions. In some systems of production, one or two tubers of the plant are harvested for domestic consumption without uprooting the plant.

Cassava is a source of good quality starch, which lends itself to a wide variety of uses: basic human nutrition, modern baking, traditional beer making, industrial uses, and animal nutrition. It ranks fifth worldwide among food plants produced, at 166 million t (FAO, 1998). However, genetic improvement of cassava has begun only recently (McKey and Beckerman, 1996; Raffaillac and Second, 1997).

The root of cassava produces hydrocyanic acid in variable quantities depending on the variety and the environment. Some varieties are dangerous from the nutritional point of view if techniques of transformation are not employed to eliminate most of the hydrocyanic acid. Cyanogenesis is known to be a system of chemical defence in many plants (Jones, 1998).

BOTANY AND TAXONOMY OF THE GENUS *MANIHOT*

Morphology

Cassava plants grown from cuttings simultaneously develop one or several principal stems from buds, while plants grown from seed have a single stem. The vegetative apparatus of cassava is characterized by two types of orthotropic ramification (Medard et al., 1992). The proleptic ramifications, or secondary branches, result from the development of lateral buds after the breaking of apical dominance. The sylleptic ramifications, or floral branches, come from the transformation of the vegetative meristem into floral meristem: two to four branches develop simultaneously. The aptitude for flowering, which determines the architecture of the plant at the end of the cycle, is one of the criteria of identification of varieties. In Fig. 5 (in the Appendix), which presents the major morphological characters by which varieties are identified, the final shape of the stem is illustrated by examples drawn from the works of Cours (1951).

Cassava leaves are highly heteroblastic: during the life of the plant, the number of lobes, an odd number, is greater at the beginning of the cycle, then diminishes to end in a single lobe at the end of the cycle in certain varieties. Leaf morphology is one of the criteria of identification (Table 1, in the

Appendix). Cours (1951) emphasizes that a comparison is valid only if made between varieties planted in the same place and under the same conditions.

The root system is made up of nodal roots and the more numerous basal roots. The latter give the largest tubers. The weight of tubers from a single plant often varies, but their dry matter content is the same. Any root can develop into a tuber. The percentage of roots that tuberize varies greatly depending on the varieties and on environmental factors and cultivation practices. A plant grown from seed puts forth a seminal taproot, which tuberizes first but is too fibrous to be easily consumed. It then produces secondary roots, attached to the taproot, that develop into tubers.

Physiologically, cassava is a C_3 plant, but it has some characteristics of a C_4 plant (El-Sharkawy and Cock, 1990). The tuberization of roots is triggered during the first weeks of growth. The number of tubers is fixed between the second and fourth months of the cycle, barring some damage to the plant. It does not depend on the photoperiod (Keating et al., 1985).

The shape of wild species ranges from a herbaceous rosette form to creepers, to shrubby or bushy forms, to that of a tree more than 15 m high.

Reproduction

All species of *Manihot* are perennial, monoecious, and allogamous. Their pollination is entomo-anemophilous. Flowering may be highly precocious and frequent during plant growth, or late and occurring only once, or nonexistent. Generally, the first floral axes abort. The inflorescences are bunches of 20 to 60 unisexual and monoperianthate flowers. The female flowers, located at the base of the inflorescence, open before the male flowers, which are 10 to 20 times as numerous (Cours, 1951). For each inflorescence, one to six fruits or capsules mature in three to five months. They burst during the dry period and release three seeds having a mottled tegument, brown to grey, with a caruncule.

Taxonomy and Distribution of *Manihot*

The genus *Manihot*, distributed naturally throughout the tropics of the New World, belongs to the family Euphorbiaceae, which is divided into five subfamilies. It is placed in the subfamily of Crotonoidae, with hevea (Webster, 1975).

The limits of the genus are well defined, but the limits between the species are very difficult to establish. The last monograph on the subject (Rogers and Appan, 1973; Rogers and Fleming, 1973) reduced the number of species from 171 to 98 on the basis of multivariate analysis of 44 vegetative characters observed in the herbarium, in which the leaf characteristics were significant. The distribution of species is very clear. Seventeen sections are described, one of which comprises only cultivated cassava. This last section is close to

two others: the Central America section, Parvibracteatae, which includes the species *M. aesculifolia*, thus considered the species most closely related to cassava, and the South America section, Heterophyllae, which comprises most of the taxa now included in the species *M. esculenta* by Allem (1994), such as cassava, ssp. *esculenta*, and the wild forms, ssp. *flabellifolia* and ssp. *peruviana*, considered by that author to be the direct ancestors of cassava.

From 77 known species present in Brazil and described by Rogers and Appan (1973), only 38 were retained as non-synonyms by A.C. Allem (personal communication). These are classified into 16 groups depending on affinity. In comparing these two classifications, we observe great differences in the grouping by affinity, which suggests enormous difficulties in defining clear discontinuities within the genus, at least in its principal area of diversity (Second et al., 1997).

In its zone of origin, the genus *Manihot* extends from southern Arizona, in the United States, to northern Argentina. The species are never dominant in the vegetation but rather sporadic. Most of them are found in regions with a long dry season, but some of them are also found in the wet forests or, most often, along the edge of or in clearings that are natural or a result of deforestation. Some species also have adventitious distribution, in disturbed zones along highways, for example.

The natural distribution of the genus does not go beyond 2000 m altitude, but some species are found in subtropical zones. One species, *M. brachyloba*, has a distribution spanning Central and South America and is also found in the Dominican Republic. This exceptional distribution could be linked to its ability to be transported in moderately saline water; some viable seeds were recovered on the coast of French Guyana, where a strong inflow from the Amazon and its local rivers was observed (D. Loubry, personal communication).

The introduction of cassava in the Old World dates from the 16th century and that of *M. glaziovii* from the end of the 19th century. *Manihot glaziovii*, the manicoba, was the object of a development trial in Africa in the 1930s for the production of latex and it has persisted for other reasons (Lefevre, 1989; Serier, 1989).

Since the works of Rogers and Appan (1973), the distinction between *M. utilissima*, bitter varieties, and *M. dulcis* or *M. aipi*, sweet varieties, has been rejected, and it is acknowledged that several environmental factors have a significant impact on the presence of two glucosides responsible for the production of hydrocyanic acid and thus bitterness.

Genome Structure

The 20 species of *Manihot* observed have the same number of chromosomes as cassava, or 18 pairs (Lefevre, 1989; Bai et al., 1993), and this is probably so for all the species in the genus. From the examination of chromosomes with

pachytene, Magoon et al. (1969) suggested that cultivated cassava was a segmentary allopolyploid. The observation of proportions of duplicated loci, for RFLP as well as for microsatellites, did not corroborate this hypothesis (Chavarriaga-Aguire et al., 1998). This presumed allopolyploidy could, however, be ancient and correspond to the entire genus, not to the cultivated species in particular. The genome size of cassava is small; it is estimated at 1.68 pg per diploid genome (Marie and Brown, 1993).

GENETIC RESOURCES

The entire *Manihot* genus could be considered a reserve of genetic resources useful for improvement of cassava by sexual means. No strong barrier to hybridization is known to exist. Moreover, interspecific hybridization has frequently been obtained or observed in nature. Nevertheless, most of the *ex situ* collections are made up of cultivars conserved generally in the collections maintained in field and regenerated vegetatively every year. Part of these cultivar collections is maintained *in vitro*. Seeds are used for conservation and exchange mainly of hybrids and wild species that have been collected recently.

It is estimated that nearly 25,000 accessions are conserved in collections throughout the world, all species included. But this number is probably an overestimation because of the loss of several collections and the duplication of others. At present, the two major collections are those of Brazil and of the CIAT (Centro Internacional de Agricultura Tropical) in Colombia. A combination of networks, formal and informal, work to conserve and use the genetic resources of cassava, on each continent and on the global scale (Second and Iglesias, 2001). A very small proportion of this genetic material has been used in crossing for improvement of varieties.

A bibliography of research prior to 1973 (Cab, 1974) pointed out the existence of old collections of the Belgian Congo (INEAC, Institut National pour l'Etude Agronomique du Congo Belge), in West and Central Africa (IRAT, Institut de Recherches Agronomiques Tropicales et des Cultures Vivrières), Madagascar (IRAM, Institut de Recherches Agronomiques à Madagascar), Kenya (EAAFRO, East African Agricultural and Forestry Research Organization), Tanzania, Indonesia, India (CTCRI, Central Tuber Crops Research Institute), Malaysia (MARDI, Malaysian Agricultural Research and Development Institute), Costa Rica (IICA, Instituto Interamericano de Ciencias Agrícolas), Venezuela, Mexico, Brazil, Colombia, Argentina, and elsewhere. Material was sometimes exchanged without rigorous sanitary control. Each research station used different techniques to manage the collections—mode of planting, quality of cuttings, duration of cycle, fertilization, planting density—and followed its own classification of varieties.

