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Diversity within American cassava germ plasm based on RAPD markers

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Abstract

This work focuses on the genetic diversity of American cassava through RAPD molecular markers. The 126 genotypes studied were distributed on four geographical levels ranging from local to continental. Samples included ethnocultivars from the Santa Isabel community in the Brazilian Amazon, local cultivars collected in the State of São Paulo, native accessions from very diverse Brazilian regions, and representative accessions from the Centro Internacional de Agricultura Tropical (CIAT) core collection. Eighty-eight polymorphic bands were analyzed. Results revealed the weak genetic structure of the cassava analyzed. The pattern formed by the first two axes of the principal coordinates analysis (PCoA) revealed an overlapping of the São Paulo State genotype, the Brazilian group and the core collection accessions. The Santa Isabel ethnocultures formed a separate group. The weak genetic structure of cassava can be explained by the common practice of exchanging botanical material among small producers as well as by recombinations among genotypes. When the genotypes were analyzed using climatic data, the sample sites were found to be structured according to temperature and precipitation. RAPD markers proved very useful in the genetic diversity study, resulting in important implications for cassava germ plasm collections and genetic breeding.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is one of the most important food crops in the tropics. It is a valuable source of carbohydrates, a staple in several developing nations in Africa, South America and Asia, and has the highest production potential of calories per hectare per day among tropical crops (De Bruijn and Fresco, 1989). Cassava is a shrubby species originated in the American continent approximately 3000 to 7000 years ago in several different regions: semi-arid and "cerrado" regions in Brazil (Rogers, 1972), savannas in the Orinoco River Basin between Colombia and Venezuela (Sauer, 1952), which originally belonged to adept cassava farming Aruak Indian tribes (Schmidt, 1951); hot, dry regions in Mexico and Guatemala (Rogers, 1972), and deserts along the Peruvian coast, which descended from the savannas created after glaciation (Ugent *et al.*, 1986).

The large number of cultivars represented in the main germ plasm collections of several research centers indicates the high genetic diversity of this species. According to Martins (1994), cassava's great diversity can be explained by life history components and species dynamics as well as the horticultural practices of different Indian and traditional farmer ("caboclo" and "caiçara") communities. Cultivars are typically randomly arranged in traditional plots, which favors intercrossing. Moreover, the seeds produced in these plots disperse, fall to the ground and lie dormant for a long time.

Another possible source of new variation is mutations or interspecific crosses between wild and/or weedy *Manihot* species with those being cultivated. The wild and/or weedy species may be found surrounding or inside the plot. Mutations or crosses could be fixed and propagated vegetatively (Martins, 1994).

According to Cohen *et al.* (1991), *ex situ* preserved cassavas represent about 16,000 accessions. Hershey (1994) estimated about 7,000 different cultivars. Furthermore, due to the large number of cultivars represented in collections, Boster (1985) proposed that some new cultivars were produced by human populations who traditionally consume this tubercle. The author cited new cultivars created by native populations of the Amazon Basin using visual selection criteria. Jeffrey (1968) stated that high polymorphism rates could be explained partly by vast, varied cultivation areas and conscious and unconscious breeding decisions, which accelerated this plant's evolution.

Agronomical and botanical descriptors (Pereira *et al.*, 1992; Cury, 1993) and isoenzyme analysis (Ramirez *et al.*, 1987; Léfèvre *et al.*, 1993) have been developed to evaluate genetic diversity. However, a limited number of isoenzymatic systems (Pasteur *et al.*, 1987) and botanical descriptors (Cury, 1993) are available; therefore, polymorphisms are restricted.

Molecular markers are a more stable and informative alternative to isoenzymes. According to Cohen *et al.* (1991), these markers can more efficiently be used to examine the genetic diversity of collections. In fact, many molecular markers are currently being used to study the genetic diversity of several different species. Marmey *et al.* (1994), Bonierbale *et al.* (1995) and Colombo (1998) have used molecular markers to study genetic diversity in cassava; however, only a limited number of genotypes were studied.

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A survey of a large number of cultivars from different regions is needed to better understand the organization and extent of genetic diversity, structuring factors, degrees of relationship between genotypes and their characterization in cassava. Consequently, conservation strategies could be developed to better exploit this germ plasm in the future. This study used RAPD molecular markers to determine the genetic diversity of American cassavas from different ecological and geographical zones.

MATERIAL AND METHODS

Material

One hundred and twenty-six assessions of *M. esculenta* were studied (Table I). Genotypes were grouped into the following four geographic levels, ranging from local to continental: a) 22 cassava ethnocultivars from a small community of "caboclos" in Santa Isabel, Amazon region (middle Negro River); b) 19 cultivars from the State of São Paulo; c) 52 accessions from different Brazilian geographical zones; d) 33 accessions from the CIAT collection selected to represent world diversity (based on a preliminary RAPD study).

