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Molecular marker (AFLP)-based *Manihot* and cassava numerical taxonomy and genetic structure analysis in progress: Implications for their dynamic conservation and genetic mapping

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Abstract/Résumé

We are using multilocus molecular markers, with samples representing the geographic and ecological range of distribution of *Manihot* spp., along with classical botany and ecology, to characterize: 1) the genetic structure of cassava in relation to its wild relatives and 2) the domestication process of cassava. Not only a numerical taxonomy of the genus is expected but also the definition of strategies for the conservation and the utilization of these genetic resources. Amplification fragment length polymorphism (AFLP™) is used on: 1) 276 plants representing a large part of the diversity of the wild species of the genus, and some presumed natural inter-specific hybrids, and 2) 82 plants of cassava representing a) the cultivars in the world collections, and b) the varieties present in one Amazonian field with ethnobotanical record. The two first axis of variation (PCo of the matrix of similarity in pair-wise comparisons) extract, from the total, cassava along with several species that are closely related to it, including hybrids between *M. glazovii* and cassava. Although domestication appears to have involved primarily *M. esculenta* spp. *flabellifolia* and *peruviana*, it seems that some other species have also contributed. The question of the possible role of inter-specific hybridization in the domestication process of cassava is then raised again, particularly since the importance of inter-specific hybridization and also the high ratio of intra/inter-specific variation in the genus are confirmed. The genetic diversity of cassava itself is high, but a diversity nearly equal, although slightly divergent, is found in a single Amazonian field at the level of AFLP. It is shown that a traditional variety can include several related genotypes and the hypothesis of a dynamic management of the diversity of cassava according to the Amzonian tradition is confirmed. The results validate the methodology adopted but it should be desirable to apply it to a sampling even better representative of the genus. They also validate the choice of a strategy of dynamic conservation for the wild species. The important genetic recombination suggested to be at the origin of the diversity of cassava gives a favorable perspective for various strategies of genetic mapping and gene tagging, for this crop usually multiplied vegetatively.

Key wors/Mots clés: *Manihot*, cassava, AFLP, molecular markers, genetic resources, dynamic conservation, numerical taxonomy

Développement d'une taxonomie numérique et de analyse de la structure génétic de genre Manihot et du manioc par les marquers moléculaire (AFLP): Implications pour leur conservation dynamique et cartograhie génétique

Nous utilisons des marqueurs moléculaires multi-loci, sur un échantillonnage de plantes représentant autant que possible la distribution géographique et écologique des espèces considérées et en complément de la botanique et de l'écologie, pour caractériser: 1) la structure génétique du manioc en relation avec les espèces sauvages du genre *Manihot* et 2) le processus de domestication du manioc. Non seulement une taxonomie numérique du genre en est attendue mais aussi la définition de stratégies pour la conservation et l'utilisation de ces ressources génétiques. L'analyse du polymorphisme de longueur de fragments amplifiés (AFLP™) est utilisée. Les résultats présentés concernent un ou deux PK, sur: 1) 276 plantes représentant des une grande part de la diversité des espèces sauvages du genre, ainsi quelques hybrides inter-spécifiques spontanés et 2) 82 plantes de manioc choisies pour représenter a) les cultivars en collection dans le monde et b) les variétés présentes dans un seul champ amazonien. Les deux premiers axes de variation (PCo de la matrice de similarité en comparaisons 2 à 2) extraient, de l'ensemble du genre, le manioc et plusieurs espèces qui lui sont étroitement apparentées, dont les sous-espèces *M. esculenta* spp. *flabellifolia* et *peruviana* et des espèces moins étroitement apparentées mais qui forment des hybrides avec le manioc. La question du rôle des hybridations inter-spècifiques dans la domestication du manioc est ainsi reposée, d'autant que l'existance d'hybridations interspécifiques spontanées dans le genre *Manihot* est confirmée, ainsi qu'un rapport élevé de la diversité intra/inter-spécifique. La diversité génétique du manioc est forte mais une diversité sensiblement égale, bien que légèrement divergente, est trouvée dans un seul champ amazonien au niveau de ces marqueurs AFLP. Il est montré qu'une variété traditionnelle peut inclure plusieurs génotypes apparentés et l'hypothèse d'une gestion dynamique de la diversité du manioc selon la tradition indienne est confortée. Les résultats valident la méthodologie adoptée qu'il faudrait pouvoir appliquer à un échantillonnage encore plus représentatif de l'ensemble du genre. Ils confortent le choix d'une stratégie de conservation dynamique pour les espèces sauvages. La recombinaison génétique importante suggérée à l'origine de la diversité du manioc donne par ailleurs une perspective intéressante pour diverses stratégies de cartographie génétique ou de marquage de genes, pour cette plante généralement multipliée végétativement.

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Introduction

Using genetic multi loci molecular markers, with a plant material representative, as far as possible, of the geographical and ecological distribution of the species concerned, and in conjunction with botanical, ecological and artificial hybridization information, our work is directed towards understanding 1) the genetic structure of cassava in relation with the wild *Manihot* spp. and 2) the process of the domestication of cassava. We aim to derive important information to propose strategies for the conservation of *Manihot* genetic resources but also for their utilization, including modern concepts and biotechnology such as dynamic conservation, genetic mapping and gene tagging, as well as more traditional ones.

When we started this work in 1994, it was becoming clear (see CIAT 1995) that the molecular data are in favor of a Brazilian centered origin of cassava involving *M. esculenta* Crantz ssp *flabellifolia* (Pohl) Ciferri and ssp *peruviana* (Müller Argoviensis) Allem, as proposed by Allem (1994). However, the role of the intra- and inter-specific hybridization in the process of domestication of cassava had not been evaluated. We thus set as a goal to investigate the possibility that other species had played a role in the domestication of cassava and we favored a population biology approach (rather than a typological one), as well as a multi-loci approach.