From the early 1980s onward, the IITA (International Institute of Tropical Agriculture) in Nigeria attempted to supervise the activities of several countries in Africa and to collaborate with the CIAT on cassava collection and exchange. In this way, a large part of the cassava collection of the CIAT was transferred to Nigeria. On its part, Thailand did the same to take advantage of the great diversity of clones in the CIAT collection.

Wild genetic resources are useful in the improvement of cassava in various ways: as a source of resistance to diseases and parasites—*M. glaziovii* is a good example because it has been widely used in the search for resistance to bacteriosis and African viral mosaic (Hahn, 1984)—and to stresses such as low temperatures; an architecture of cultivars in a given environment and leaf morphology adapted to cultural constraints such as concurrence with weeds and associated cultivation; starch content and physicochemical properties, which are of great importance but have seldom been studied in the wild species.

In this chapter, we attempt to show how the use of molecular markers complements the studies done earlier on a morphological and physiological basis. Through molecular markers, the genetic origin of cassava can be more precisely defined: the most direct wild parent, other closely related parental species, and other species that are involved in the genetic constitution of cassava by introgression. The technique can help us understand the processes of domestication and management of genetic diversity observed in a traditional environment. Finally, it allows us to comprehend the scope and structure of the genetic diversity of cultivars and the entire genus *Manihot*, the principal centre of which, Brazil, will be particularly explored.

ORGANIZATION OF THE DIVERSITY

Agromorphological Variability

Traditional farmers, particularly the Native Americans, distinguish a very large number of cultivars (Emperaire et al., 1998). They use several criteria to classify clones: taste, linked to concentration of cyanogenic glucosides in the root cortex, for which there is a continuum of variation from very low to extremely toxic concentrations (McKey and Beckerman, 1996); precocity—varieties called short-cycle are harvested in 6 to 8 months while others have a cycle of more than 18 months; and appearance of the plant—e.g., the shape of the aerial part, the coloration of leaf, stems, and roots, and their shape.

A large number of morphological and biochemical markers are available for cassava. The most remarkable description, one that has inspired subsequent works in this field, is that of Cours (1951). Cours proposed a classification of varieties in Madagascar into eight botanical sections. The characters used by Cours and in later works are indicated in Table 1 (in the Appendix). Gulick et al. (1983) compiled them in a document for the IBPGR

(International Board for Plant Genetic Resources) and proposed a universal list. Thus, there is an exhaustive catalogue, which comprises classic descriptors that can be used for the identification of cultivated varieties of cassava.

Following the classification of cassava varieties established by Rogers and Appan (1973) from multivariate analysis of 44 characters, all the specific and subspecific denominations of cassava were made synonymous: *M. utilissima* became *M. esculenta*. Ultimately, 19 'similarity groups' were constituted, which were intended to facilitate the work of the breeder but proved to be difficult to use. In effect, apart from genetic factors, cultivated cassava was subjected to two types of significant factors of variation: one, the stage of the cutting and the way in which it is cut and planted; two, soil fertility and cultivation practices. These factors complicate agromorphological evaluation and the comparison of cultivars that have grown in different environments or during different years.

Agromorphological plasticity in relation to the cutting

The quality of the cutting affects the structuration of the plant population. It determines the rate of renewal, and therefore the density of the population and the number of stems per plant. Moreover, the primary rooting is also conditioned by the stage of the cutting, at the nodal level (number of nodes in contact with the wet soil) as well as at the basal level (orientation and number of primary roots linked to the nature of the cut and the position of the cutting in the soil).

In comparison to a cutting taken from the upper part of the stem, a base cutting has a better rate of regrowth, faster growth initiation, more stems per plant, and better nodal and basal rooting (Raffaillac, 1992). Similarly, for a base cutting, the age of the stem affects the quality of the cutting. In Côte d'Ivoire, more than 13,900 stems per hectare were achieved with stem cuttings of 6 months and 27,400 with stem cuttings of 12 months. A cutting of 6 months yields an average of 17.2 roots whereas a cutting of 12 months yields 32.7 (Raffaillac, 1998).

The stems cut at harvest can be stored for several weeks before being planted in a new field. This storage modifies the behaviour of the young plants. A cutting taken from a stem stored for four to eight weeks roots more quickly. Moreover, the number of stems per plant increases (Raffaillac and Nedelec, 1988). These few examples show that it is advisable to make a rigorous selection of cuttings if one wishes to study varietal behaviour on the basis of criteria such as rate of renewal, initial vigour of the plantlet, structuration of the plant population, and root potential.

Agromorphological plasticity in relation to management and environment

The number of stems per plant depends on the way the cutting is planted

and the climatic conditions that prevail in the weeks that follow (rain, overall radiation). Certain varieties develop more stems per plant if the plantation is horizontal rather than oblique. When the overall radiation received at the beginning of planting is limited, the number of stems per cutting declines (Raffaillac, 1998). Nevertheless, for a single variety, a field of cassava is always planted with a combination of single stem plants and plants with two or more stems, most often with one type being dominant. An oblique planting combined with a slanted cut allows location of the primary root axes in one soil sector; on the contrary, a vertical planting with a straight cut favours a radial dispersal of roots around the plant (Raffaillac, 1992). The grouping of roots, and thus of future tubers, in a single sector makes it easier to harvest the tubers. It is pointless to study ways to improve varieties for this character, since it can easily be controlled by planting techniques.

The varieties of cassava present differences in their aptitude to develop secondary branches, the presence of which remains linked to environmental factors. These branches are more numerous when the soil is rich and the radiation high. On the contrary, small differences between plants or the simultaneous presence of other plants (adventitious or associated) reduce the number of branches.

Flowering may be nonexistent during the cultivation cycle or may occur several times depending on the environmental factors: on potassium-deficient soils the number of flowerings is higher than that observed on potassium-rich soils (Howeler, 1990; Raffaillac, 1998). This effect of soil fertility is found for the morphology and quality of tuberized roots. At equal weight, a tuber obtained from a potassium-deficient soil is longer and thinner than one from a fertilized soil (Raffaillac, 1997). Potassium fertilization considerably increases the yield but reduces the dry matter content of the tuberized roots. The rainfall received during the last weeks before harvest also modifies the quality of the tubers: the starch content is low when the quantity of water received is high (Raffaillac, 1985).

The plasticity of wild species is also known. We can recall two examples of this. The first pertains to the wild forms of *M. esculenta*, which naturally has two biological types according to whether it grows in the forest or in the savannah (or on the edge of forests). In the first case, it grows as a false creeper, which climbs on tree branches. In the second, it grows in a bush shape. Seeds taken from the creeper form and cultivated in full light or seeds that have germinated on the edge of the forest give bushes without any traces of creeper-like growth. The second example pertains to *M. quinquepartita*, which forms whole leaves or strongly indented leaves depending on whether it grows in full light or in shade, as has been observed in Saul, French Guyana. The morphological differences are such that, in a herbarium, the two forms may be thought to be different species, even by an expert.

Molecular Diversity

RAPD ANALYSIS OF CULTIVATED CASSAVA

RAPD analysis has been done on 126 accessions (Colombo, 1997). These consist of 71 accessions from the collections of IAC (Instituto Agronômico de Campinas) and EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) and originating from different geographic zones of Brazil, 33 accessions from a core collection of world diversity at CIAT (Hershey et al., 1994), and 42 plants representing 18 ethnovarieties from a single clearing along the middle of the river Negro in the Amazonian basin. This sample can be considered representative: Brazil probably covers a good part of the zone of origin of cassava and is the source of a majority of cultivars, but the collections there are deficient in Amazonian origins. However, the sample remains limited, with only 7 accessions of Central America and the Caribbean and 6 non-American accessions.

From 21 primers, 193 RAPD bands were observed, of which 88 are polymorphic in presence-absence, which indicates the great diversity of the species. The Jaccard coefficient of similarity varies from 0.99 to 0.45 with a mean of 0.67 (the polymorphic bands alone are taken into account). The distribution of 126 genotypes on the two primary factorial axes of a principal coordinates analysis (PCoA) is given in Fig. 2a. The Lebart test (Lebart et al., 1977) on a correspondence analysis (CA) was used to characterize the bands that contribute significantly to the primary axes: 46 bands, or only 52%, contribute to the five primary axes of the CA.

The variation is continuous and no strong structure appears. However, the ethnovarieties from the Amazonian clearing are separated from the rest of the collection by the primary axis. The other varieties that are closest to it originate from the Amazonian region of Brazil, from countries that share the Amazonian basin (Venezuela, Colombia), or countries that have regions with a similar climate, such as Malaysia and Thailand.

