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Assessment of genetic variability in a traditional cassava (*Manihot esculenta* Crantz) farming system, using AFLP markers

M. ELIAS*†, O. PANAUD‡ & T. ROBERT‡

†*CEFE-CNRS, 1919 Route de Mende, 34293 Montpellier Cedex 5, France,* ‡*Evolution et Systématique, bâtiment 360, Université Paris-Sud, 91405 Orsay cedex, France*

Despite the urgent need to conserve domesticated plant genetic resources, and developing 'on farm' strategies of conservation, the impact of traditional farming practices and of their interaction with ecological factors on the structure and evolutionary dynamics of the genetic variability of crop populations has been little documented. We assessed the genetic variability of 31 varieties of cassava (*M. esculenta* Crantz) traditionally grown by Makushi Amerindians from Guyana, using AFLP markers. We used a sample of 38 varieties from an *ex situ* core collection as a reference. Accessions of wild cassava were also included. While clonality of the varieties was expected due to the vegetative propagation of cassava, 21 varieties presented intravarietal polymorphism. Among the varieties from a single site in Guyana, genetic diversity was the same as that in the accessions from the core collection. We suggest that incorporation of volunteer seedlings, produced by sexual reproduction, into the stock of varieties grown by the Makushi plays a major role in explaining both intravarietal polymorphism and the high level of genetic diversity. No correspondence was found between the structure of molecular diversity and variation observed for agronomic traits that are targets for selection by cultivators. As found in previous studies, all wild forms of cassava clustered together and separately from the cultivated varieties in a Neighbour-Joining dendrogram. These results are consistent with the hypothesis of a limited domestication event in a restricted area, followed by rapid diffusion of cultivated phenotypes and convergent evolution. Our results show that local varieties are an important source of genetic diversity, and highlight the importance of the interaction between human and ecological factors in the dynamics of this diversity.

Keywords: AFLP, cassava, domestication, genetic variability, *Manihot*, traditional farming.

Introduction

Defining the scientific bases from which efficient strategies for conserving biological diversity can be developed, still remains a challenge for the scientific community. A wide range of theoretical and empirical knowledge about wild plant and animal populations, has led to conservation strategies, but equivalent information is missing for populations of domesticated crops. Defining plans for 'on farm' or *in situ* conservation of plant genetic resources requires assessment of the pattern of both molecular and phenotypic diversity and of processes that drive evolution of crop populations (Miller *et al*., 1995). Traditional agrosystems are of particular interest because crop diversity within them is generally high. It is common to find numerous varieties in the same field. Moreover, related and interfertile wild forms sometimes occur sympatrically. Such situations offer opportunities for studying the effects of gene flow, disruptive selection and genetic drift in crop populations, which often are enmeshed in crop/wild/weed complexes (De Wet & Harlan, 1975). Conservation of traditional landraces must take into account the peculiarities of man-made environments, in which human action not only shapes landscapes but also is an important selective factor acting on populations of landraces.

Our research programme aims to evaluate the impact of traditional agricultural practices on varietal and genetic diversity, using cultivation of cassava (*Manihot esculenta*) by Amerindians as an experimental model. Cassava, a vegetatively propagated crop, is widely cultivated in traditional farming systems in Amazonia (McKey & Beckerman, 1993; Salick *et al*., 1997;

*Correspondence. E-mail: elias@cefe.cnrs-mop.fr

Emperaire *et al*., 1998) where it exhibits a huge varietal and genetic diversity (Colombo, 1997; Second *et al*., 1997). This paper reports an assessment, using molecular markers, of the organization of genetic variability of cassava landraces in a single village of Makushi Amerindians in Guyana, South America.

Cassava is the Makushis' staple crop. Makushi Amerindians practice slash-and-burn agriculture, with a cycle of two years of cultivation (2 crops) followed by one year of fallow. Every year, each farmer prepares a new field from secondary forest or fallow, and burns it. Cassava is propagated by means of stem cuttings, which are planted in the new field (first crop). In this study, AFLP markers were used to characterize the genotypes of a sample of the varieties present in the village studied, in order to: (i) assess the level of intravarietal diversity; (ii) study the relationship between the organization of genetic variability and the local taxonomy, and also the relationship between genetic variability and the diversity of four agronomic traits (degree of bitterness, colour of the root, dryness of the root and length of cultivation cycle), which are targets for selection by farmers; (iii) compare the diversity found in the village with that of a sample from a worldwide *ex situ* core collection [from the International Center for Tropical Agriculture (CIAT, Cali, Colombia)]; (iv) assess the level of genetic similarity of the local varieties and local and exogenous wild forms of cassava.