Methodology

DNA isolation and amplification

DNA was isolated from silica gel dried leaf material according to the following methodology: 0.5 g dry leaves were ground in liquid nitrogen and transferred to 20-ml plastic tubes. Ten milliliters of extraction buffer (0.1 M Tris HCl, pH 8.0, 1.25 M NaCl, 0.02 M EDTA, 2% MATAB (mixed alkyltrimethylammoniun bromide)) and 1% ß-mercapto-ethanol was added just before use. After 90-min incubation at 65°C with slow stirring, an equal volume of chloroform/isoamylalcohol (24:1) was added twice and the resulting supernatant transferred to a clean plastic tube. RNAse (100 µl of a 10 mg/ml solution) was added immediately after these extractions and the solution subsequently incubated at 37°C for 30 min. DNA pellets were obtained by adding 0.8 vol. of isopropanol. After washing with 70% ethanol, the DNA pellet was vacuum dried and dissolved in 200 µl of TE buffer (10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA). The quality and concentration of the DNA fragments were evaluated by electrophoresis in 0.8% agarose gels.

PCR conditions

PCR was carried out in a 25-µl reaction containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001 gelatin, 10 ng template DNA, 0.4 µM primer, 100 µM of each dNTP and 0.5 units Taq polymerase (Appligene). DNA was amplified in a thermocycler (PTC-100 MJ Research) programmed as follows: 95°C for 4 min, followed by 45 cycles of 1 min at 95°C, 1 min at 35°C, and 2 min at 72°C, a final stage of 7 min at 72°C, and then maintained at 4°C prior to analysis. After adding 3 µl buffer (0.5% bromophenol/blue/glycerol, 1:2:1), the amplification products were submitted to electrophoresis on 1.8% agarose gel in 1X TBE buffer, stained with ethidium bromide and photographed under UV light with Polaroid film.

Data analysis

Only clearly amplified polymorphic fragments were analyzed. Scores of 1 (present) or 0 (absent) were used to form a matrix. Simple matching coefficients (Sokal and Michener, 1958) were obtained to perform cluster (UPGMA) and principal coordinate analyses (PCoA). Canonical correlations (Hotelling, 1935) were used to investigate the relationship between two variable sets. The first set was represented by PCoA scores (90% total variation) based on RAPD markers, and the second set used the average temperature and humidity values from the geographical zones of the cassava's origin. NTSYS-pc software (Rohlf, 1993) was used for the calculations. Nei's (1973) index of genetic diversity (*Ho*) was calculated for the four cassava cultivar groups based on allele frequencies of dominant RAPD "loci":

$$Ho = \sum 1/N\, p \text{ (present) } x\, q \text{ (absent)}.$$

RESULTS

Twenty-one of the 100 primers tested were selected for their quality, quantity and reproducibility capacity of fragments amplified. Eighty-eight of the 193 bands observed were polymorphic (Table II). The average number of fragments amplified per primer and their polymorphism rate were 9.5 (ranging from 6 to 13) and 3.8 (ranging from 2 to 8), respectively. Size of amplified fragments varied from 300 to 2000 pb with an average of 900 pb. An example of PCR amplification is shown in Figure 1. Only the polymorphic bright bands that were clearly amplified were analyzed and the faint bands were not scored.

Genetic similarity coefficients were calculated between all samples taken two by two. Values varied between 0.99 and 0.45, with an average of 0.67. No cultivars had similar profiles. Similar minimal genetic similarity values have been obtained using RAPD markers with other allogamous plants, such as 0.49 for cocoa clones (Wilde *et al.*, 1992), 0.57 for tea (Wachira *et al.*, 1995) and 0.45 for *Picea sitchensis* (Van de Ven and McNicol, 1995). Furthermore, the minimal similarity value observed among cassava cultivars of this study was greater than that of Marmey *et al.* (1994), which was 0.64 between the two most divergent African cassava clones.

Similarity coefficients of the four cultivar groups (Santa Isabel, São Paulo, Brazil and the World) were determined (Table III). The Santa Isabel group was significantly different from the other groups. The World group

Table I - Plant samples used to investigate the genetic relatedness among cassava cultivars.