The PCoA done on a 'non-Amazonian' group (Fig. 2b), constituted by eliminating varieties of the traditional field and those closest to them, indicates, from three primary axes, a set of 13 varieties, which have some remarkable characteristics. Their origins are varied: 6 originate from Brazil (4 of those from northeastern Brazil), 2 are artificial Brazilian crosses included in the sample, and 5 come from various countries. Among the varieties originating from northeastern Brazil, variety SRT1316 has a remarkable affinity with interspecific hybrids between cassava and *M. glaziovii*. Among the varieties originating from various countries, NGA2 was artificially introgressed by *M. glaziovii* to acquire a resistance to African viral mosaic and bacteriosis; it is one of the parents of the cross used to construct the first genetic map for cassava (Fregene et al., 1997). Another, Col 22, is a traditional variety, which is involved in the genealogy of several recently selected varieties (see table 2 in Second et al., 1998b). The hypothesis that an introgression of

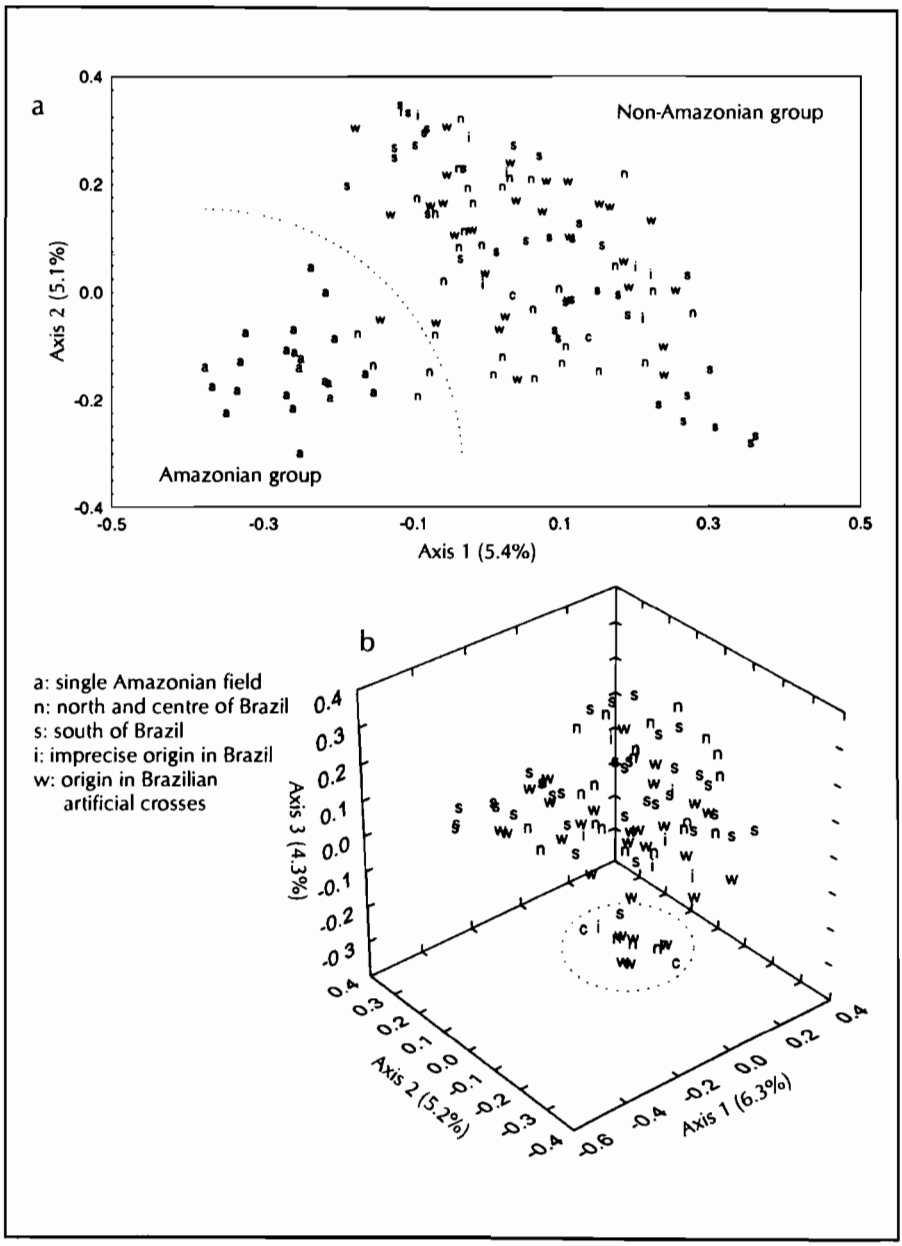


Fig. 2. Principal coordinates analysis of the matrix of similarity (Jaccard) based on the presence or absence of 88 polymorphic RAPD fragments in comparisons of genotypes two by two: plane or volume defined by the 2 or 3 primary axes. a: distribution of 126 genotypes representing a world collection and a single Amazonian field (the dots indicate the separation of an Amazonian group from the rest of the collection). b: analysis of the non-Amazonian group alone (the dotted circle indicates a remarkable group).

M. glaziovii would be at the origin of this group of varieties is worth confirming.

Other peculiarities can be detected by examining certain axes that prove significant despite the low values of total variance extracted. Low values of axis 4 (3.7%) isolate the four varieties from Thailand and Malaysia, the New World varieties closest to them originating from Colombia.

Analysis of Amazonian ethnovarieties and of a collection of cultivars by AFLP and RAPD markers

The collection of ethnovarieties from the clearing may clarify the question of the nature of an ethnovariety denoted by a name. Does the multiplicity of names reported by anthropologists cover a real genetic diversity and does a name identify a clone or a family of clones? More generally, it could direct us in verifying the hypothesis of a dynamic traditional management of cassava diversity, which could reveal to us the process of its domestication (Emperaire et al., 1998; Second et al., 1998).

AFLP markers have been used to analyse the 42 plants representing the 18 varieties sampled in the same clearing, at a ratio of 1, 2, or 10 plants per variety, and 40 accessions of distinct geographic origin, coming from the world collection and representing the variability described earlier. The two combinations of primers used enable us to observe 60 polymorphic bands out of a total of 132. The analysis reveals a structuration of diversity similar to that observed on the same samples using RAPD markers. A global analysis has thus been done by combining the polymorphic bands observed with the two techniques, or 143 bands.

The UPGMA dendrogram of 82 plants studied, established by means of the Jaccard coefficient of similarity (Fig. 3), reveals the presence of two groups. One corresponds to the world collection, the other to the clearing. A single variety of the world collection is found in the group of plants from the clearing. It originates from the Brazilian state of Para in the Amazon basin. The two groups, with a widely differing geographic origin, have clearly equivalent total diversity. Only 10 bands (7% of the total), the frequency of which is less than 0.45 in the world collection, are not observed among the varieties from the clearing. A significant coefficient of correlation of 0.7 is found between the frequencies of bands observed in the clearing and in the world collection. An analysis of molecular variance (Amova) reveals that 80% of the total genetic diversity is found within the groups and 20% between the two groups.

All the names correspond to different genotypes and, in general, plants having the same name cannot be differentiated: they probably represent clones. There are, however, four exceptions for which a single name—F, Mn, Bu, and Sn in Fig. 3—corresponds to several genotypes. The varieties Fino (F) and Manipeba (Mn) are each represented by two plants that correspond to two genotypes, with respectively 16 and 26 different bands out of the 143