Ethnobotanists have related that the diversity of cassava is very high in particular in the Rio Negro area of the Amazon basin and that it is classified by the Indians (and the Caboclos i.e. mixed blooded Indians), according to a large number of different varietal names. For example, Chernela (1987) found that the Tukano use 137 different names and Kerr (1980) reported 40 names with the Desana. They have also stressed a dynamic management of cassava diversity that involves exchanges or gifts of cuttings among Indian tribes, over long geographic distances, as well as the integration in varieties vegetatively propagated of plants originated from seeds. Recently, Emperaire et al (1997) observed that a single "roça" (traditional field) included 26 groups of plants with varietal names and that some plants were from direct germination of seeds, as recognized by their pivotal root. Some groups of plants supposedly originated from seeds are called "without name" by Caboclos, presumably until they are characterized.

The taxonomy of *Manihot* ssp. is contentious as can be judged by comparing the classification by A.C. Allem (unpubl.) and by Rogers and Appan (1973) as shown in Table 1 in annex. There is no doubt, however, that Brazil represents the main center of the diversity of the genus *Manihot*.

With the aim to be able to use precious herbarium specimens, we restricted our choice of molecular techniques to the PCR based ones and we concentrated on extracting DNA from dried leaves. Among PCR based techniques, AFLP™ is the technique of choice when it comes to interspecific comparison, because it is more specific than PCR primed in a purely arbitrary way such as in the RAPD technique. However, to be efficient in comparing many individuals from different experiments, in particular in the scoring of bands from different gels, AFLP needs a computer aided scoring of the bands. Analysis are still underway but the results of the first one or two primers enzymes combinations (PK) will be presented here.

Materials and Methods

Wild plant material successfully analyzed includes 264 plants representing all Brazilian species (> 40) and 18 plants representing 9 species from Paraguay, French Guyana, Venezuela, Colombia and Central America. Brazilian and Guyanean plants were all directly collected from the wild and either maintained as live plants or herbarium specimens (about 5/9 and 4/9, respectively). They were chosen so as to represent, as far as available, not only the various subspecies known, but also their range of geographical and ecological distribution. They included also 5 presumed natural inter-specific hybrids from 4 different populations, with representatives of the presumed parental species from the same populations. Other accessions were from the collections maintained in CIAT or Washington University that were introduced in Brazil.

Cultivated cassava includes 82 plants comprising 1) 40 plants chosen to be representative of a world collection according to Colombo (1997) 19 are from the "core of the core collection" as provided by Dr. M. Bonierbale from CIAT, 21 are extracted from collections held in Brazil after a larger representative set was analyzed for morphology and RAPD polymorphism), and 2) 42 plants representing a single Amazonian field in the Middle Rio Negro, including 18 varieties with a name (1 with 10 plants, 12 with two plants and 5 with 1 plant) and one variety "without name" with 3 plants.

DNA was extracted from dried young fresh leaves or from herbarium specimens, basically according to the method described in Colombo (1997), using 4% CTAB.

AFLP was performed according to Vos et al (1995) using the enzymes EcoRI and MseI by the commercial laboratory Linkage Genetics (Salt Lake City, USA). The software from Keygene was used to score the bands. The arbitrary sequences of the primers were chosen after screening 8 different +3/+3 combinations. The following ones were used: AGA/CAG when a single PK was analyzed and AGT/CTC for the other PK.

Data analysis. The bands were scored as present or absent and NTSYS-PC (Exeter software) was used for the statistical analysis using Simple Maching or Jaccard's similarity indexes and UPGMA or Principal Coordinate analysis of the matrices of similarities. STATISTICA (StatSoft) was used to draw part of the graphics.

Results

AFLP patterns and band scoring. DNA was successfully extracted from most plant material used, whether from freshly dried leaves or herbarium material kept for up to 20 years. It could be submitted with only a few exceptions to AFLP analysis and the DNA from a total of 364 plants was analyzed with one PK. Ninety three bands were scored and no band was found monomorphic across all samples, although several were present in nearly all samples. This lack of monomorphic bands was a difficulty for the computer assisted scoring of the presence-absence of the bands and the reproducibility of the scoring fell to around 85-90% for the 8 test samples repeated on each half gel (including each 46 samples in total). Only 4% of the scored points were considered as missing data.

***Manihot* spp diversity in relation to cassava.** The complete matrix of presence-absence of bands was used to compute both Jaccard and Simple Maching coefficient in pair-wise comparison of the genotypes. The resulting matrices of similarities were submitted to principal coordinate analysis (PCo) with the aim to identify the wild species related to cassava. Both coefficients are higly correlated but the Simple Matching one tends to result in a better evidenced structure when comparing the complete set of accessions. The Figure 1A shows the plan defined by the two first axis when using Simple Maching coefficient. The first axis extracts 15.3% of the total diversity while > 20 axis extract a part of the total variance larger than expected at random. It separates cassava from the rest of the genus analyzed. Five species appear very closely related to cassava, at least for some of the accessions. The wild forms of *M. esculenta* (spp. *flabellifolia* and