No.	Code	Name of cultivar/variety	Origin/locality	Group*	Precip.*	Temp.*
1	E8189-1	Manipeba	Santa Isabel/AM/Brasil	A	2000	27
2	E8190-1	Mucura branca	Santa Isabel/AM/Brasil	A	2000	27
3	E8191-2	Maraquiri	Santa Isabel/AM/Brasil	A	2000	27
4	E8192-1	Japura	Santa Isabel/AM/Brasil	A	2000	27
5	E8194-1	Lito	Santa Isabel/AM/Brasil	A	2000	27
6	E8196-1	Fino	Santa Isabel/AM/Brasil	A	2000	27
7	E8200-1	Uapixuma	Santa Isabel/AM/Brasil	A	2000	27
8	E8201-1	Mucura Roxa	Santa Isabel/AM/Brasil	A	2000	27
9	E8203-1	Taboquinha	Santa Isabel/AM/Brasil	A	2000	27
10	E8204-1	Samaúma	Santa Isabel/AM/Brasil	A	2000	27
11	E8205-1	Barcelos	Santa Isabel/AM/Brasil	A	2000	27
12	E8207-2	Baixinha	Santa Isabel/AM/Brasil	A	2000	27
13	E8208-1	Juruna	Santa Isabel/AM/Brasil	A	2000	27
14	E8209-1	unnamed	Santa Isabel/AM/Brasil	A	2000	27
15	E8210-1	unnamed	Santa Isabel/AM/Brasil	A	2000	27
16	E8211-1	unnamed	Santa Isabel/AM/Brasil	A	2000	27
17	E8215-1	Seis Meses Branca	Santa Isabel/AM/Brasil	A	2000	27
18	E8214-1	Malave	Santa Isabel/AM/Brasil	A	2000	27
19	E8216-2	Esteio Branco	Santa Isabel/AM/Brasil	A	2000	27
20	E8221	Buia 3	Santa Isabel/AM/Brasil	A	2000	27
21	E8223	Buia 5	Santa Isabel/AM/Brasil	A	2000	27
22	E8225	Buia 7	Santa Isabel/AM/Brasil	A	2000	27
23	F1089	Lavoura	Monteiro Lobato/SP/Brasil	B	1250	24
24	F1117	Santista Branca	Ubatuba/SP/Brasil	B	2000	22
25	F1135	Pão de Ló	Maresias/SP/Brasil	B	1750	22
26	F1153	Cacau I	Praia Grande/SP/Brasil	B	1850	22
27	F1162	unnamed	Peruibi/SP/Brasil	B	1850	22
28	F2023	unnamed	Jacupiranga/SP/Brasil	B	1650	22
29	F2030	Pão do Céu II	Iporanga/SP/Brasil	B	1650	22
30	F2072	unnamed	Itapetininga/SP/Brasil	B	1350	20
31	F3039	Canela de Urubu	Franca/SP/Brasil	B	1500	20
32	F3075	unnamed	Barretos/SP/Brasil	B	1350	21
33	F4025	Santa Catarina	Catanduva/SP/Brasil	B	1250	22
34	F4048	unnamed	Fernandópolis/SP/Brasil	B	1250	22
35	F4072	unnamed	Araçatuba/SP/Brasil	B	1250	22
36	F4113	Mato Grosso	Bariri/SP/Brasil	B	1250	20
37	F4130	unnamed	Sta Bárbara D'Oeste/SP/Brasil	B	1250	20
38	F5075	Vassourinha XII	Ouro Verde/SP/Brasil	B	1250	22
39	F5096	Amarela VI	Pirapozinho/SP/Brasil	B	1250	22
40	F5115	unnamed	Assis/SP/Brasil	B	1350	21
41	F5129	Vassourinha XIV	Chavantes/SP/Brasil	B	1350	21
42	SRT1	Vassourinha Pta	Unknown/Brasil	C	-	-
43	SRT59	Branca de Santa Catarina	Volta Grande/SC/Brasil	C	2150	17
44	SRT120	Santa	Ubatuba/SP/Brasil	C	2000	22
45	SRT454	Guaxupé	Guaxupé/MG/Brasil	C	1500	19
46	SRT521	Carape II	Unknown/Brasil	C	1500	18
47	SRT1012	Guaxo	Araquari/SC/Brasil	C	1750	20
48	SRT1105	Mico	Rio do Sul/SC/Brasil	C	1500	19
49	SRT1116	Cigana Preta	Cruz das Almas/BA/Brasil	C	1100	24
50	SRT1196	Cavalo	Brasília/DF/Brasil	C	1600	20
51	SRT1197	Paiza	Brasília/DF/Brasil	C	1600	20
52	SRT1214	Unha	São Mateus/ES/Brasil	C	1250	24
53	SRT1256	unnamed	Venceslau Brás/PR/Brasil	C	1350	19
54	SRT1273	Azulona	Gentil do Ouro/BA/Brasil	C	750	22
55	SRT1275	Aipim Branco	Gentil do Ouro/BA/Brasil	C	750	22
56	SRT1293	unnamed	Itumbiara/GO/Brasil	C	1600	20
57	SRT1312	Izabel de Souza II	São João/PE/Brasil	C	750	21
58	SRT1313	Cambadinha	Tabira/PE/Brasil	C	750	22
59	SRT1315	Cravo	Moreno/PE/Brasil	C	1800	25
60	SRT1316	Pacare	São João/PE/Brasil	C	750	21
61	SRT1317	Pipoca	Afogados do Ingazeira/PE/Brasil	C	750	23
62	SRT1318	Pai Antonio	São João/PE/Brasil	C	750	21
63	SRT1319	Lagoa	São João/PE/Brasil	C	750	21