Materials and methods

Plant material from Guyana and sampling procedure

Varietal diversity and traditional farming practices were studied in Rewa, a 30 household Makushi village in Guyana (Fig. 1) which grows 76 cassava varieties. Hereafter the term 'variety' refers to the unit identified by farmers under a single name. Each variety is recognized by farmers on the basis of morphological features (Elias, 2000). Interviews and questionnaires were conducted, in which farmers were asked to

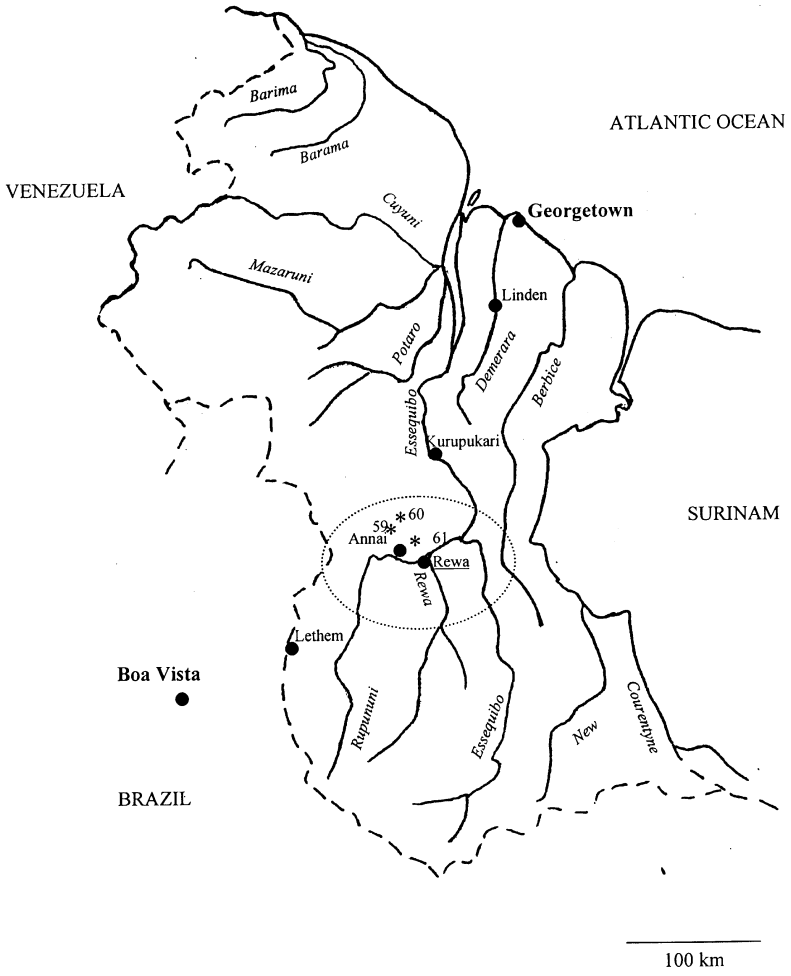

Fig. 1 Map of Guyana, showing location of Rewa, the Makushi community where the study was conducted, and of the three populations of wild cassava (*M. esculenta* ssp. *flabellifolia*) included in the analysis (symbolized by *, followed by the population code number).

characterize each variety they grew (Elias, unpubl. data). Based on the answers, we classified each variety by delimiting *a priori* classes for each of the following traits, which constitute four major agronomic groups: the colour of the root; the starch content of the root; the degree of bitterness (associated with cyanide content); and the length of the cultivation cycle (Table 1).

Of the 76 varieties grown in Rewa, 31 were chosen for AFLP analyses (Table 2). The varieties chosen represent each of the major agronomic groups defined above, and include both frequent and rare varieties. For each variety, three to five plants were collected, from one or several farmers depending on the variety. The sampling strategy therefore focused mainly on intervarietal diversity. Wild cassava populations (*M. esculenta* ssp. *flabellifolia*) (A.C. Allem, EMBRAPA Recursos Genéticos e Biotecnologia, Brazil, pers. comm.), are also present in the same region. Plants from three very small