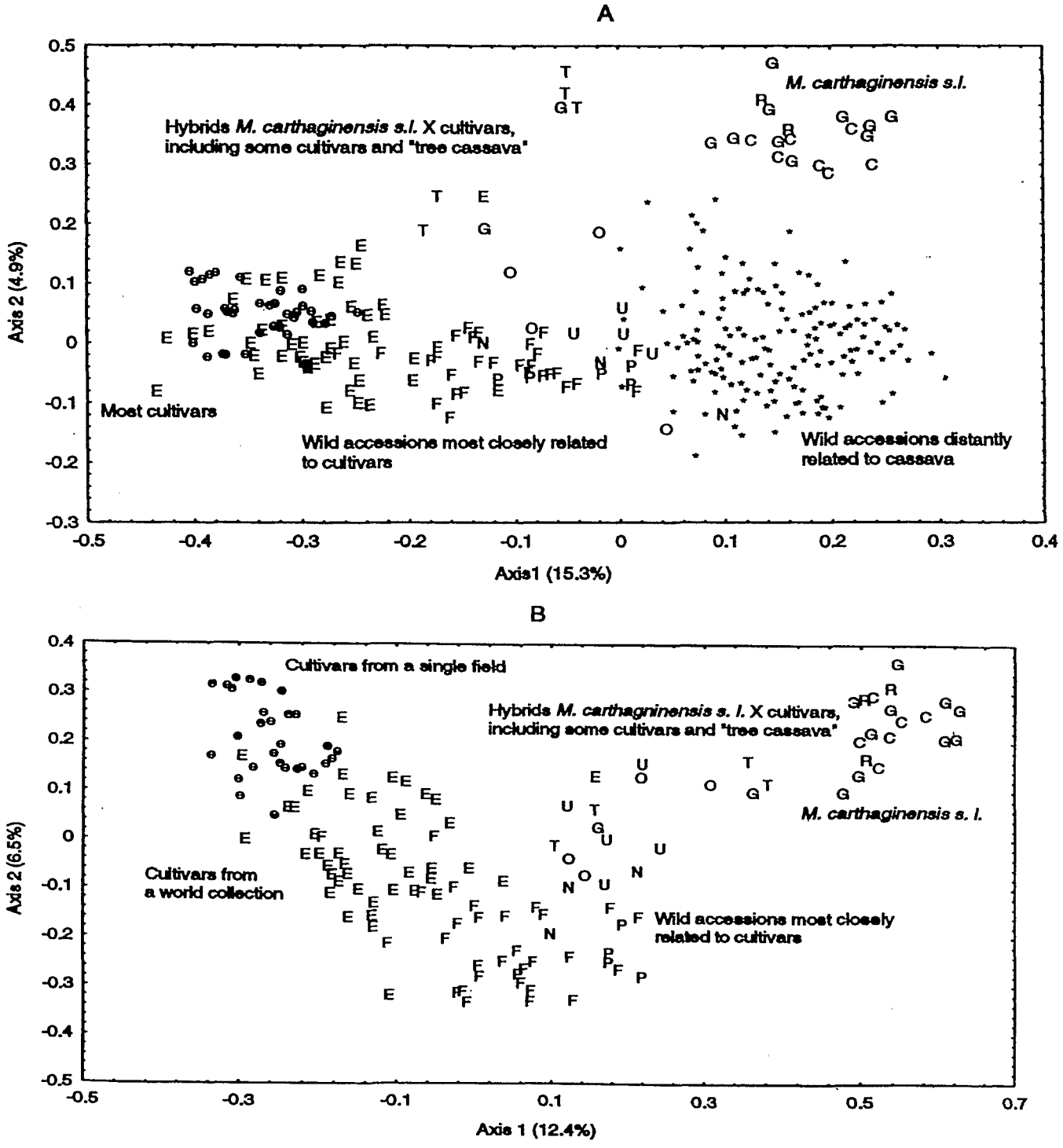

Figure 1. Distribution in the plan defined by the first two eigen-vectors (the percentage of variance extracted is indicated on the axis) of a principal coordinate analysis of the Simple Matching matrix of similarities in pair wise comparison of 364 accessions representatives of the genus *Manihot*, including cassava. Symbols represent species as follows: E and e = cassava from a world collection and a single Amazonian field, repectively; others symbols = wild species or hybrids. C = *M. carthaginensis*, F = *M. esculenta* ssp. flabellifolia and peruviana, G = *M. glazovii*, N = *M. pentaphylla*, O = *M. procumbens*, P = *M. pruinosa*, R = *M. epruinosa*, U = *M. fruticulosa*, T = "tree cassava" (see text). * = 196 accessions representing 49 other species included, plus 5 natural interspecific hybrids. In part A, all accessions are included. In part B, only the 168 accessions with specific symbols in part A, are included.

peruviana) were most closely related but *M. pruinosa*, *M. fruticulosa* (excluding the "caiaponia" form), one herbarium accession classified Cf. *M. pentaphylla* and three herbarium accessions of *M. procumbens* (from the state of Parana in Brasil and Paraguay) also appear closely related to cassava.

The second axis extracts 4.9% of the total variance and isolates the following species considered as conspecific by A.C. Allem (unpubl.): *M. carthaginensis*, *M. glaziovii* and *M. epruinosa*. Interestingly, some accessions of *M. glazovii* as well as some "tree cassava" and apparently one cassava accession ("manipeba de corda") appeared to be intermediate between cassava and the *M. carthaginensis* group, strenghtening the view of their presumed origin from hybridization with cassava. The rest of the genus was not resolved in Figure 1 and appeared, on further statistical analysis, difficult to classify in well isolated, robust groups of species. Accessions from the same species usually group relatively closely, but clear-cut separation between some of the species is difficult to find. We hope that the analysis of additionnal PKs will allow a clearer structuring. The Figure 2. shows, as an example, the diversity found within the *M. caerulescens* group, including 4 species. The UPGMA dendrogram shows that it involves a tremendous diversity while the PCo shows this diversity is in continuum between species. Interestingly, two accessions classified as intermediate between *M. caerulescens* and *M. quinquepartita* on the basis of the available morphological evidence are also found intermediate at molecular level, as shown in the PCo analysis. It should be also noted that the only one accession *M. aesculifolia* (H.B.K.) Pohl included was the most divergent from all the accessions studied.