Continued on next page

Table I - Continued

No.	Code	Name of cultivar/variety	Origin/locality	Group*	Precip.*	Temp.*
64	SRT1326	Mandiocaba	Belém/PA/Brasil	C	2350	26
65	SRT1327	Sutinga III	Belém/PA/Brasil	C	2350	26
66	SRT1328	Uapixuna	Belém/PA/Brasil	C	2350	26
67	SRT1333	Amarela	Coxim/MS/Brasil	C	1250	24
68	SRT1336	Olho Roxo	Jaceara/MS/Brasil	C	1500	24
69	SRT1337	Pão XIII	Jaceara/MS/Brasil	C	1500	24
70	SRT1338	Paraguainha	Jaceara/MS/Brasil	C	1500	24
71	SRT1340	Buião	São Francisco/MG/Brasil	C	1000	24
72	SRT1341	Bambu	São Francisco/MG/Brasil	C	1000	24
73	SRT1342	Manteiga IV	São Francisco/MG/Brasil	C	1000	24
74	SRT1344	Jaburu	Ceará State/Brasil	C	-	-
75	SRT1345	Saracura	Santa Cruz/RJ/Brasil	C	1350	24
76	SRT1351	Apronta a Mesa	Rio Grande do Sul State/Brasil	C	-	-
77	BGM276	IAC 5-36	Crossing/Brasil	C	-	-
78	BGM243	EAB-105	Crossing/Brasil	C	-	-
79	BGM95	Paraguainha	Canavierias/BA/Brasil	C	1800	24
80	BGM82	Branca SC	Unknown/Brasil	C	-	-
81	BGM128	Embuzeiro	Alagoa/RS/Brasil	C	1600	20
82	BGM134	Palmeira Preta	Irara/BA/Brasil	C	1500	24
83	BGM252	Manteiga	MacedoCosta/BA/Brasil	C	-	-
84	BGM42	Brava de Padua	unknown/Brasil	C	-	-
85	BGM21	Cachimbo	unknown/Brasil	C	2350	26
86	BGM739	Cambraia II	unknown/Brasil	C	1250	24
87	BGM32	Cidade Rica	Tangara/RN/Brasil	C	1200	24
88	BGM1269	Engana Ladrão	Petrolina/PE/Brasil	C	600	26
89	BGM441	Taquari-SRT 1099	Rio Grande do Sul State/Brasil	C	1500	18
90	BGM1014	Mico	Itajaí/RS/Brasil	C	1600	19
91	BGM1013	Mandim Branca	Itajaí/RS/Brasil	C	1600	19
92	BGM81	Mameluca	Pará State/Brasil	C	2350	26
93	BGM19	Xingu	Belém/PA/Brasil	C	2350	26
94	ARG11	Duro do Valle 30	Argentina	D	1000	21
95	BOL3	Rasada de Bolivia	Bolivia	D	1400	25
96	BRA12	unknown	Brasil	D	-	-
97	BRA110	Pangola	Araua/Sergipel	D	-	-
98	BRA383	Vassourão	Brasil	D	-	-
99	BRA881	Branca de Santa Catarina	Cruz das Almas/BA	D	1100	24
100	COL22	Uvita	Cordoba-Ayapel	D	1500	25
101	COL1438	Llanera	Colombia	D	1800	27
102	COL1468	Mantiqueira	Colombia	D	-	-
103	COL1505	unknown	Venezuela	D	-	-
104	COL1522	Algodonera Amarilla	Cauca-Morales/Colombia	D	1500	23
105	COL1684	unknown	Colombia	D	-	-
106	COL2061	Regional morada	Popayan/Colombia	D	1910	17
107	COL2066	Chiroza Gallinaza	Caicedonia/Colombia	D	-	-
108	COL2215	Venezolana 1	Pivijay/Colombia	D	1000	28
109	CR32	Yuca Mangi	Costa Rica	D	2500	-
110	CUB51	Pinera	Cuba	D	1400	26
111	CUB74	Señorita	Cuba	D	1400	26
112	ECU41	De tres meses	Manabi-Chone/Equador	D	370	30
113	ECU82	Blanca	Loja-Loja/Equador	D	1500	20
114	HMC1	unknown	Unkown	D	-	-
115	IND33	unknown	Bogor/Malaysia	D	2600	27
116	MAL2	Black Twig	Malaysia	D	-	-
117	MAL48	Red Twig	Kuala Lumpur/Malaysia	D	2400	26
118	MEX59	unknown	Chiapas/Mexique	D	-	-
119	NGA2	Hybrid (TMS30572)	Ibadam/Nigeria	D	-	-
120	PAN51	Unknown	Ocu-Ocu/Panama	D	3000	27
121	PAR110	Tacuara Sayyu	Colonia Liberation/Paraguai	D	1400	23
122	PTR19	NO.9588	Puerto Rico	D	1550	26
123	TAI1	Rayong 1	Bangkhen-Bangkok/Thailand	D	1500	28
124	THAI2	unknown	Thailand	D	-	-
125	VEN25	Querepa Amarga	San Rafael Maraimo/Venezuela	D	2500	22
126	VEN45-A	unknown	Venezuela	D	-	-

* Cassava germplasm from Santa Isabel (A), São Paulo (B), Brazil (C) and World collection (D) and local average precipitation (Precip.) and temperature (Temp.).

Table II - Number and frequency of RAPD polymorphism products amplified by the primer in cassava.

Primer (Operon)	Number of RAPD products by primer	Number of polymorphic RAPD products by primer	Polymorphic product percentage	Number of polymorphic RAPD products by groups			
				Santa Isabel	São Paulo	Brazil	World
H6	10	7	70	7	7	7	7
H7	7	2	29	1	2	2	2
I1	9	3	33	3	3	3	3
I8	7	2	29	2	2	2	2
J9	11	2	18	1	2	2	1
J10	11	4	36	4	2	2	3
K12	11	5	45	4	4	4	5
K14	10	7	70	5	5	6	7
L7	13	6	46	6	6	6	6
M10	10	5	50	3	3	3	5
M12	6	3	50	2	2	2	3
N5	9	5	56	3	5	5	5
N20	9	5	56	4	3	3	5
X5	7	5	71	5	2	3	4
Y11	9	4	44	3	3	4	4
Y14	11	3	27	3	3	3	3
Y16	6	4	67	3	3	3	4
Z4	11	8	73	7	6	7	8
Z6	7	3	43	2	3	3	3
Z9	9	2	22	1	2	2	2
Z17	10	3	30	2	3	3	3
Totals	**193**	**88**	**46**	**71**	**71**	**75**	**85**
% of polymorphism				**81**	**81**	**85**	**97**