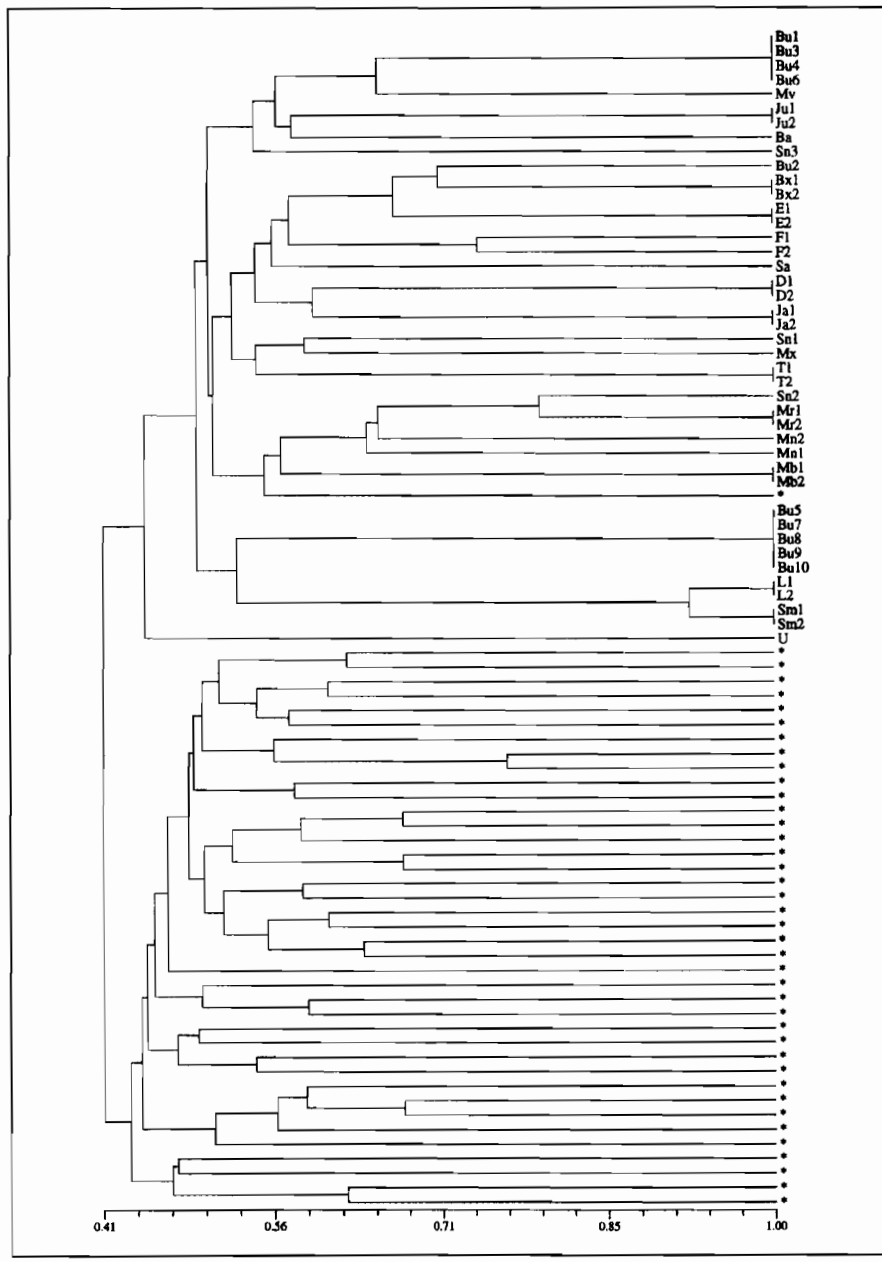


Fig. 3. Comparative molecular diversity of 42 plants from a single Amazonian field (the abbreviations represent the names of varieties, except Sn, which represents nameless varieties; the values denote the order of plants of the same name) and of 40 plants representing the world collections (symbolized by an asterisk). UPGMA dendrogram from the matrix of similarity calculated for 143 polymorphic bands (60 AFLP and 83 RAPD).

studied. The variety Buia (Bu) is represented by three genotypes, which differ by 31, 38, and 40 bands; despite the dispersal of Buia genotypes in the dendrogram of Fig. 3, a PCoA, not presented, confirms the tendency to group genotypes that have the same name in relation to the total diversity of the field. Each of the three 'nameless' varieties (Sn) corresponds to one genotype, which agrees with their presumed origin. They come from three plants grown from seed, in the course of evaluation by the cultivator (Emperaire et al., 1998); their relative grouping agrees with that of genotypes observed respectively in the three varieties F, Mn, and Bu.

Thus, these preliminary results confirm the existence of a dynamic management of cassava diversity: grouping of a considerable diversity in a single field, recombination linked to sexual reproduction, and selection of high-performing genotypes collected subsequently under a single name according to their affinities.

From this perspective, a traditional variety can be considered a family of clones that share certain characteristics of direct interest for cultivators, such as productivity, quality, and resistance to diseases, parasites, and various stresses, as well as characters of less immediate interest by which varieties can be recognized and given a name, for example, colour, shape, or general appearance of the plant. These characteristics do not suffice in themselves to characterize a variety, which must be evaluated up to the point of consumption.

For the sample representative of the world collection, AFLP analyses can only confirm—with a much smaller number of accessions—the results obtained with the RAPD analyses, i.e., the absence of strong structuration in a considerable global diversity. However, taking into account the grouping of certain genotypes on a geographical basis, it seems to us that with a study of a large number of genotypes and more loci, one could determine the loose structuration that can be predicted for this diversity.

Analysis of diversity of *Manihot*

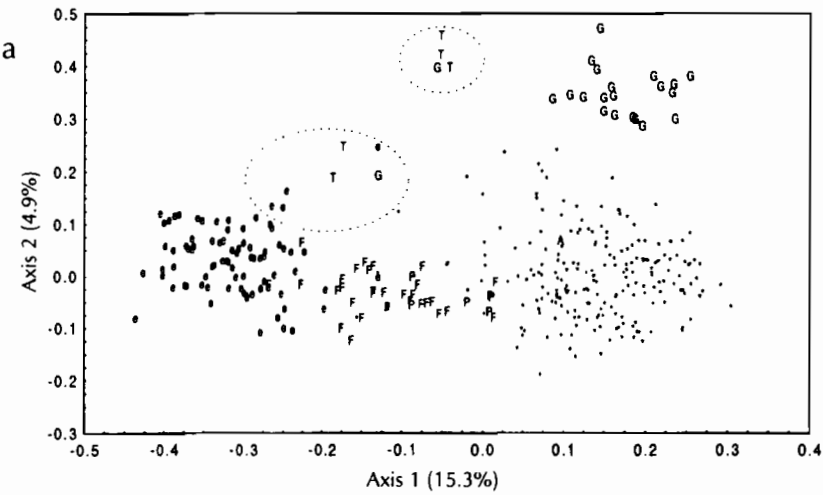
The first molecular characterization of the genus *Manihot* was that of Bertram (1993). Based on an RFLP (on Southern blots) of chloroplast and ribosomal DNA, it was limited to six species of Central America, cassava, and a South American species, *M. carthaginensis*. The results show relatively low divergence (0.1% at most for chloroplast DNA) and maximal divergence between the species of Central America, on the one hand, and *M. carthaginensis* and cassava, on the other. These results contradict the hypothesis of a maximal relationship of cassava with *M. aesculifolia*, proposed by Rogers and Appan (1973). On the other hand, they suggest that South America could be the zone of origin of cassava. The study of some species of South and Central America by RAPD (Colombo, 1997) confirms the conclusions of Bertram (1993).

AFLP studies (Roa et al., 1997) and microsatellite studies (Roa et al., 1998) of cassava, of four wild species of South America—wild forms of *M. esculenta*, *M. tristis* (a species related to *M. esculenta* ssp. *flabellifolia*), *M. carthaginensis*, and *M. brachyloba*—and of *M. aesculifolia* confirm the hypothesis of Allem (1994) that the wild forms of *M. esculenta* were the direct ancestors of cassava. Moreover, they show that the diversity of wild forms is greater than that of cassava. In the case of microsatellites, the number of alleles per locus varies from 4 to 22 with a total of 124 alleles for the 10 loci analysed (one or two loci were not detected in *M. aesculifolia*, *M. brachyloba*, and *M. carthaginensis*). The group of species formed by *M. aesculifolia*, *M. brachyloba*, and *M. carthaginensis* (species related to *M. glaziovii*) shows less than 20% similarity with cassava. *Manihot esculenta* ssp. *flabellifolia* and ssp. *peruviana* and *M. tristis* form a group closer to cassava, with 35% to 50% similarity. It is interesting to note, in relation with the exchange of genetic diversity that we have observed in the traditional management of cassava, that the wild species have a deficit of heterozygotes while in cultivated cassava the rates of heterozygosity observed are equivalent to the values expected.

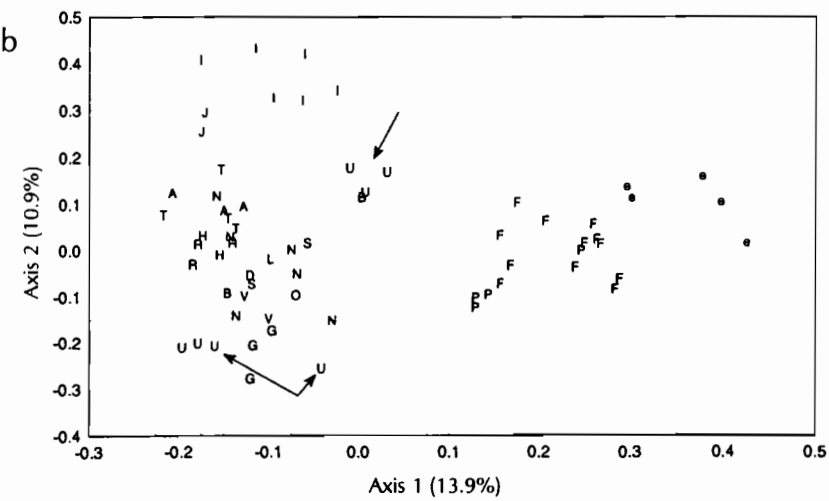
Our study, based mainly on AFLP markers and conducted at the same time as the one just mentioned—independently but with certain common samples—is, to date, the most exhaustive on the genus *Manihot*. It includes all the Brazilian species and four species of Central America; it involves totally 282 wild forms and 82 varieties of cassava. The analyses were done on the presence or absence of 93 polymorphic bands revealed from a single pair of primers.

A UPGMA analysis of similarities (simple matching) shows that the genotype most divergent from the group is that which represents the Central American species *M. aesculifolia*. The distribution of 364 accessions in the plane defined by the two primary axes of a PCoA is represented in Fig. 4a. The first axis isolates most of the cultivars from all the wild species. However, there is a continuum between these two groups, where one finds the wild forms of *M. esculenta* and *M. pruinosa*. The other wild species are distributed along axis 1, while axis 2 allows us to separate these wild species from a group corresponding to *M. glaziovii* in the wide sense, as A.C. Allem has defined it, considered synonymous with *M. carthaginensis* (table 1 in Second et al., 1997). Two groups of forms in an intermediate position between *M. carthaginensis* and *M. esculenta* (isolated by the circles in Fig. 4a) are indicated. They comprise forms classified as *M. glaziovii*, forms of 'tree cassava' frequent in northeastern and central Brazil, and, in the group closest to the cassava group, four cultivars, including SRT1316, originating from northeastern Brazil. We consider these forms hybrids between *M. glaziovii* and *M. esculenta*.