Natural interspecific hybridization. Several presumed interspecific natural hybrids were included along representatives of the parental species from the same populations. As shown in Figure 3, the results so far confirmed the hybrid nature of those plants, in line with earlier claims of putative hybridization in this genus (Cruz 1968; Rogers & Appan 1973).

Diversity in *M. esculenta*. The Figure 1B gives more detail on the relationships among the species extracted by the two first axis in Figure 1A as most closely related to cassava, but the conclusions are the same as in Figure 1A. However, since we distinguished on Figure 1B the cassava accessions originated from a single Amazonian field, we notice that those accessions appear relatively very variable for being from the same field. Moreover, these Amazonian accessions appear to be the most divergent from the wild accessions considered, as if accessions, or maybe species, not represented in the present work would be necessary to explain their slight divergence.

In Figure 4 are shown 100 accessions representing only the wild and cultivated forms of *M. esculenta*. Two PKs were analyzed and 153 bands were consisdered. It confirms that the subspecies *flabellifolia* and *peruviana* do not seem to represent a strong divergence to one another and moreover that they both show a slight divergence with cassava. Though closely related to cassava, they do not explain alone its whole genetic diversity. Also, the large molecular diversity of the cassava from a single Amazonian field and its slight divergence from the rest of the world collection of cassava is confirmed. Cassava appears as a genetically diverse crop but with no strong genetic structure (in a PCo of a set of cassava cultivars, no eigen-vector extracts more variance than expected).

Diversity of cassava from a single Amazonian field. In Figure 5, only the cassava from the single field is analyzed with the same two PKs and with the aim of answering the two following questions: a) can two individual plants recognized under the same name present a genetic differentiation, and b) in the cases where they present a genetic differentiation, is it equivalent to the differentiation found in the field or is there a greater genetic relationship among plants grouped under the same name? To avoid considering a difference in banding

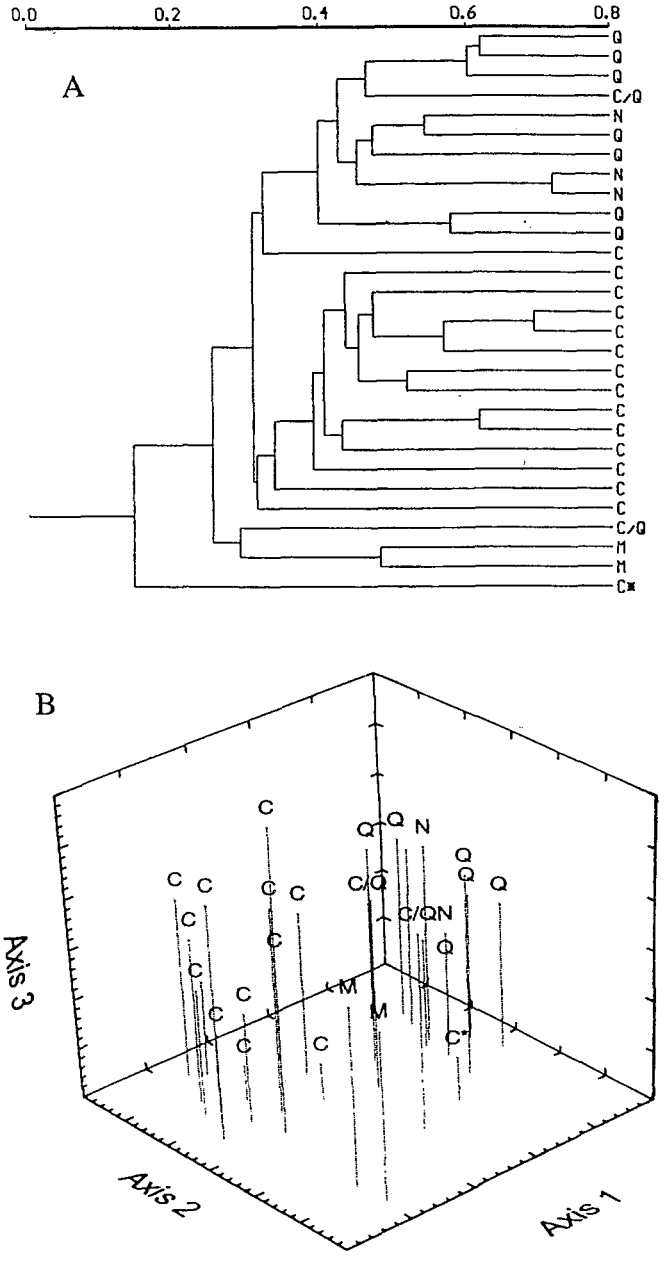

Figure 2. Analysis of the matrix of similarity (Jaccard's index) between 28 accessions representative of the *M. caerulescens* complex. C = *M. caerulescens* (* indicates an apparently divergent accession from state of Mato Grosso, near Bolivia), Q = *M. quinquepartita*, M = *M. compositifolia*, N = a form being described as a new species (Allem, unpubl.), C/Q: intermediate C and Q, based on the available morphological evidence. Part A: a UPGMA dendrogram (the values of Jaccard's index is indicated on the scale). Part B: a three dimensional plot of a principal coordinate analysis.