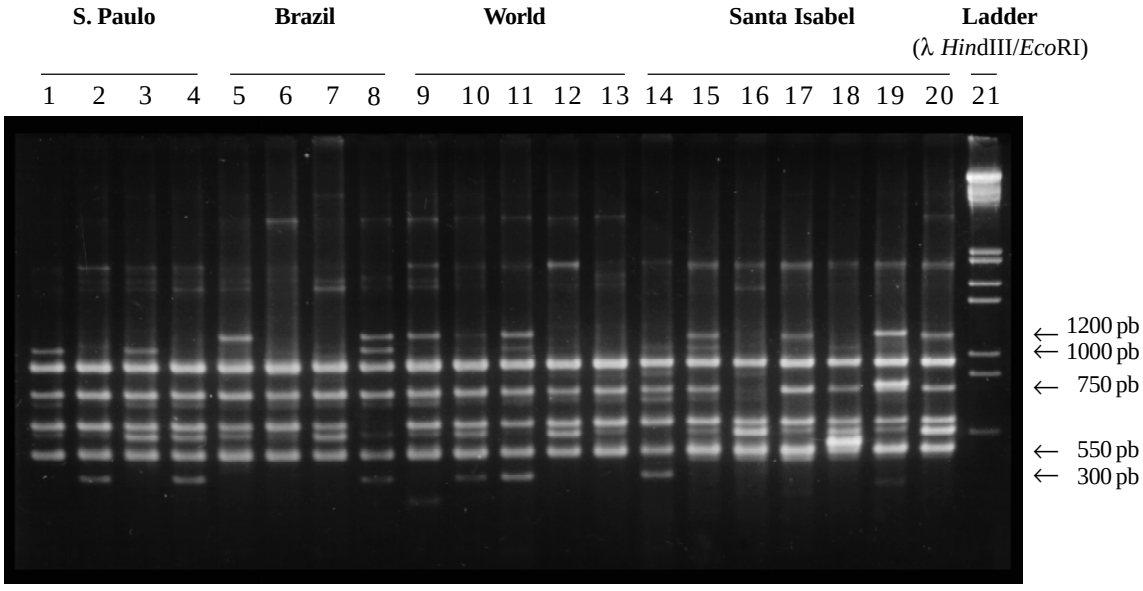

Figure 1 - Example of cassava RAPD amplification (primer N20 Operon) in agarose gel 1.8%. Only the clearly amplified polymorphic bright bands were analyzed (arrows signal the length of these fragments in pairs of bases). The faint bands were not scored.

was the most diverse. Surprisingly, similar results were found for the cultivars from the State of São Paulo. Local cultivars collected in this state were almost as diverse as those of the world collection.

A dendrogram of genetic relationships was established from the similarity coefficient matrix (Figure 2). The hierarchical agglomeration of cultivars was constructed according to the UPGMA method. No strong structure was found among major groups. Nevertheless, most genotypes regrouped on three main branches (A, B and C), or a fourth branch (D), consisting of four samples (Tai1, Mal48, Mal2 and BGM739). All local cultivars from the Amazon (E) belonged to branch A. Cultivars from the State of São Paulo (F), all of Brazil (SRT and BGM) as well as those from the CIAT core collection were well distributed on nearly all branches of the dendrogram.

The first two axes of the principal coordinate analysis (PCoA) (Figure 3) were used to structure the four geographically different cassava groups. The percentage of total inertia of axes 1 and 2 was about 14%. Distribution of three of the four cassava groups (São Paulo, Brazil and World) overlapped. Neither axis 1 nor 2 was capable of distinguishing these groups. However, the Santa Isabel group was defined by axis 1.

Shannon genetic diversity indices (*Ho*) were calculated with all RAPD markers (Table IV). A comparison of the averages revealed significantly less genetic diversity in the Santa Isabel cultivar that the other groups. World collection cultivars were the most diverse (0.93), followed by the State of São Paulo and Brazil, both with 0.91. However, when these differences were examined by comparing averages, they were not statistically significant.

Canonical analysis (Figure 4) was performed on 90 of the 126 cassava genotypes that had climatic data available (Table I). In addition, factors calculated by PCoA were based on 88 RAPD markers. The first discriminant function was statistically significant by itself ($R = 0.82$, $P < 0.0052$). The two variable groups studied, climatic data and PCoA factors, were statistically correlated. Root coefficients were -0.25 and 0.92 for the variables precipitation and temperature, respectively.

Discriminant analysis was performed on the precipitation data of 90 cassava cultivars (Figure 5). Each variable was correlated with PCoA factors obtained with RAPD markers. Cultivars distributed by the two discriminant functions structured around the variable precipitation. Group P1 consisted of cultivars from weak precipitation zones, group P2 cultivars from average precipita-

Table III - Simple matching coefficient values for cassava cultivar groups: Santa Isabel local cultivars, São Paulo State cultivars, accessions of the Brazilian group and accessions of the World collection.