Table 1 Classification in major agronomic groups and intravarietal genetic diversity among the cassava (*Manihot esculenta*) varieties grown by the Makushi in Rewa. Classes of all major groups are given for each variety, as well as the number of plants studied. The three last columns show, respectively, the number of different AFLP patterns detected for each variety (a variety is monomorphic when only one pattern was detected), the heterogenous polymorphic varieties (see text), and the varieties that present intravarietal polymorphism even within a farm

	Major groups*							
Variety	Degree of bitterness (4 classes)	Colour of the root (4 classes)	Starch content (5 classes)	Cultivation cycle (3 classes)	Number of plants	Number of different AFLP patterns	Heterogenous polymorphic varieties	Intravarietal intrafarm polymorphism
E 5	2	2	3	1	4	2		x
E 6	1	0	1	0	5	4	x	x
E 7	2	1	2	0	4	4	x	x
E 12	1	2	2	1	3	2	x	
E 14	2	3	0	1	5	2		
E 15	2	2	2	1	4	3	x	x
E 16	2	3	0	0	3	1		
E 17	2	2	2	1	5	2		x
E 18	2	3	0	1	3	2	x	x
E 19	1	0	3	1	4	1		
E 21	2	2	4	2	5	2	x	x
E 22	3	0	2	0	5	1		
E 23	2	2	2	2	4	2		
E 25	1	2	3	0	5	1		
E 26	2	2	3	2	5	1		
E 27	2	1	2	1	5	1		
E 28	2	?	2	1	5	1		
E 32	1	2	2	2	4	4	x	x
E 34	2	1	2	0	3	2	x	x
E 35	2	1	2	1	5	2		
E 39	1	0	2	0	5	1		
E 40	1	2	3	2	4	4		x
E 41	3	1	4	1	5	1		
E 43	2	0	2	1	4	2	x	
E 45	2	1	2	2	5	2	x	
E 47	2	0	3	0	3	2	x	
E 48	1	0	2	2	4	2		
E 49	2	3	0	0	4	3		x
E 50	2	2	2	1	5	1		
E 53	2	3	2	0	3	3		x
E 58	0	0	2	2	5	4		

* Degree of bitterness: 0, sweet; 1, half bitter; 2, bitter; 3, very bitter.
Colour of the root: 0, white; 1, cream; 2, yellow; 3, very yellow.
Starch content: 0, very watery; 1, watery; 2, half watery; 3, dry; 4, very dry.
Length of cultivation cycle: 0, short; 1, medium; 2, long.

Table 2 Accessions of cultivated cassava (*M. esculenta* ssp. *esculenta*) from Guyana used for AFLP analysis