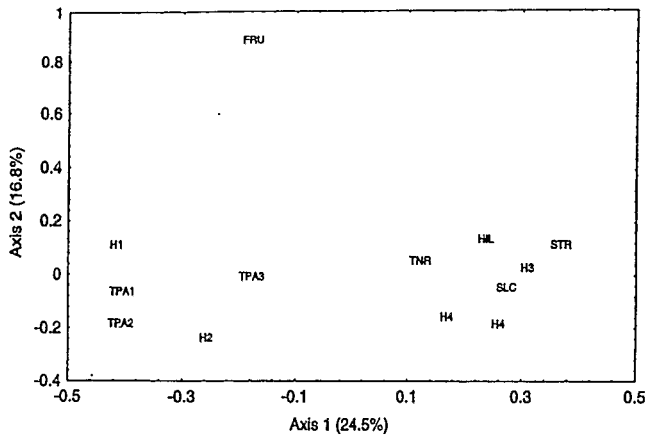

Figure 3. Distribution in the plan defined by the first two eigenvectors (the percentage of variance extracted is indicated on the axis) of a Principal Coordinate analysis of the Jaccard's matrix of similarities in pair wise comparison, of 13 accessions including 5 putative natural Brasilian interspecific hybrids found in 4 populations and a representative for each of their putative parental species collected from the same populations. FRU= *M. fruticulosa*, HIL= *M. hilariana*, TNR= *M. tenerrima*, TPA= *M. tripartita*, SLC= *M. salicifolia*, STR= *M. stricta*. H = putative hybrids: H1 = FRU and TRA1, H2= TEN and TRA2, H3= SLC and HIL, H4= two plants STR and TRA3.

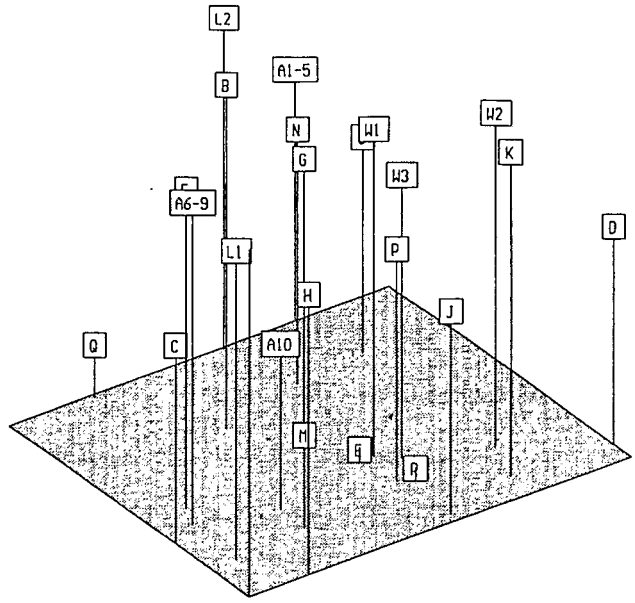

Figure 5. Distribution, in a three dimensionnal space defined by the three first eigenvectors of a principal coordinate analysis of the Jaccard's matrix of similarities in pair-wise comparison, of 23 genotypes distinguished in an Amazonian field. The genotypes represent 18 traditionnal varieties named and one variety "without name". The letters stand for the names of the varieties (Hidden letters are F and I, W = "without name"). Three varieties include more than one genotype: A, L and W. The figures stand for the plant numbers representing each genotype (see text).

patterns due to a trivial reason (such as a missing data, a poorly amplified band, a "spurious band" etc.), data were re-scored by eye and only bands clearly segregating among accessions were considered (50 polymorphic bands in total). One individual for each of the 23 distinguished genotypes was used in the analysis. Some 11 varieties with two plants each could not be distinguished from clones in this way. However, 2 named varieties (A and L in Figure 5, with respectively 10 and 2 plants each) were composed of three and two genotypes each, respectively. The variety "without name" (W in Figure 5) had three genotypes for three plants. In Figure 5, there is a tendancy for a higher similarity between genotypes with the same name than could be expected at random.

Discussion

While still in progress, the above results validate the choice of the methodology adopted: it was valuable to include as