In northeastern Brazil, a similar situation is found to that described in West Africa (Lefevre, 1989). Cultivars of cassava have undoubtedly been introgressed by *M. glaziovii*, probably by the intermediary of forms of tree cassava, even though these are often largely sterile and propagated vegetatively.



e: cassava, the same genotypes as in Fig. 2. F: the wild forms of *M. esculenta*. P: *M. pruinosa*. G: *M. carthaginensis* in the wider sense (*M. carthaginensis, M. glaziovii, M. epruinosa*). C: *M. compositifolia*. *: all the other species. T: cassava tree of northeastern and central Brazil.



e, F, P as in Fig. 4a, A: *M. hassleriana*. B: *M. brachyloba*. D: *M. pseudo-pruinosa*. E: *M. tenerrima*. G: *M. gracilis*. H: *M. hilariana*. I: *M. pilosa*. J: *M.* sp. nov. L: *M. longepetiolata*. N: *M. nana*. R: *M. noguerae*. O: *M. procumbens*. S: *M. stipularis*. T: *M. triphylla*. U: *M. fruticulosa* (the arrows indicate the two subspecies distinguished). V: *M. paviaefolia*.

Fig. 4. Diversity of the genus *Manihot*: principal coordinates analyses based on similarities (simple matching) of genotypes two by two in the analysis of 93 polymorphic AFLP fragments. a: distribution of 364 plants representative of the genus. b: distribution of genotypes of *M. esculenta* (wild and cultivated forms), of *M. pruinosa*, and Brazilian species morphologically most closely related that are found on a single AFLP gel. Data of Fig. 4a reduced to this single gel.

Our analysis thus confirms that the wild forms of *M. esculenta* are the closest to cassava among all the species of South America. Moreover, it shows that *M. pruinosa* is not distinguished at the molecular level from the wild forms of *M. esculenta*. This proximity is also found in the morphology (A.C. Allem, personal communication). Our study further suggests that *M. carthaginensis*, and particularly the form recognized as *M. glaziovii*, is part of the genetic constitution of certain cultivars. This contribution needs to be placed in the context of successful introduction of *M. glaziovii* in the Old World and its use as a genetic resource in modern efforts to improve cassava varieties. Finally, certain species, particularly *M. fruticulosa*, could be genetically close to cassava, even though their direct contribution to its domestication is not proved.

The PCoA limited to the species *M. esculenta* and to Brazilian species considered *a priori* the closest to cassava (Fig. 4b) shows a clear separation between *M. esculenta* and *M. pruinosa* on the one hand and the other Brazilian species on the other. *Manihot fruticulosa* splits up into two subspecies in agreement with the morphological analysis that recognizes a subspecies *caiaponia* (A.C. Allem, personal communication). The form *M. fruticulosa* in the strict sense seems closest to *M. esculenta*. *Manihot fruticulosa*, being a herbaceous species, could be used in varietal improvement to modify the architecture of cassava.

The structure illustrated in Fig. 4a is accurate despite the dispersal of samples across six gels and the difficulty of transcribing the gels in terms of presence or absence of specific bands associated with them: AFLP analysis of a subsample of DNA used in Fig. 4a, for three combinations of primers, all placed on the same gel for each primer, confirms this structuration. The three groups suggested by Fig. 4a are found: *M. esculenta*, with its wild forms ssp. *flabellifolia* and *peruviana*, and *M. pruinosa*; *M. carthaginensis* in the wider sense; a group including all the other South American species represented, among which only *M. compositifolia* appears as an intermediate between this group and *M. carthaginensis*. Also it is noted that grouping according to molecular similarity does not always correspond to the taxonomic classification, which illustrates again the difficulty of classifying this genus.

Manihot carthaginensis (including *M. glaziovii*) appears in this study to be the most divergent species of cassava in South America. The fact that it has indisputably contributed to the domestication of cassava indicates that all the species of *Manihot* could *a priori* be considered direct genetic resources for cassava—of the primary or secondary pool according to the terminology of Harlan.

RELATIONS BETWEEN MORPHOLOGICAL AND MOLECULAR DIVERSITY

The relations between morphological and molecular diversity have not been the subject of rigorous experiments on a significant scale. We have emphasized

the agromorphological plasticity of cassava, which complicates this comparison. The plasticity of wild species is also considerable. It is nevertheless possible, on the basis of what has been reported earlier, to make the following parallels between the observations made for these two levels of diversity.

For 31 of the accessions studied, all Brazilian, one description has been drawn from nine characters—colour of three root tissues and roughness of the root, colour of apical bud, colour of young stem, colour of petiole, width and sinuosity of the central lobe—or in total 27 states of characters. This description has enabled a comparison with the diversity observed using RAPD (74 polymorphic bands). The PCoA done independently for morphological descriptors and molecular markers shows similar structures with, notably, a grouping of varieties that present industrially useful characteristics (Colombo, 1997).

More generally, for the cultivated forms, the absence of subspecific division revealed by botanical or agromorphological analysis is found at the molecular level, where the variability seems continuous, without a large linkage disequilibrium, the major part of the diversity of the species being found in a single Amazonian clearing. The Native Americans recognize in fact a large number of varieties, which is consistent with the wide molecular diversity observed, but these varieties are dynamic, in perpetual evolution, notably because of genetic recombination. Even though there is agreement between the two scales of observation, the molecular analysis seems better designed to describe reliably the genetic diversity of cassava, to the extent that it is insensitive to environmental variations.

Classification of the genus *Manihot* into well-defined species is difficult both by morphological and by molecular markers. However, for the genus *Astrocaryum* (palm), molecular AFLP markers unambiguously reveal the large subdivisions and suggest a new one that is not in contradiction with the morphological analysis (Kahn and Second, 1998). Used in the oil palm, RFLP and AFLP markers clearly indicate the cryptic subspecies (Barcelos, 1998; Barcelos et al., 1998). The difficulty of classifying the genus *Manihot* thus appears to be the result of its biological characteristics.

The species *M. aesculifolia*—classified in morphological terms as the closest to cassava (Rogers and Appan, 1973) but highly divergent in molecular terms—represents nevertheless a notable case of disagreement between the two levels of observation. It illustrates the utility of molecular markers from the phylogenetic point of view: the Central American species are geographically isolated, which could explain their phylogenetic distance despite the morphological resemblances that are more subject to natural selection than to molecular diversity.

Moreover, the forms classified as intermediate between the closely related species *M. caerulescens* and *M. quinquepartita* according to the morphological criteria are also found in intermediate position in the molecular analysis.

Besides, several cases of spontaneous hybridization, suspected during the morphological study, were confirmed by molecular analysis (Second et al., 1997). These hybridizations partly explain the difficulties of delimiting the species in the genus *Manihot*.

As with cassava, there is generally no contradiction between the results of the two levels of observation for the entire genus *Manihot*. It is by a combination of morphological observation and molecular characterization that one comes to a better understanding of this difficult genus. It already seems confirmed that entities considered species according to classical botany have generally conserved the possibility of fertile hybridizations. Under these conditions, it is probably inappropriate to represent the relations between these species by a phylogenetic tree. A 'network' of relations gives a more realistic picture. It would be impossible to chart this network exhaustively; it could be illustrated at best by multivariate analysis on several axes of variation. Once we recognize the reality of this situation, we can see that a model of dynamic conservation of the genus is particularly appropriate.

APPLICATIONS IN GENETIC RESOURCE MANAGEMENT

The biological characteristics of the genus *Manihot*, as well as considerations of complementarity with approaches of *in situ* and *ex situ* conservation, have led to a proposed procedure for dynamic conservation for the wild species of the genus (Second et al., 1998; Second and Iglesias, 2001). Artificial populations can be collected in their biotopes of origin by grouping individuals that belong to a single species considered in the strict sense. In biotopes or continents new to these species, the grouping could be done by species in the wider sense. Thus, we end up with a manageable number of species that, by exchange of genetic diversity, have a greater chance of adapting to new conditions. A periodic exchange of seeds between the populations of the same species—excluding populations of the zone of origin, which must not be genetically 'contaminated'—would ensure the maintenance of a high genetic diversity at low cost. This diversity is directly subjected to natural selection and available for integration in breeding programmes. Such a mechanism of dynamic conservation would come to complement *in situ* conservation, when that is applicable, and *ex situ* conservation by seed, which remains to be optimized.

As for cultivated cassava, its status as a plant generally propagated by vegetative means has favoured a procedure of conservation by clones *in vitro*, which has at least two major disadvantages: high cost and the associated risks of transmission of parasites, particularly viral parasites, during exchanges of genetic material.