Variety code	Makushi and English names	Individual code*	Variety code	Makushi and English names	Individual code	Variety code	Makushi and English names	Individual code	Variety code	Makushi and English names	Individual code
E 5	*amuru pîye* thick stick	E 5-1 (ne) E 5-3 (ne) E 5-4 (ne) E 5-5 (ne)	E19	*kasiri pîye* cassiri stick	E 19-1 (dh) E 19-2 (dh) E 19-3 (dh) E 19-4 (dh)	E 28	*maka pîye* maggah stick	E 28-1 (je) E 28-2 (je) E 28-3 (je) E 28-4 (je) E 28-5 (je)	E 43	*saprî pîye* fine fish stick	E 43-1 (ta) E 43-2 (ta) E 43-3 (ta) E 43-4 (ma)
E 6	*anra pîye* crane stick	E 6-1 (ne) E 6-2 (ne) E 6-3 (ne) E 6-4 (ma) E 6-5 (va)	E 21	*kiniˊ pîye* dry stick	E 21-1 (fp) E 21-2 (va) E 21-3 (va) E 21-4 (ta) E 21-5 (ta)	E 32	*meekoro pîye* black man stick	E 32-2 (ne) E 32-3 (ne) E 32-4 (jm) E 32-6 (jm)	E 45	*siya pîye* Shea stick	E 45-1 (ne) E 45-2 (ne) E 45-3 (ta) E 45-4 (ta) E 45-5 (ta)
E 7	*fatpoi pîye* Fat Boy stick	E 7-1 (wa) E 7-3 (dh) E 7-4 (dh) E 7-5 (ne)	E 22	*kuratuma pîye* caiman stick	E 22-1 (fp) E 22-2 (ta) E 22-3 (ta) E 22-4 (va) E 22-5 (fp)	E 34	*naman pîye* Naman stick	E 34-1 (wa) E 34-2 (va) E 34-3 (va)	E 47	*siment pîye* cement stick	E 47-1 (fp) E 47-2 (ta) E 47-3 (ja)
E 12	*eti pîye* Eddie stick	E 12-2 (wa) E 12-3 (ph) E 12-4 (va)	E 23	*kurarî pîye* curral stick	E 23-1 (va) E 23-2 (va) E 23-3 (va) E 23-4 (ch)	E 35	*papîro ye* Pablo stick	E 35-1 (am) E 35-2 (ta) E 35-3 (va) E 35-4 (va) E 35-5 (va)	E 48	*supra pîye* cutlass stick	E 48-2 (ma) E 48-3 (ch) E 48-4 (ne) E 48-5 (ta)
E 14	*eri pîye* Ely stick	E 14-1 (ne) E 14-2 (ne) E 14-3 (ne) E 14-4 (ne) E 14-5 (wa)	E 25	*isman pîye* Isman stick	E 25-1 (ph) E 25-3 (fp) E 25-4 (fp) E 25-5 (fp) E 25-6 (va)	E 39	*pîrîkwa pîye* bird stick	E 39-1 (ta) E 39-2 (ta) E 39-3 (va) E 39-4 (va) E 39-5 (ch)	E 49	*tarekaya pîmoi pîye* water turtle egg stick	E 49-1 (ch) E 49-2 (fp) E 49-3 (fp) E 49-5 (va)
E 15	*esekwipo ye* Essequibo stick	E 15-1 (jh) E 15-2 (jh) E 15-3 (va) E 15-5 (va)	E 26	*paranakîrî pîye itakon ye* white man stick cousin	E 26-1 (ne) E 26-2 (ne) E 26-3 (ne) E 26-4 (ne) E 26-5 (ne)	E 40	*paranakîrî pîye* white man stick	E 40-1 (ma) E 40-3 (je) E 40-4 (je) E 40-5 (ch)	E 50	*tikîrî pîye* ?	E 50-1 (dh) E 50-2 (dh) E 50-3 (dh) E 50-4 (dh) E 50-5 (dh)
E 16	five months stick	E 16-2 (ta) E 16-3 (ta) E 16-4 (ta)	E 27	*lio pîye* Lio stick	E 27-1 (wa) E 27-2 (wa) E 27-3 (wa)	E 41	*reni pîye* Reni stick	E 41-1 (dh) E 41-2 (dh) E 41-3 (dh)	E 53	*u'wi pîye* farine stick	E 53-1 (jm) E 53-2 (va) E 53-5 (fp)
E17	*sona pîje* Johna stick	E 17-1 (ne) E 17-2 (ne) E 17-3 (ne) E 17-4 (ne) E 17-5 (ne)			E 27-4 (wa) E 27-5 (wa)			E 41-4 (dh) E 41-5 (dh)			
E18	*kaima pîye* pumpkin stick	E 18-1 (ma) E 18-3 (ma) E 18-6 (ma)							E 58	*kana* sweet cassava	E 58-1 (ja) E 58-2 (ma) E 58-3 (fp) E 58-4 (fp) E 58-5 (fp)

* The two letters in parentheses are initials of the name of the farmer in whose farms the plant was collected.

populations of wild cassava located near Rewa were also analysed (Table 3). Their map locations are given in Fig. 1.

Material from the CIAT core collection

CIAT provided us with 38 accessions from a core collection of cultivated cassava, chosen to maximize the extent of diversity (Roa *et al*., 1997). We also obtained from CIAT 14 accessions of wild cassava (*M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana*, which Roa *et al*. (1997) considered synonymous with *M. esculenta* ssp. *flabellifolia*), originating from different locations (Table 4).

Analyses of genetic variability with AFLP markers

DNA extraction of *Manihot* accessions from Guyana was performed on leaves dried for 48 h at 35°C, using the protocol described by Colombo (1997). We followed the AFLP protocol described by Vos *et al*. (1995) and the adaptation for silver staining detection of Le Thierry

Table 3 Accessions of wild cassava (*M. esculenta* ssp. *flabellifolia*) from Guyana

Population code	Population	Individual code	Population code	Population	Individual code	Population code	Population	Individual code
W 59	bush	W 59-1	W 60	savanna	W 60-1	W 61	Rockview	W 61-1
		W 59-2			W 60-2			W 61-2
		W 59-3			W 60-3			W 61-3
		W 59-4			W 60-4			W 61-4
		W 59-5			W 60-5			W 61-5
					W 60-6			W 61-6
					W 60-7			W 61-7