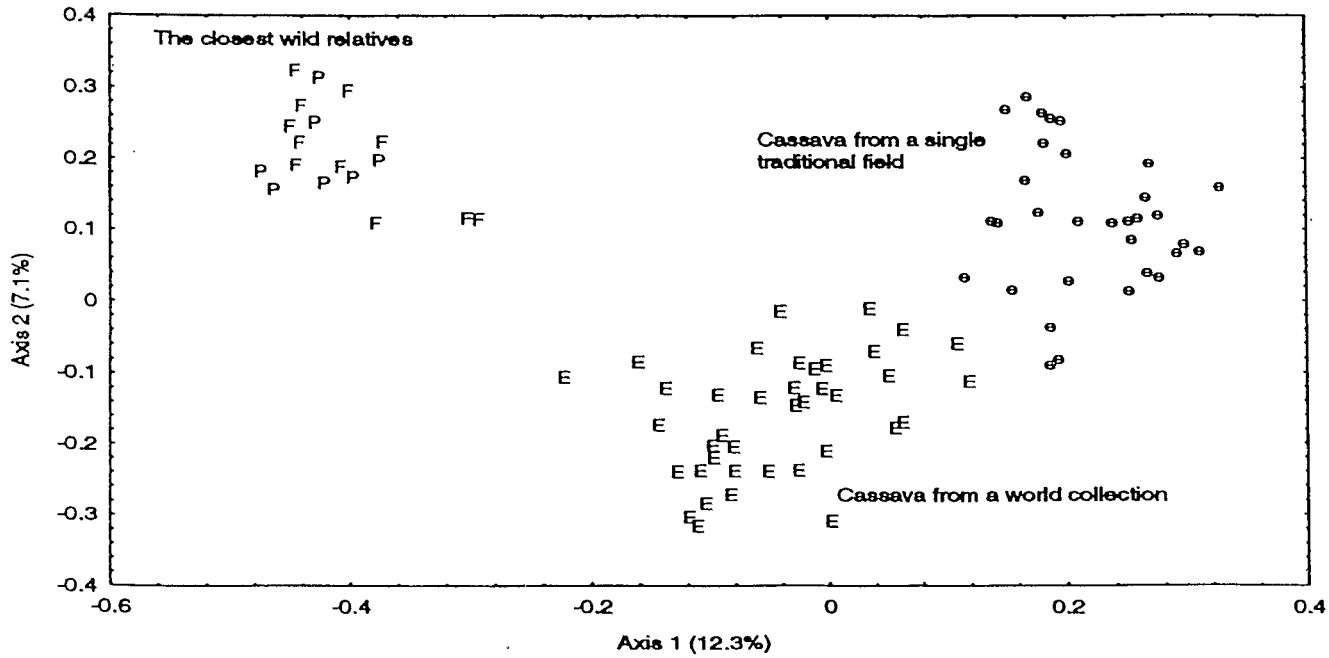

Figure 4. Distribution in the plan defined by the first two eigenvectors (the percentage of variance extracted is indicated on the axis) of a Principal Coordinate analysis of the Jaccard's matrix of similarities in pair wise comparison, of 100 accessions of the cultivated and wild forms of *M. esculenta*. E and e = same as in Figure 1, F and P = *M. esculenta* ssp. *flabellifolia* and *peruviana*, respectively.

much as possible a representative set (in term of taxonomy, geographical and ecological origins) of accessions for the whole genus *Manihot*, because some species appeared to be more closely related to cassava than previously assumed. These include in particular *M. procumbens*, a rare species tested at the moment only from herbarium specimen, *M. fruticulosa* and maybe *M. pentaphylla*, in addition to *M. pruinosa* and the wild strains of *M. esculenta* already known to be closely related. Moreover, we face the possibility that the collected diversity of the wild species cannot explain all of the diversity of cassava. It should thus be valuable to pursue the effort of gathering diversity from the whole genus, in particular from the Amazonian area and from other countries, both in South and Central America. An effort on collecting more of the most closely related species should be made.

Also, a population and a multi-loci approach seems appropriate to evaluate the structure of the genetic diversity in the genus *Manihot*, because of the large intra-specific diversity and of the possibility of interspecific hybridization playing a role in the evolution of this genus. Hopefully this work will help in deriving an authoritative taxonomy.

The molecular marker technology chosen was probably the best available at the moment. The reproducibility of AFLP allows to analyze newly collected material (or to verify doubtful results) in the same way and to compare the results of different experiments. Computer assisted scoring allows to read across various gels, which is nearly impossible by eye. An effort is however to be made to still increase the reliability of this scoring. It should then be relatively easy to built up a data base of the AFLP polymorphism of *Manihot* spp. to assist in the species determination, whether from fresh material or poorly representative herbarium specimen, or from *in vitro* material. Such a tool should be invaluable to assist both, the research on the genome of *Manihot* and the *ex-situ* conservation of the various species, given the difficulty of the taxonomic determination by non specialists (and also by a specialist when the material is out of its natural environment and/or poorly represented).

Even if preliminary, the genetic analysis of the diversity of the traditional varieties from a single Amazonian field is consistent with the ethnobotanical record, both in the accumulation of a large diversity and in the use of genetic recombination through the integration, in the varietal set, of plants originated from seeds (Emperaire et al. 1997). Apart from this validation, these observations provoke three remarks:

1. the power of molecular markers to evidence poorly represented areas in a world collection of cultivars was evidenced;
2. the limit of the molecular markers AFLP (we have verified that the results are the same with RAPD, C. Colombo 199) is, however, evidenced, to compare at a fine scale the genetic diversity present in various collections or sub-collections of cassava. This has also a bearing on the validation of a core collection, when using the same markers, as follows:

While a very large part of the genetic diversity present in a representative set of a world collection was also present in a single roça at the level of the molecular markers, it cannot be concluded that the roça represents anything close to a core of the world collection from the standpoint of useful variation. Although the morphological diversity, as present in the roça, is sufficient for the Indians (or "caboclos") to distinguish a large number of varieties, this morphological diversity is smaller in the roça than in a world collection (unpublished). We propose the following interpretation. The mutations evidenced by AFLP (restriction polymorphism) originated in ancient events in the phylogenetic lineages that lead to cassava. Hence, they do not tag, for the most part, more recent mutations that have been selected in the course of domestication. To better tag a finer genetic differentiation, mutations occurring more frequently (i.e., from an adaptatively neutral diversity point of view, that are showing a larger allelic diversity) are necessary. Microsatellites, with generally a high allelic diversity, look a fine prospect in this regards.

3. We would like to point out a possible unexpected outcome of the present study. Since an Amazonian field represents nearly a world diversity for cassava at the level of AFLP and since this diversity is managed in a way that includes genetic recombination, it should be possible to use such a population of genotypes to derive a molecular genetic map that would include a large part of all allelic variants in a world collection. AFLP easily allow to score a very large number of markers in a given mapping population. A degree of genetic linkage could be derived from a high frequency of co-occurrence, even if the mapping population is not segregating in a simple fashion. The validity of this approach could be tested by using markers closely linked from the currently available genetic map (Fregene et al, this meeting) before proceeding with a large number of unmapped markers.

More generally, the recurrent genetic recombination suggested to be at the origin of the domestication process of cassava gives a perspective, for gene tagging strategies in particular, that is far from the perspective envisionned previously for this vegetatively propagated crop. It also validates a possible strategy of conservation and distribution of the cultivar diversity through seeds.