The confirmation that diversity is dynamically managed in traditional practices—which probably represent the process of domestication of cassava—has led to revived interest in conservation of cassava diversity by seed: the genotypes are modified but diversity is conserved. For dynamic conservation of cultivated cassava in the field (Second and Iglesias, 2001), we must note a peculiarity linked to the consumption of roots. Unlike with cereals, for which the seed-harvest cycle represents a selection pressure in favour of a productive cultivated variety in field collections, selection pressure in a plant cultivated for its roots is imposed directly by the cultivator. Here lies, probably, the explanation of the traditional field model of cassava, where the clones of a single variety are often cultivated in clumps in the field, so that they can be evaluated up to the time they are consumed. Thus, to perpetuate this system in an effective and justifiable manner, the association between conservation and improvement must be maintained. When a large diversity is organized in groups of clones in a single field, the clones can be evaluated in the context of domestic agriculture. It is thus possible, as a function of scientific knowledge acquired, to assist this process to the end and to render it more effective, notably through production of hybrids, backcrosses, and genetic transformation. The use of biotechnology such as microchips or DNA chips may make it relatively easy in such a procedure to follow the evolution of the frequency of certain loci in DNA extracts from combinations of individuals in the population. It would thus be possible to associate conservation and improvement of genetic diversity on the farm with the advantages that would result from technologies of genetic manipulation.

CONCLUSION

Use of molecular markers to analyse the scope and organization of genetic diversity of cassava and of the genus *Manihot* yields a set of results that are generally consistent with the morphological data, with just one notable exception. These results have enabled us to make rapid progress in our understanding of the genus.

Molecular analysis unambiguously confirms the relationship between cultivated cassava and its presumably ancestral forms. It also indicates proximity between *M. pruinosa* and *M. esculenta*. *Manihot pruinosa* would also be a wild form of *M. esculenta*, adapted to the Brazilian savannah.

This analysis indicates the singularity of the species *M. carthaginensis* in the wide sense, the validity of its classification with *M. glaziovii* being verified. It suggests the introgression of *M. glaziovii* in certain cultivated varieties, while this species is, among the Brazilian species, the most divergent from *M. esculenta*. *Manihot glaziovii* is thus, since its diffusion in the Old World at the end of the 19th century, an example of dynamic conservation of the genetic diversity associated with varietal evolution on the farm (Second, 1998).

The complex genetic nature of the genus *Manihot* is confirmed by the analysis of molecular diversity; it seems to imply several spontaneous hybridizations.

From these results, new concepts can be advanced. The varieties distinguished traditionally correspond to families of clones resulting from a dynamic management of the diversity, which must be encouraged. This process of dynamic management could include varietal improvement on the farm, which would benefit from inputs of modern genetics. To the extent that the structure of the genus *Manihot* is better described by a network of relations between the species than by a phylogenetic tree, it also seems appropriate to consider a dynamic management for the conservation of this genus.

Cassava is an important food plant for the tropical regions, and the studies we have undertaken are therefore essential. A more representative sample of cassava varieties needs to be examined that includes, apart from the collections already studied, the entire Amazonian basin and the African and Asian continents. The traditional process of management of the diversity must be thoroughly analysed, and the nature of the traditional varieties must be examined from a larger sample, structured according to the field, the village, the region, and so on. At the same time, the perfection of microsatellite and cytoplasmic markers must be pursued. For the genus *Manihot*, it will be necessary to assemble all the Central and South American species in a single analysis. Case studies must be undertaken on the organization of the diversity and gene flows in the species complexes close to the genus *Manihot* as a function of their distribution and their ecology. From the analysis of forms of *M. glaziovii* diffused throughout the world for more than a century and varietal selections, cultivated and spontaneous, that have resulted from them, we can begin to evaluate the possibilities of dynamic conservation of the genus.

APPENDIX

Plant Material

The analyses were carried out on 130 varieties of cassava from collections of Brazil and CIAT and on 278 plants representing all the South American species and four species from the Central American species of the genus *Manihot*. A living collection of all the Brazilian species, such as has been considered by A.C. Allem at CENARGEN (Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia, Brazil; table 1 in Second et al., 1997), was constituted from cuttings, transplants, and seeds collected directly in the natural populations. This collection of wild species was supplemented by samples from the herbarium of the CENARGEN collection. Most of the forms distinguished morphologically for which there presently exist known populations have been represented. Six presumed hybrids were included as well as their presumed parents from the same populations. About a third of the samples come from herbarium, the rest, with only a few exceptions, are from direct collections from nature or plants obtained from seeds collected in nature. Seven plants, of which four represent species of Central America, come from *in vitro* collections of CIAT, in Colombia, and from the laboratory of the University of Washington, in the United States. For the analysis, samples of healthy young leaves were dried in a ventilated oven at 50°C for 20 h, then conserved dry in the presence of silica gel.

DNA Extraction

A modified protocol of the CTAB technique (cetyl-trimethyl ammonium bromide) was used (Dellaporta et al., 1983; Colombo, 1997).

RAPD Analysis

For the RAPD analysis, 208 decamer primers were tested and 22 were retained for the quality of profiles of bands obtained (see Colombo, 1997, for details on the primers and PCR amplification).

AFLP Analyses

All the AFLP analyses were entrusted to the commercial laboratory of Linkage Genetics at Salt Lake City, in the United States (presently PE AgGen Inc.). The laboratory used the AFLP technique as published and the Keygene program for computer-assisted reading of the gels. For the arbitrary part of the primers, the AGA/CAG combinations and, eventually, AGT/CTC and AGA/CAA combinations were used, when two or three combinations were used among the 12 combinations tested.

Statistical Analyses

The matrixes of presence or absence of bands were used to calculate the coefficients of similarity (Jaccard coefficients or simple matching) in the comparisons of genotypes two by two or for the purpose of a CA. The matrixes are compared using the Mantel test. The matrixes of similarities are used to construct dendrograms (UPGMA) or to conduct principal coordinates analyses (PCA, PCoA). To construct a UPGMA dendrogram from the value of the coordinates on the primary axes, a Euclidean distance is used.

For all the calculations above, the Ntsys program of Exeter Software (Rohlf, 1998) was used. The analysis of molecular variance is calculated with the Amova program (Excoffier et al., 1992). Ntsys and Statistica were used for the graphics (Statsoft).

Table 1. Characters of vegetation, inflorescence, and roots of cassava used to differentiate varieties (Cours, 1951). The criteria in italics are from later bibliographical courses. The arrows refer to illustrations in Fig. 5

Organs	Type
Wood (stems)	1. green
• dominant colour of leaves that are not fully developed on the terminal shoot (equilibrium between chlorophyll and anthocyanin pigmentations)	2. dark green with highlights 3. light purple 4. dark purple
• time of impregnation (duration of coloration expressed in number of leaves coloured)	0 to 12 (generally 5 or 6)
• colour of the young part of the stem that is not harvested	1. green 2. yellow-green 3. green and beginning of petiole red 4. green and beginning of petiole red with red ribs 5. green and red in equal area 6. some traces of green 7. entirely light red 8. dark red to purple-brown
• colour of stem (old part) at 1 and eventually at 2 years	1. ash grey to dark green 2. olive green 3. mahogany 4. dark brown
• shape of stem at the end of the cycle in relation with ramification linked to flowering of the terminal vegetative meristem	1. rampant (more than 6 flowerings) 2. spread out (4 to 6 flowerings) → A1 3. tall and spread out (2 or 3 flowerings) → A2 4. erect (1 late flowering) → A3 5. cylindrical (no flowering)
• lateral ramifications (development of lateral buds by lifting of apical dominance)	1. absence 2. presence with:

(Contd.)

(Contd.)

Organs	Type
	2a. erect shape (parallel to stem) 2b. drooping (stem spread out and drooping)
• *average number of branches developed at each flowering*	1. *2 branches* 2. *3 branches* 3. *4 branches* 4. *more than 4 branches*
• *angle of gap between branches developed by flowering*	1. *none* 2. *15° to 30°* 3. *45° to 60°* 4. *75° to 90°*
• *total height of the main stem*	*in cm*
• *time taken for the first inflorescence to appear*	*in days after planting*
• *height of first branch at flowering*	*in cm*
• *number of stems per plant obtained by cutting (noting the mode of planting)*	*number*
Nodes and internodes on stem	
• colour of eye (bud) at latent stage	1. green 2. coloured base and green scales 3. entirely coloured
• emergence of eye (bud) at latent stage	1. deep → B1 2. projecting → B2
• small cushion (bulge that bears leaf scar at the spot where petiole was inserted) with dentate stipules on both sides	1. ephemeral teeth → B3-B4 2. persistent teeth → B3-B4
• dimension of stipular roll (swelling of stipules and cushion)	in cm → B3-B4
• alignment of internodes (young part)	1. zigzag 2. straight
• striations on the young part of stems and ribs	1. ephemeral ribs 2. caducal ribs 3. persistent ribs
• vigour of stems (measured by the base diameter between two nodes)	in cm
Leaf	
• general shape	1. palmipartite (normal shape) 2. palmisequate (the lobes totally separate)
• dimension of petiole	1. sessile type (absent or less than 1 cm) 2. intermediate type (associated with the palmisequate form) 3. long and cylindrical type

(Contd.)