	Total	Santa Isabel (1)	São Paulo (2)	Brazil (3)	World (4)
Number of cultivars	126	22	19	52	33
Maximal	0.987	0.987	0.896	0.909	0.948
Minimal	0.468	0.519	0.532	0.519	0.494
Average	**0.663**	**0.717**	**0.677**	**0.679**	**0.663**
Standard deviation	0.005	0.006	0.007	0.005	0.006
CV	0.089	0.093	0.102	0.081	0.096

Comparison of averages by the *t*-test (Student-Fisher)
*t*1x2 = 2.25 (P < 0.05) *t*1x3 = 4.88 (P < 0.05) *t*1x4 = 4.62 (P < 0.05) *t*2x3 = 0.02 *ns* *t*2x4 = 1.22 *ns* *t*3x4 = 1.13 *ns*

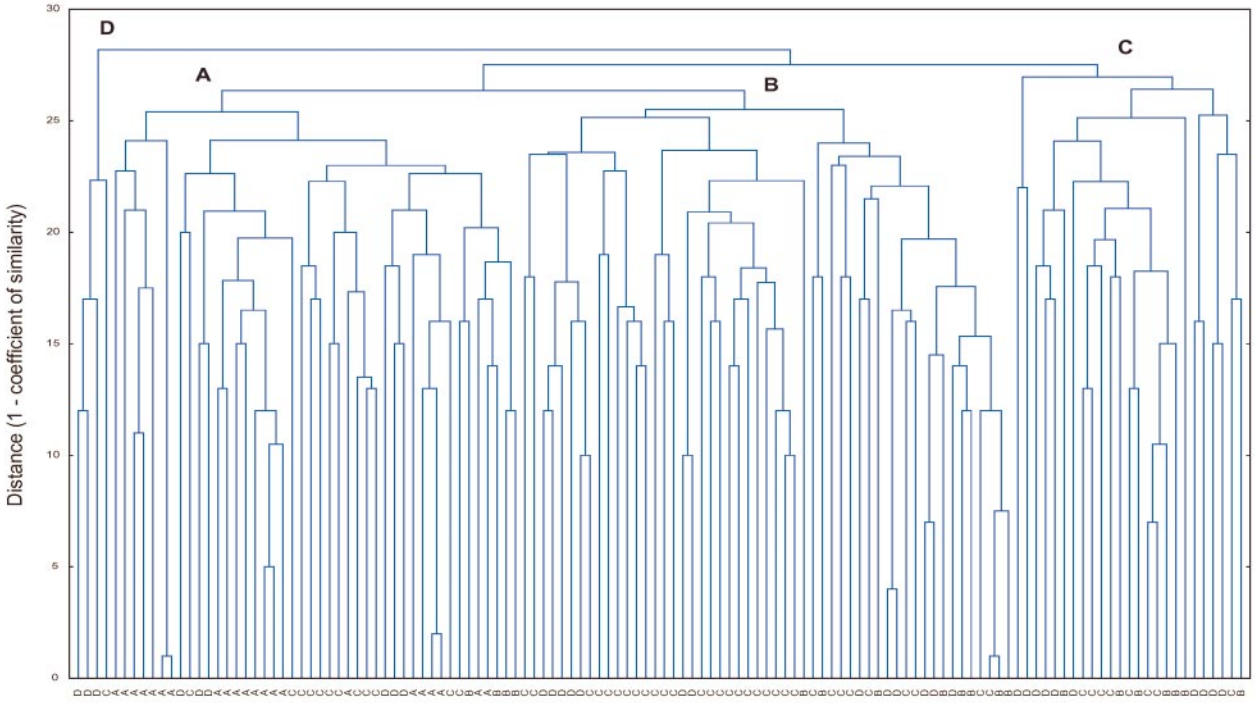

Figure 2 - Dendrogram based on UPGMA model calculated from genetic distances among American cassava genotypes from Santa Isabel (A), São Paulo (B), Brazil (C) and world collection (D), employing 88 RAPD markers.