The results strenghten the view that the process of domestication of cassava has involved a dynamic management of its diversity, building its genetic base to a level allowing its present success in various agricultural systems of the tropical world. In the same way, a dynamic conservation strategy for the wild species of *Manihot* (Second and Mendes 1996) not only appears as a valid practical approach but also is in line with the build up of the genetic structure of the genus so far evidenced: large intra *vs* inter-specific diversity, interspecific hybridization. The management of a dynamic approach for conservation can be adapted to various situations. For example, in the geographical area of origin of the species, the mixing in the collection fields of accessions from various populations can be restricted toaccessions originated a relatively small area with homogeneous ecological conditions. On the other hand, when it comes to adapting various species to different continents or numerous ecological situations widely different from the original ones, a much larger mixing of genetic diversity could be crucial to a successful establishment of the population in the long term.

Annex Table 1. Comparison of the systematics of genus *Manihot* (species found in Brasil only) as classified i sections by Rogers & Appan (1973) and in groups by A. C. Allem (in preparation).

Rogers & Appan species (76) (subspecies)	A. C. Allem species (40) (ssp/var)	Groups
M. sect Heterophyllae		
M. zehntneri	*M. caerulescens*	*XIV*
M. marajoara	*M. esculenta (flabellifolia)**	*V*
M. surinamensis	*M. esculenta (flabellifolia)**	*V*
M. tristis (tristis)	*M. esculenta (flabellifolia)**	*V*
M. tristis (saxicola)	*M. esculenta (flabellifolia)**	*V*
M. tristis (surumuensis)	*M. esculenta (peruviana)**	*V*
M. janiphoides	*M. janiphoides*	*VI*
M. grahami	*M. grahamii* +	*XIV*
M. inflata	*M. grahamii* +	*XIV*
M. pilosa	*M. pilosa**	*VI*
M. corymbiflora	*M. pilosa**	*VI*
M. leptopoda	*M. pilosa**	*VI*
M. quinquefolia	*M. caerulescens*	*XV*
M. jolyana	*M. janiphoides*	*VI*
M. handroana	*M. janiphoides*	*VI*
M. pohlii	*M. pohlii*	*IV*
M. sect Anisophyllae		
M. anisophylla	*M. carthaginensis*+	*XIV*
M. guaranitica (guaranitica)	*M. carthaginensis*+	*XIV*
M. guaranitica (boliviana)	*M. carthaginensis*+	*XIV*
M. sect Carthaginenses		
M. carthaginensis	*M. carthaginensis*+	*XIV*
M. filamentosa	*M. carthaginensis*+	*XIV*
M. sect Quinquelobae		
M. acuminatissima	*M. sagittato-partita*	*IX*
M. sagittato-partita	*M. sagittato-partita*	*IX*
M. xavantinensis	*M. tripartita (xavantinensis)*	*X*
M. sparsifolia	*M. sparsifolia*	*XII*
M. falcata	*M. hilariana*	*I/II*
M. pruinosa	*M. pruinosa**	*V*
M. quinqueloba	*M. quinqueloba*	*VIII*
M. alutacea	*M. alutacea*	*XII*
M. violacea (violacea)	*M. violacea*	*XI*
M. violacea (recurvata)	*M. violacea*	*XI*
M. jacobinensis	*M. violacea (jacobinensis)*	*XI*
M. divergens	*M. violacea (divergens,* var)	*XI*
M. irwinii	*M. irwinii*	*XI*
M. cecropiaefolia	*M. violacea (cecropiaefolia,* var)	*XI*
M. mossamedensis	*M. mossamedensis*	*IV*
M. sect Graciles		
M. flemingiana	*M. flemingiana*	*IV*
M. hunzikeriana	*M. hassleriana*	*III*
M. hassleriana	*M. hassleriana*	*III*
M. triphylla	*M. triphylla**	*II*
M. fruticulosa	*M. fruticulosa*	*II*
M. pentaphylla (pentaphylla)	*M. pentaphylla*	*XI*
M. pentaphylla (rigidula)	*M. pentaphylla?*	*XI*
M. pentaphylla (tenuifolia)	*M. pentaphylla?*	*XI*
M. pentaphylla (tenuifolia)	*M. tenerrima*	*II*
M. tenella	*M. ?*	*?*
M. gracilis (gracilis)	*M. gracilis*	*II*
M. gracilis (varians)	*M. tripartita*	*X*
M. paviafolia	*M. pentaphylla (paviafolia)*	*XI*
M. sect Sinuatae		
M. anomala (pubescens)	*M. anomala*	*X*
M. anomala (anomala)	*M. anomala*	*X*
M. anomala (cujabensis)	*M. anomala*	*X*
M. anomala (glabrata)	*M. anomala*	*X*
M. anomala (pavoniana)	*M. anomala*	*X*
M. warmingii	*M. tomentosa*	*IV*
M. sect Variifoliae		
M. mirabilis	*M. anomala*	*X*
M. variifolia	*M. anomala*	*X*
M. sect Glaziovianae		
M. glaziovii	*M. carthaginensis*+ *(glaziovii)*	*XIV*
M. pseudoglaziovii	*M. carthaginensis*+ *(glaziovii)*	*XIV*
M. epruinosa	*M. carthaginensis*+ *(glaziovii)*	*XIV*
M. brachyandra	*M. ?*	*?*
M. maracasensis	*M. pohlii*	*IV*
M. catingae	*M. carthaginensis*+ *(glaziovii)*	*XIV*
M. dichotoma	*M. dichotoma*+	*XIV*
M. sect Peruvianae		
M. brachyloba	*M. brachyloba*	*VII*
M. leptophylla	*M. ?*	*V*
M. quinquepartita	*M. quinquepartita*	*XV*
M. peruviana	*M. esculenta (peruviana)**	*V*
M. sect Crotalariaeformes		
M. procumbens	*M. procumbens*	*III*
M. affinis	*M. ?*	*?*
M. reptans	*M. Cf pruinosa**	*V*
M. crotalariaeformis	*M. tripartita*	*X*
M. sect Stipulares		
M. stipularis	*M. stipularis*	*I*
M. pusilla	*M. longepetiolata pusilla*	*I*
M. oligantha	*M. ?*	*?*
M. longepetiolata	*M. longepetiolata (longepetiolata)*	*I*
M. nana	*M. longepetiolata (nana)*	*I*
M. sect Grandibracteatae		
M. tomentosa (tomentosa)	*M. tomentosa*	*IV*
M. tomentosa (araliaefolia)	*M. tomentosa*	*IV*
M. sect Brevipetiolatae		
M. stricta	*M. stricta*	*XIII*
M. purpureo-costata	*M. purpureo-costata*	*XIII*
M. salicifolia	*M. salicifolia*	*XIII*
M. attenuata	*M. salicifolia*	*XIII*
M. weddelliana	*M. salicifolia*	*XIII*
M. orbicularis	*M. purpureo-costata*	*XIII*
M. sect Peltatae		
M. peltata	*M. peltata*	*VIII*
M. populifolia	*M. anomala*	*X*
M. reniformis	*M. reniformis*	*VIII*
M. sect Tripartitae		
M. tripartita (tripartita)	*M. tripartita*	*X*
M. tripartita (humilis)	*M. tripartita*	*X*
M. tripartita (vestita)	*M. tripartita*	*X*
M. tripartita (laciniosa)	*M. tripartita*	*X*
M. sect Caerulescentes		
M. caerulescens (caerulescens)	*M. caerulescens*	*XV*
M. caerulescens (paraensis)	*M. caerulescens*	*XV*
M. caerulescens (macrantha)	*M. caerulescens*	*XV*
	New species	
	M. compositifolia	*XV*
	M. diamantinensis	*?*
	M. gabrielensis	*IV*
	M. nogueirae	III