Table 4 Accessions of cultivated and wild cassava obtained from CIAT*

Manihot subspecies	Accession code	Country of origin	*Manihot* subspecies	Accession code	Country of origin
M. esculenta ssp. *esculenta*	ARG 11	Argentina	*M. esculenta* ssp. *esculenta*	IND 33	Indonesia
	BOL 3	Bolivia		MAL 2	Malaysia
	BRA 12	Brazil		MAL 48	Malaysia
	BRA 97	Brazil		MEX 59	Mexico
	BRA 110	Brazil		PAN 51	Panama
	BRA 383	Brazil		PAR 110	Paraguay
	BRA 881	Brazil		PTR 19	Puerto Rico
	BRA 885	Brazil		TAI 1	Thailand
	BRA 900	Brazil		VEN 25	Venezuela
	BRA 931	Brazil		VEN 45	Venezuela
	COL 1468	Brazil		COL 1505	Venezuela
	COL 22	Colombia		CM 2177–2	CIAT
	COL 1438	Colombia		CM 3306–9	CIAT
	COL 1522	Colombia		NGA	IITA†
	COL 1684	Colombia	*M. esculenta* ssp. *flabellifolia*	EF 423–6	Brazil
	COL 2061	Colombia		EF 427–3	Brazil
	COL 2066	Colombia		EF 427–7	Brazil
	COL 2215	Colombia		EF 430–6	Brazil
	HMC 1	Colombia		EF 443–9	Brazil
	CR 32	Costa Rica	*M. esculenta* ssp. *peruviana*	PER 40–1	Brazil
	CUB 51	Cuba		PER 412–4	Brazil
	CUB 74	Cuba		PER 415–5	Brazil
	ECU 41	Ecuador		PER 417–6	Brazil
	ECU 82	Ecuador			

* Adapted from Roa *et al*. (1997).
† International Institute of Tropical Agriculture, based in Nigeria.

d'Ennequin (1999). We used A and G as selective nucleotides during pre-amplification for *Eco*RI and *Mse*I primers, respectively. Two primer combinations selected for their polymorphism and readability (Y. Gutiérrez, CIAT, Cali, Columbia, personal communication) were used for final amplifications: *Eco*RI + AAC × *Mse*I + GTA, and *Eco*RI + ACG × *Mse*I + GGT.

Data analysis

AFLP data were recorded in terms of the presence or absence of each band level for each plant. Shannon indices of phenotypic diversity (Bowman *et al*., 1971) were calculated for the set of varieties from Guyana, and for the sample from the CIAT core collection. The similarity index (S) of Nei & Li (1979) and the distance matrix ($D = 1 - S$) were calculated on the basis of interindividual pairwise comparisons. Neighbour-Joining dendrograms were constructed using the PHYLIP software (Felsenstein, 1993). Robustness of nodes was estimated by using 100 bootstrap resamplings. In order to evaluate how genetic variability was distributed among major agronomic groups and among varieties, analyses of variance on the molecular diversity were performed (variety level nested in the group level) using version 1.55 of the WINAMOVA software (Excoffier *et al*., 1992). Φ statistics (analogous to F statistics (Wright, 1965)) were also computed. Non-parametric tests for variance components and Φ statistics were conducted with 500 random permutations of the data set.

Results

Polymorphism revealed by AFLP markers

119 AFLP bands were detected (50 with AAC × GTA combination, and 69 with ACG × GGT combination), among which 112 were polymorphic within the whole sample (94% polymorphism). Considering only cultivated accessions, 99 AFLP bands were detected, among which 80 were polymorphic (81% polymorphism). Among wild accessions, 107 bands were detected, of which 98 were polymorphic (92% polymorphism). Twenty-three of these bands were found only in wild accessions, and 14 were specific to wild accessions from Guyana.

Organization of genetic variability in the local varieties

The classification in major agronomic groups, the number of plants studied and the number of AFLP patterns detected for each variety are given in Table 1. Both monomorphic and polymorphic varieties can be identified. All the plants of a monomorphic variety have exactly the same AFLP patterns. However, the small sample size does not allow the conclusion that each of these varieties is clonal. The plants from the monomorphic varieties E22, E25, and E39 were collected from several farmers. In contrast, other varieties collected from different farmers displayed different genotypes among the farmers (e.g. polymorphic varieties E7, E12, E43). For the majority of polymorphic varieties, polymorphism was detected even within a single farm (e.g. individuals E40-3 and E40-4, which were collected from the same farm and display different AFLP patterns). This result is similar to those obtained by Second *et al*. (1997) and Mkumbira *et al*. (personal communication) in fields grown by Caboclos from Brazil and farmers from Malawi, respectively.