(Contd.)

Organs	Type
• angle at which petiole is attached to stem	1. less than 20° 2. 20° to 55° 3. 55° to 70° 4. 70° to 90° 5. 90° to 120° 6. more than 120°
• area in which deviation of petiole appears	1. submedian 2. median 3. subterminal 4. terminal
• coloration of petiole	1. entirely green 2. entirely yellow-green or light green 3. cross red at the base and the rest green 4. submedian part green and the rest red 5. submedian part green (representing half the length) and the rest coloured 6. red except part of the green submedian zone 7. vivid red, darker than the cross 8. dark red or purple-red
• length and diameter of petiole	5 to 60 cm and 1 to 5 mm
• pubescence of leaf hilum, part at the end of the petiole where principal lobes of the nerve converge	1. absent 2. slight 3. moderate 4. great
• dimension of leaf hilum	1. shrunken → C1 2. spread out → C2
• shape of leaf hilum	1. concave → C3 2. convex → C4
• number of lobes constituting limb, calculated on an average over 12 months of plant growth	1. less than 3 2. 3 to 5.5 3. 5.5 to 7 4. more than 7
• shape of lobes (L/W, ratio between length and width, measured on median lobe)	1. very narrow (L/W > 20) → C5 2. with parallel edges (L/W 6 to 20) 3. normal (L/W 4.5 to 6) 4. wide (L/W 3 to 4.5) → C6 5. rounded (L/W less than 3) with two widening points
• form of lobes defined by position of maximal widening point along median lobe (for types 3 and 4)	1. proximal (in first basal half of lobe) → C7 2. median (in middle of lobe) 3. submedian (between medium and three quarters)

(Contd.)

(Contd.)

Organs	Type
	4. terminal (beyond the last quarter towards the tip) → C8
• ornamentation of lobes	1. presence or absence of median swelling 2. presence or absence of spurs 3. incurved lamina 4. gondolate margins
• colour of upper surface of lamina	1. white without chlorophyll (partial or total albinos) or with motley colours 2. light green 3. yellow-green 4. dark green 5. purplish
• colour of underside of lamina	1. green 2. whitish-green 3. yellow-green 4. purple or red
• coloration of lamina nerves	1. always green 2. red on underside of young leaves 3. red on upper surface of young leaves 4. red on upper surface of adult leaves 5. red on underside of adult leaves 6. red on both sides of young leaves 7. red on upper surface of young or adult leaves 8. red on underside of leaves of any age 9. on both sides of young leaves and subsisting on underside of adult leaves 10. always on both faces
• lobe sinus (empty space separating two lobes)	closed to the extent that the number of lobes is high → C9
• basiliar sinus (angle formed between the central nerves of the two extreme or lower lobes taken on both sides of petiole)	1. closed (angle less than 180°) → C10 2. open (lobes at extreme rising, angle greater than 180°) → C11
• dimension of velum (surface of lamina at the confluence of extreme lobes at the level of basiliar sinus	1. band of 0.5 mm 2. less than diameter of petiole tip → C12 3. greater than diameter of petiole tip 4. wider than base of petiole 5. greater than twice the diameter of base of petiole → C13
• shape of velum	1. straight → C14 2. spread out

(Contd.)

(Contd.)

Organs	Type
	3. retracted 4. wrinkled → C15
• ornamentation of velum	1. none 2. dentate 3. stipulate → C16 4. fringed → C17
Inflorescence → D1	
• fructification of inflorescence	1. continuous (the first flowers are functional) 2. late (abortion of first inflorescences, fructification of later ones)
• form of sepals in calix of female flower	1. wide 2. medium 3. narrow
• colour of sepals of flower → D2	1. entirely green 2. green and coloured nerves 3. red and green 4. red to purple
• torus, roll with nectaries between calyx and pistil	1. yellow or yellowish 2. reddish 3. brown-red
• shape of six wings on the ovary with three carpels (corresponding to points of suture of carpels)	1. straight at any stage of fruit development 2. straight then sinuous → D3 3. sinuous then straight at maturity → D4 4. always sinuous
• colour of wings on the day the flower opens	1. entirely green 2. red
• colour of ovary body	1. green 2. red
• colour of stigmata on the day the flower opens	1. absent (white or light pink) 2. red
• presence of pollen in the male flower with calyx and torus (not used for classification) and androcate with 10 stamens	1. male flower sterile 2. male flower fertile
• colour of fruit (or capsule) with swelling of peduncle	1. green 2. light to red purple 3. predominantly red 4. entirely red
• *dimension of fruit (length and diameter)*	*in mm*
• colour of seed tegument → D5	1. grey 2. brown

(Contd.)

(Contd.)

Organs	Type
• mottles on seed tegument → D5	1. few 2. dense
• colour of caruncle of seed	1. white or cream 2. pink or red 3. purple
• *dimension of seed (length and diameter)*	*in mm*
Root	
• point of attachment on parent cutting	1. sessile 2. pedunculate 3. long pedunculate (greater than 10 cm)
• length of root	1. short (less than 40 cm) 2. normal (40 to 80 cm) 3. long (greater than 80 cm)
• diameter of root at its maximum	1. thin 2. medium 3. thick
• shape of root	conical → E1 fusiform → E2 cylindro-conical → E3 cylindrical → E4
• *constriction in roots*	1. *absent* 2. *present*
• direction of root	1. horizontal 2. vertical
• number of roots	number
• *homogeneity of weight of roots (numbers per weight class)*	*in %*
• *texture of root surfaces*	1. *smooth* 2. *medium* 3. *rough*
• appearance of external bark (cork)	1. grey and thin 2. brown and thick
• colour of phelloderm	1. white 2. pink 3. purple
• *detachment of phelloderm from central cylinder*	1. *easy* 2. *difficult*
• colour of pulp (or flesh, central cylinder)	1. white 2. yellow
• *index of precocity (dry matter yield of roots at 6-8 months in relation to dry matter yield of roots at 12-14 months)*	*in %*

(Contd.)

(Contd.)

Organs	Type
• *index of harvest (dry weight of tuberized roots in relation to total biomass of plant)*	*in %*
• presence of fibres	1. absent 2. some fibres visible 3. numerous
• taste (significance of release of hydrocyanic acid)	1. sweet 2. bitter, *or three classes defined by the intensity of coloration with picric acid: low, medium, high*

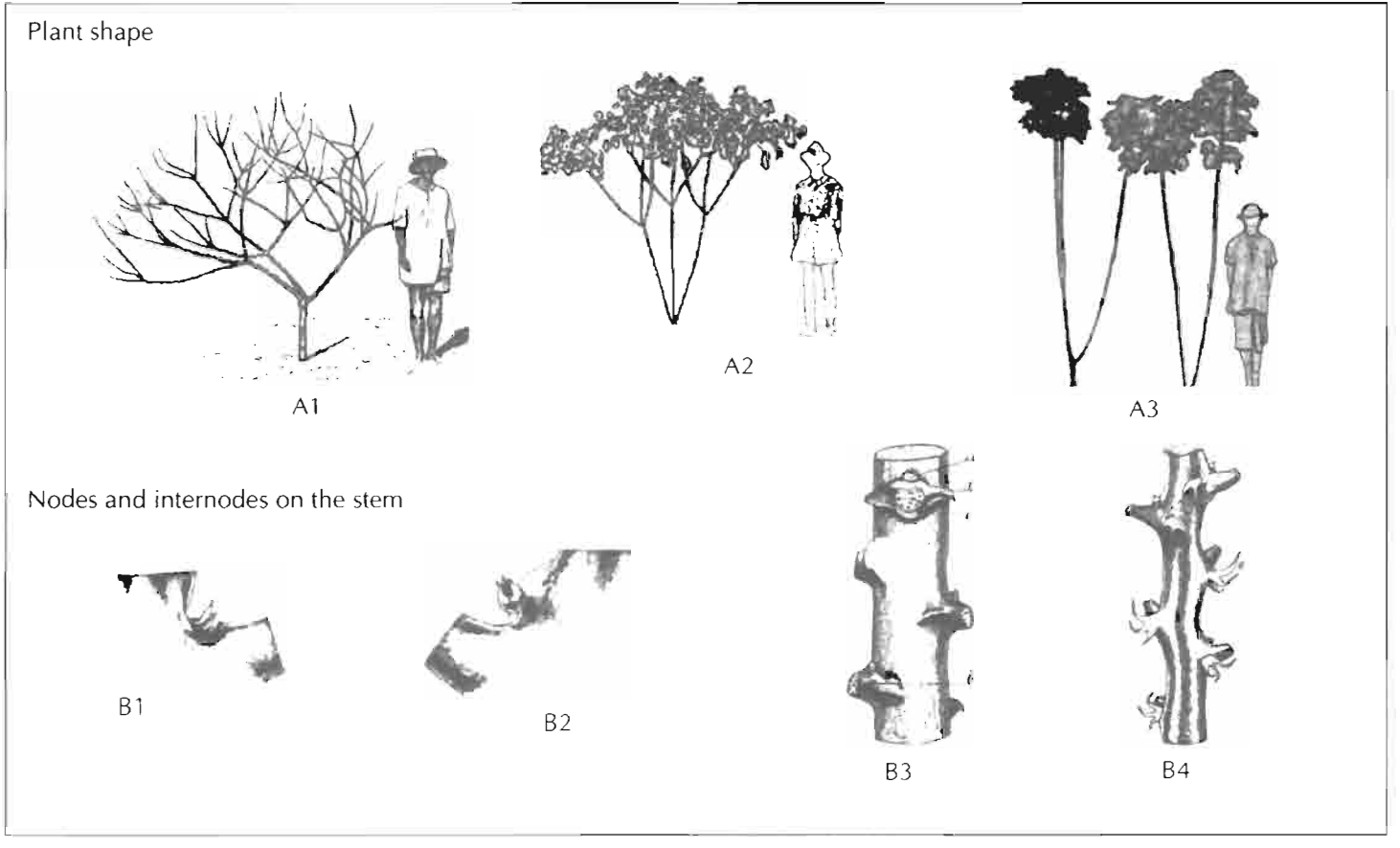


Fig. 5. Characters of vegetation, inflorescence, and roots of cassava used in differentiation of varieties (Cours, 1951)