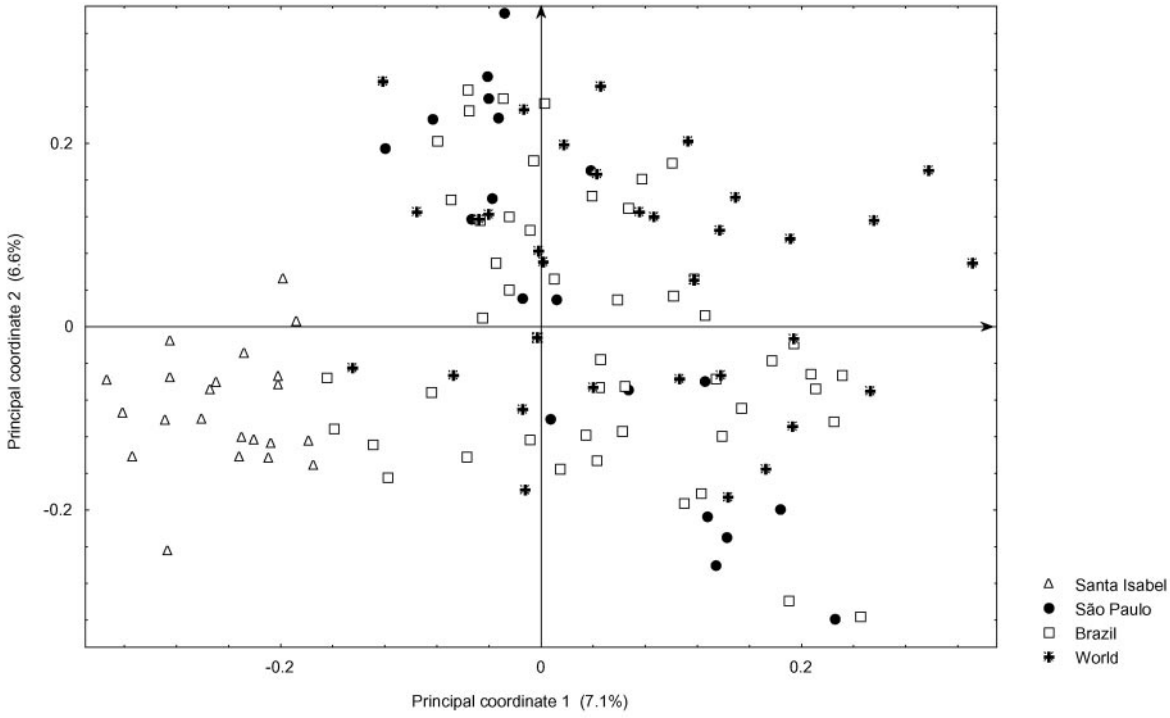

Figure 3 - Plot defined by the first two principal coordinate analysis factors based on genetic similarities (simple matching coefficients) of 126 cassava genotypes. Similarity coefficients were calculated with 88 RAPD markers.

Table IV - Shannon genetic index for the four cassava cultivar groups and comparisons of their averages by *t*-test (Student-Fisher).

	Shannon diversity (*Ho*)	Average comparison (*t*)
		t_{1x2} = 5.78 **
$Ho_{\text{Santa Isabel (1)}}$	0.80	t_{1x3} = 5.36 **
$Ho_{\text{São Paulo (2)}}$	0.91	t_{1x4} = 6.53 **
$Ho_{\text{Brazil (3)}}$	0.91	t_{2x3} = 0.00 ns
$Ho_{\text{World (4)}}$	0.93	t_{2x4} = 1.04 ns
		t_{3x4} = 0.97 ns

ns, Nonsignificant. **Statistically significant at 1%.

tion zones and group P3 cultivars from strong precipitation zones. Group P1 was composed of cultivars from northeastern Brazil, the most arid zone in the country, as well as cultivar ECU 41, the cultivar from the area with the least precipitation among the 90 cultivars included in this study. Group P3 was primarily formed by cultivars from northern Brazil, the Brazilian coast and Central American. Group P2 united cultivars from average precipitation zones, primarily central and southern Brazil.

Discriminant analysis was also performed on the variable temperature (Figure 6). As in the previous analysis, cultivars structured around the variable temperature. All groups, except groups T1 (< 19.9°C) and T2 (20°C-22.9°C), were significantly different (P = 0.05). All groups except the mainly equatorial group T4, were composed of cultivars from several different regions.

DISCUSSION

Cassava has high genetic diversity, which indicates a large genetic base. However, most of the polymorphic RAPD markers used in this study did not reveal much about cassava's genetic structure. It can be supposed that most RAPD markers were well distributed throughout the entire genome. Moreover, these markers revealed an important degree of homoplasy for the structuring of cassava, according to their degree of relationship. These results illustrate that RAPD markers could be useful for structuring the genetic diversity of collections, assessment or formation of a core collection, and especially construction of a genetic map. In addition, these markers are considered valid for identification or characterization of cultivars, establishment of genetic distance between genotypes or identification of identical lines in a collection of clones, as shown in Colombo *et al.* (1998).

Diversity of local cultivars from the State of São Paulo was identical to that of accessions from the whole country. This result is interesting because São Paulo represents only 1/32 of Brazilian territory. Cassava cultivars collected in this state came from small producers whose produce was destined for domestic consumption. Normally these cultivars have distinct agronomic features based on the producer's preference, which can explain the great diversity found among these cultivars.

Diversity among cultivars distributed throughout Brazil (Brazil group) was also just a little less than acces-