Figure 2 shows a Neighbour-Joining (NJ) dendrogram based on the Nei and Li similarity index, including all the plants from Rewa, and rooted with wild accessions from Guyana. The bootstrap values are shown for each node. The NJ dendrogram shows that two types of polymorphic varieties exist, homogeneous and heterogeneous. For the former (e.g. E5) all the plants studied cluster together in the dendrogram. For the latter (e.g. E6), the plants are dispersed in the dendrogram.

There is obviously no agreement between the classification in major agronomic groups (shown in Fig. 2) and the organization of the molecular polymorphism as revealed by AFLP. In most cases, varieties that cluster together in the dendrogram belong to different major agronomic groups. Results given in Table 5 confirm that only a very small portion of the molecular variation is distributed between groups (from 0 to 9.8% depending on the trait under consideration). It is noticeable that the percentage of the total variance explained by within-variety diversity is larger than the proportion due to differentiation between groups. Finally, most of the variation is distributed between varieties (Φ_{sc} varies from 0.79 to 0.80). Only one variety of the sample was sweet (0 degree of bitterness). Although we can conclude that there is very little differentiation in AFLP pattern among bitter varieties according to their degree of bitterness, the material used in this study does not allow generalization of this conclusion to all the sweet and bitter varieties present in Guyana.

Structure of diversity in a single Makushi village, compared to the core collection

Figure 3 shows a rooted NJ dendrogram based on the Nei and Li similarity index, including all varieties from Rewa (the monomorphic or polymorphic and

homogenous varieties are represented by one plant only), accessions from the core collection, as well as accessions of wild cassava from Guyana and those provided by CIAT. Excepting the wild accession PER417-6, considered to be a putative escapee from cultivation (C. Roa, CIAT, Cali, Columbia, pers.

Fig. 2 Neighbour-Joining dendrogram based on the Nei and Li similarity index, showing bootsrap values. Cassava varieties from Rewa, and wild accessions from Guyana are compared. For each cultivated accession, corresponding classes of the four major agronomic groups are indicated (codes are in Table 1).

Table 5 Analyses of molecular variance (AMOVA) of the local varieties of cassava, conducted with 113 plants. The varieties were classified into four major agronomic groups. The statistics include sums of squared deviations (SSD), degrees of freedom (d.f.), variance component estimates (σ^2), the percentage of the total variance contributed by each component (%), Φ statistics[a] and the probability value (*P*-value) of obtaining a more extreme component estimate by chance alone, obtained with 500 bootstrap resamplings over loci

Trait used for classification	Source of variation	SSD	d.f.	σ^2	%	Φ statistics	*P*-value
Toxicity	Between group	1.13	3	-1.10^5	−0.02	$\Phi_{ct} = 0$	0.49
	Between varieties/group	9.10	27	0.0752	79.9	$\Phi_{sc} = 0.80$	< 0.002
	Within varieties	1.93	102	0.0189	20.1	$\Phi_{st} = 0.80$	0.998
Root colour	Between group	1.32	3	0.0033	3.5	$\Phi_{ct} = 0.03$	0.002
	Between varieties/group	8.91	27	0.0727	76.6	$\Phi_{sc} = 0.79$	0.002
	Within varieties	1.93	102	0.0189	19.9	$\Phi_{st} = 0.80$	0.998
Life-cycle	Between group	0.85	2	0.0018	1.9	$\Phi_{ct} = 0.02$	0.146
	Between varieties/group	9.39	28	0.0740	78.1	$\Phi_{sc} = 0.80$	< 0.002
	Within varieties	1.93	102	0.0189	20.0	$\Phi_{st} = 0.80$	0.998
Starch content	Between group	2.10	4	0.0096	9.8	$\Phi_{ct} = 0.10$	< 0.002
	Between varieties/group	8.13	26	0.0692	70.8	$\Phi_{sc} = 0.79$	< 0.002
	Within varieties	1.93	102	0.0189	19.4	$\Phi_{st} = 0.80$	0.998

[a] Φ_{ct}, Φ_{st} and Φ_{sc} represent the differentiation among groups, among populations within the whole population and among populations within groups, respectively.

comm.), there is no interclustering between wild accessions and cultivated cassava whatever their geographical origins. The wild accessions from Guyana are distinct from the wild accessions provided by CIAT (although all have been identified as *M. esculenta* ssp. *flabellifolia*).