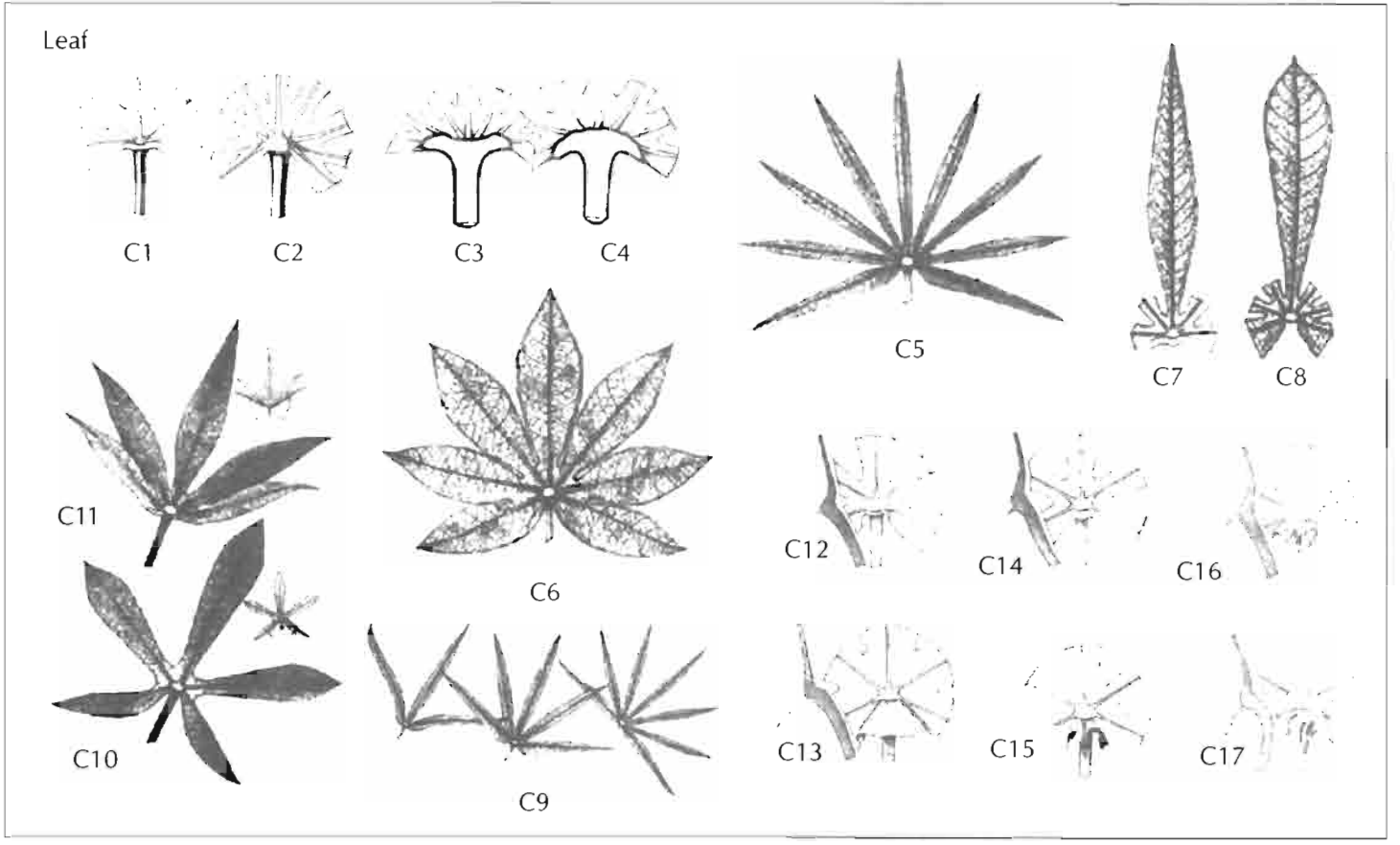


Fig. 5 (contd.)

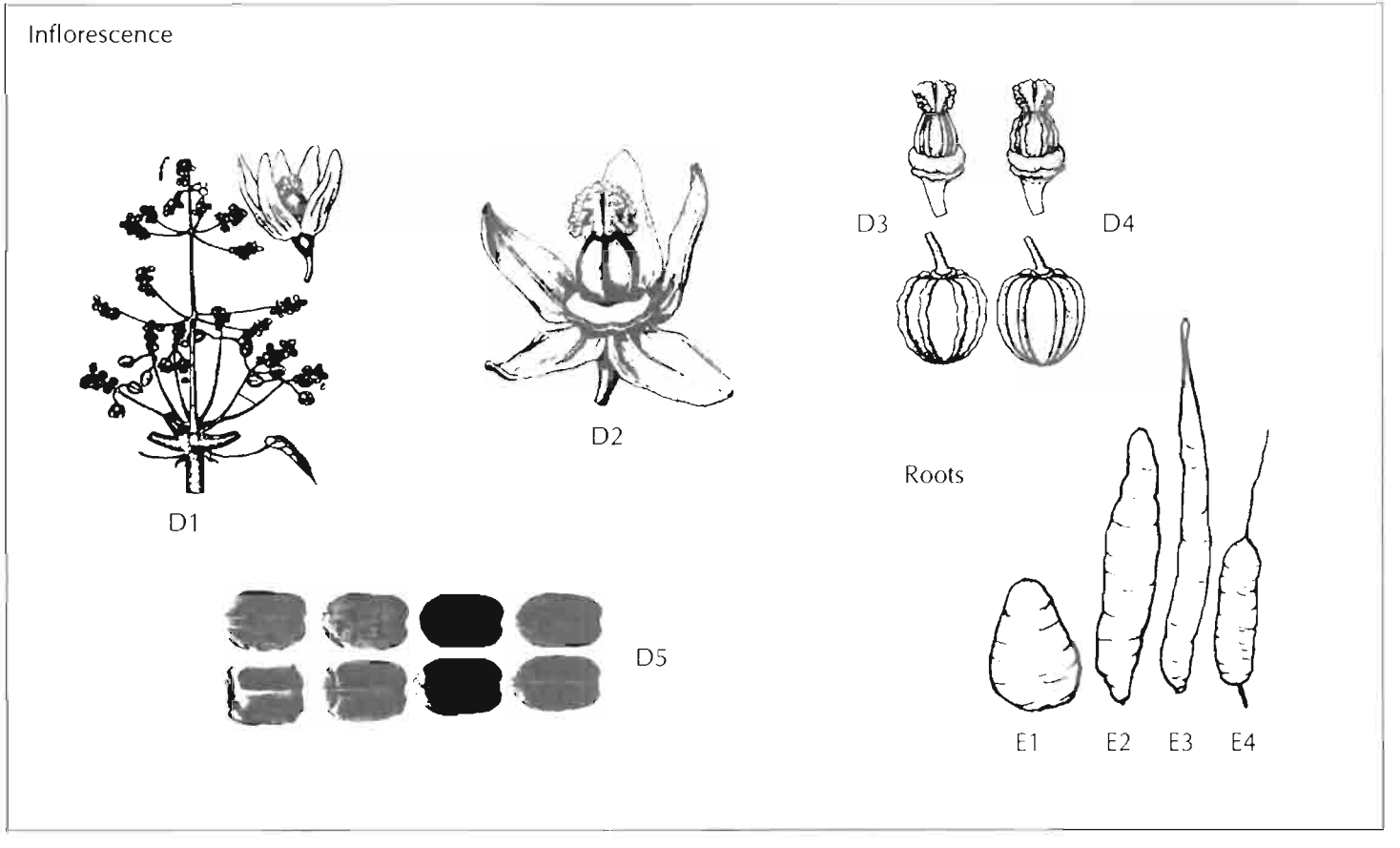


Fig. 5 (contd.)

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