N.B. The distribution is generally in accordance with Rogers and Appan (*M. caerulescens* is an exception). * According to Allem: species morphologically most closely related to cassava, + *M. carthaginensis* s. *lato* (Incl. *glaziovii* and *epruinosa*) and close relatives: large discrepancies between the two classifications can be noted, both at the supraspecific and infraspecific levels.

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Phylogenetic relationships and genetic diversity in *Manihot* species

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Abstract/Résumé

The phylogenetic relationships among species of the genus *Manihot* has been actively debated. Resolution of species affinities is of critical importance since the domesticated species, *Manihot esculenta*, cassava, provides an important source of carbohydrates for people throughout the tropics. We have investigated the species relationships within the genus *Manihot* by a molecular phylogeny reconstruction and by an analysis of RAPD data. Gene sequences used for phylogeny reconstruction include the ITS-I and ITS-2 regions of nuclear ribosomal DNA, calmodulin, linamarase, and aspartate amino transferase. Genomic DNA is isolated from either fresh or dried leaf material, DNA sequences are amplified by PCR and PCR products are directly sequenced. Sequence data are analyzed using the computer program PAUP while RAPD data are analyzed by NTSYS. DNA analysis indicates a South American origin for cassava with M. *esculenta ssp.flabellifolia* and *M. esculenta ssp. peruviana* being the nearest wild relatives. Several gene sequences are identical among these two subspecies and cassava, indicating close affinities and most likely, a recent common ancestor. Thus, particular effort should be made to collect and preserve germplasm from ssp. *flabellifolia* and ssp. *peruviana* for future use in cassava breeding programs.

Keywords/Mots clés: cassava, phylogeny, *Manihot* species, genetic diversity

Relations phylogénétiques et diversité génétique parmi les espèces de manioc

Les relations phylogénétiques parmi les espèces du genre *Manihot* ont été activement débattues. La résolution des affinités spécifiques est d'une importance cruciale du fait que l'espèce domestiquée, *Manihot esculenta*, le manioc, constitue une importante source d'hydrates de C pour les populations des régions tropicales. Les auteurs ont analysé les relations spécifiques au sein du genre *Manihot* par une reconstruction de la phylogénie moléculaire et par une analyse des données RAPD. Les séquences de gène utilisées pour la reconstruction de la phylogénie comprennent les régions ITS-1 et ITS-2 de l'ADN ribosomique nucléaire, la calmoduline, la linamarase, et l'aspartate amino transférase. L'ADN génomique est isolé de matériel foliaire frais ou séché, les séquences d'ADN sont amplifiées par PCR et les produits PCR sont directement séquencés. Les données de séquence sont analysées au moyen du programme informatique PAUP, tandis que les données RAPD sont analysées par le logiciel NTSYS. L'analyse de l'ADN indique que le manioc est originaire d'Amérique du Sud, *M. esculenta* sous-espèce *flabellifolia* et *M. esculenta* sous-espèce *peruviana* étant les parents sauvages les plus proches. Plusieurs séquences de géne sont identiques parmi ces deux sous-espèces et le manioc, ce qui indique de grandes affinités et très probablement, un récent ancêtre commun. Dès lors, un effort particulier devarit être fait pour récolter et préserver le matériel génétique des sous-espèces *flabellifolia* et *peruviana* afin de les utiliser, dans l'avenir, dans des programmes d'amélioration du manioc.