Accessions from the core collection and local varieties do not interpenetrate much in the dendrogram, and the nodes linking accessions from the core collection to local varieties are not supported by high bootstrap values. Also, the value of the Shannon index for the varieties grown in Rewa is 4.293, whereas that of the sample from the core collection is 4.289. This shows that the same amount of genetic variability is present in one small Amerindian village and in the core collection from CIAT.

Discussion

Intra-varietal genetic variability

Each generation, a very low number of plants per variety (about 10% of the initial population of a field) contribute to the stock of cuttings planted the next generation. This should lead to a very strong bottleneck effect and to genetic uniformity within varieties in a few generations. Our results are not in agreement with this expectation. However, plants of the same variety were sometimes collected in fields of different farmers. Although sample size does not allow evaluation of the level of differentiation within varieties between farmers, the intravarietal diversity could be partly due to the genetic differentiation of plant material grown by different farmers. Exchange of cuttings between farmers is a factor that should lead to the genetic homogenization of varieties and loss of genotypic diversity, as expected from classical population genetics when migration is effective between a limited number of populations (Varvio *et al.*, 1986). However, such exchanges also allow the existence of intravarietal and within-farm polymorphism in transition phases to equilibrium. In this case, the number of different genotypes present in a variety for a given farmer would depend on the frequency of introduction of cuttings from other farmers of the community or from other villages. Moreover, different farmers sometimes use the same names for different plants that differ for morphological and agronomic features. Recent inquiries seem to reveal such inconsistency of taxonomy among farmers, at least for some varieties, particularly those named after their origin, or the quality of the root (Elias, 2000).

Our results show, however, that intravarietal genetic variability is also found among plants collected from the stock of a single farmer. In this case, one could invoke confusion between plants belonging to different varieties but sharing similar phenotypes. For instance, individuals 7-4 and 7-3 are representatives of the same variety ('fat boy stick') coming from the same farmer, but they have different AFLP patterns. Genetically plant 7-3 is very close to 47-2 ('cement stick'). Morphological descriptions and ethnological observations suggest that

Fig. 3 Neighbour-Joining dendrogram based on the Nei and Li similarity index, showing bootstrap values. Cassava varieties from Rewa, wild accessions from Guyana (bold), and cultivated (italic) and wild (bold italic) accessions provided by CIAT are compared.

these two varieties have very similar features and are frequently confused by the farmers. Situations like this one could be common and would partly explain the intravarietal polymorphism observed.

Another explanation for within-variety polymorphism could be the incorporation, in the farmer's planting stock, of volunteer plants originating from seeds produced by unmanaged sexual reproduction. Our inquiries have shown that the Makushi often multiply volunteer cassavas. This has also been reported among other Amerindian tribes (Boster, 1984; Salick *et al*., 1997; Emperaire *et al*., 1998) and in Africa (Chiwona-Karltun *et al*., 1998), but it has always been considered as marginal and of rare occurrence. Sexual reproduction of cassava in Makushi fields would allow recombination between varieties, because intercropping varieties is the general rule and because cassava has a predominantly outcrossing mating system (Rogers, 1965). Volunteer cassavas could therefore correspond to new genotypes, different from the parental varieties. While most of them, because of novel combinations of morphological characters, are considered by the Makushi as new varieties, we have observed that some are deliberately assigned to a known variety with which they share most of their morphological features. Although the frequency of this process is uncertain, we suspect it could be a major factor accounting for introduction of intravarietal genetic diversity to a greater degree than previously suspected (Elias, 2000).

Finally, somatic mutations transmitted through vegetative propagation must also be considered as a factor explaining intravarietal polymorphism. It is noticeable, for example, that for variety 23 only one band of 55 amplified differentiates individual 23-4 from the other plants of this variety.

Comparison of genetic variability between local varieties and the core collection and wild forms

Genetic diversity of the varieties grown in Rewa, assessed by the Shannon index of phenotypic diversity, is as high as genetic diversity of the sample from the core collection. Similar findings have been reported previously for cassava in other traditional farming systems in the Amazon (Colombo, 1997; Second *et al*., 1997). A small number of varieties would provide for the Makushi culinary requirement. However, they cultivate more than 76 varieties of cassava. Although Makushi have a strong preference for highly productive and yellow rooted varieties, there is no absolute selection. A farmer very rarely discards even a low-yielding variety, but maintains it at a low frequency. This corresponds to a strategy of risk management in uncertain farming conditions, but the practice is also motivated by social or cultural reasons. Because diversity is prized for its own sake, Makushi cultivators are eager to acquire new varieties through multiplication of volunteer cassava or through exchanges (within the community, or with other villages). These practices ensure that genetic variability is introduced and maintained between and within varieties, as witnessed by the morphological and molecular diversity we observed.

Finally, our results show that the scarcity in the core collection of local varieties leads, to some extent, to an under-representation of the genetic variability of cassava, because high diversity and novel genotypes are characteristic of traditional farming systems in comparison to the few cultivars found in modern farming systems. These results highlight the need for redefining the biological unit for strategies of conservation of local cassava diversity based not only on the major agronomic groups or even on the variety, but also on ecological and human factors that contribute to the differentiation of the genetic stocks. Traditional practices, especially intercropping and incorporation of volunteer seedlings, would promote the contribution of recombinant genotypes to the cultivated stocks, therefore allowing selection and adaptation to continue in this mainly vegetatively propagated crop. Even though the extents of traditional practices and their consequences for the genetic diversity of local varieties must be evaluated more precisely, it is already obvious that the loss of these practices could lead to a long-term reduction of genetic variability in local cassava varieties. Conservation strategies should therefore aim to maintain such processes.

Phylogenetic studies (Schaal *et al*., 1997; Olsen & Schaal, 1999) and analyses of the structure of the genetic variability in the cassava species complex (Fregene *et al*., 1994; Colombo, 1997; Roa *et al*., 1997) tend to confirm Allem's (1994) hypothesis that *M. esculenta* ssp. *flabellifolia* is the wild progenitor of cultivated cassava. In our study, wild forms clustered independently from all cultivated varieties. Similar patterns were observed in previous studies by Colombo (1997) and Roa *et al.* (1997). Such a pattern is consistent with an hypothesis of a limited (or even unique) domestication event in a restricted area, followed by diffusion of the cultivated phenotypes, as has been suggested, for example, for wheat (Heun *et al*., 1997). Olsen & Schaal (1999) reached the same conclusion for cassava from phylogeographic analyses using molecular sequences. They proposed that cultivated cassava originated in southern Amazonia. This scenario seems all the more plausible as fixation of interesting phenotypes, and their diffusion throughout the Amazonian basin, would have been facilitated by the vegetative reproductive mode. The pattern of

genetic differentiation between varieties that shows very short internodes and long-terminal branches in the dendrogram, could therefore be interpreted as a consequence of a radiation-type divergence of these varieties for morphological features under human selective pressures, from a common ancestral domesticated stock, combined with their fixation through vegetative propagation. In addition, the very low level of genetic differentiation between the major varietal groups defined above, even with the expectation of strong linkage disequilibrium as a consequence of the vegetative reproduction mode, would suggest that convergent evolution for the agronomic features defining them has occurred. However, confirming these results requires a more precise evaluation of the agronomic features used for this classification than one based on questionnaires and interviews only.

Our results also show that domesticated cassava in Guyana is more similar (in terms of AFLP markers) to wild cassava from Brazil, which is closer to the putative centre of domestication, than it is to wild cassava in Guyana. This leads us to hypothesize that gene flow from wild cassava is not a significant evolutionary factor in cultivated populations in Guyana and that the local wild gene pool has contributed little to the diversity of the cultivated pool.

The interpretations offered here depend on the reliability of AFLP markers in reflecting evolutionary relationships between taxa at the infraspecific level. Several studies have already shown the reliability of AFLP markers for taxonomic purposes in cultivated species including lettuce (Hill *et al*., 1996) and tea (Paul *et al*., 1997). In rice, analyses of cultivated varieties of the indica and japonica groups, carried out using RFLP, AFLP and ISSR markers, have shown that similar branching patterns are obtained whatever the type of markers used (Blair *et al*., 1999). However, one cannot discard the possibility that some of the AFLP markers correspond to very rapidly evolving sequences (Reamon-Büttner *et al*., 1999), and that saturation and homoplasy could therefore occur, at least for such markers, even at the infraspecific level. However, this hypothesis is difficult to equate with the cultivated/wild dichotomy of AFLP markers found in cassava.

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