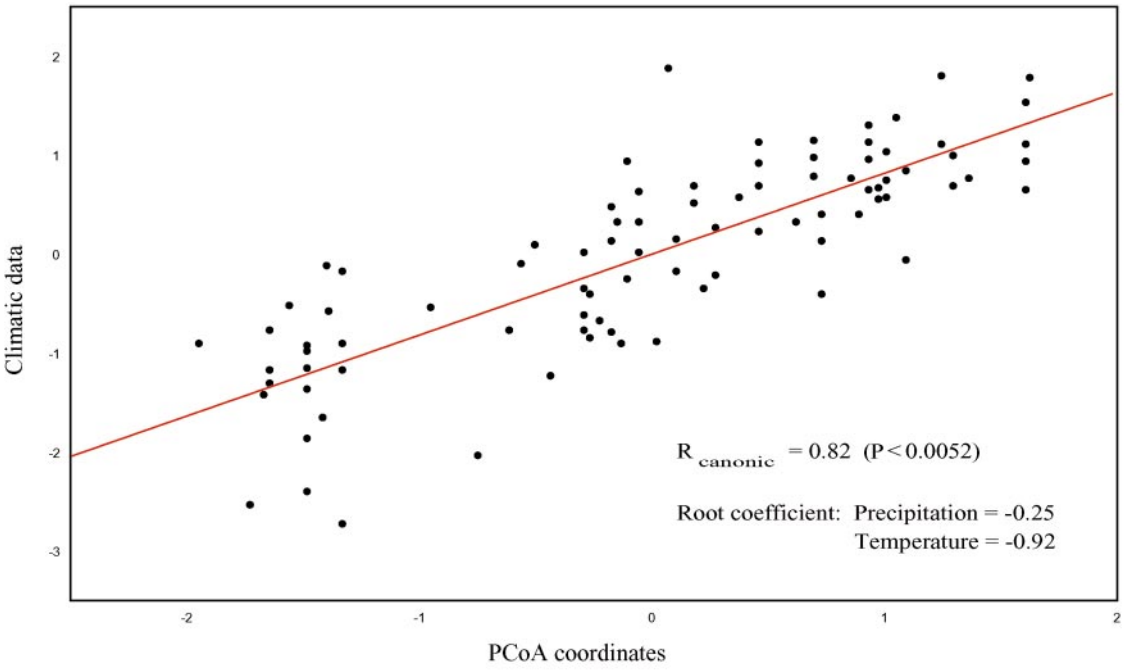

Figure 4 - Canonical analysis of climatic data (temperature and precipitation) from 90 cassava cultivar sites and principal coordinate analysis (PCoA) factors, calculated with 88 RAPD markers.

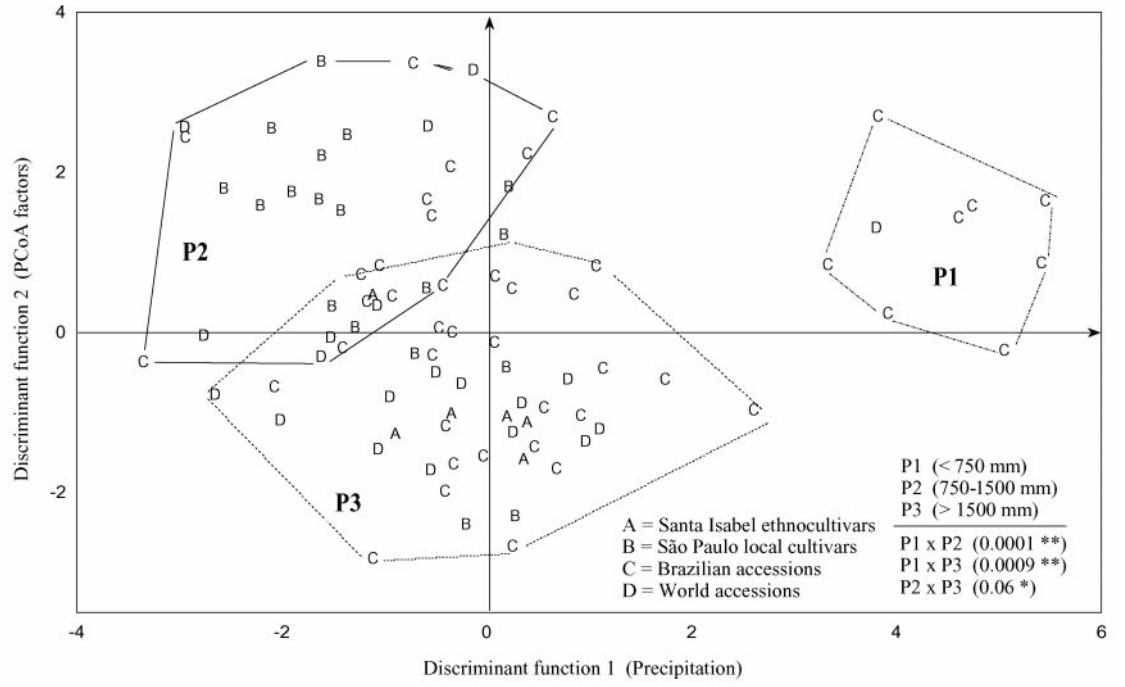

Figure 5 - Scattering of 90 cassava cultivars on a plot defined by discriminant function 1, based on precipitation (P1 to P3) of cultivar's place of origin, and discriminant function 2, based on the first 40 principal coordinate analysis (PcoA) factors. *,**Statistically significant at 5% and 1%, respectively.

sions representing the world collection, which was composed of American accessions. This result supports the hypothesis that Brazil being is a center of cassava diversity, which corroborates Nassar's report (1978). It has continental dimensions with diverse ecological conditions and is historically the largest cassava producer and consumer worldwide.

The large degree of genetic diversity of ethnocultivars from Santa Isabel was surprising. This considerable genetic diversity could be explained by the Indian agricul-

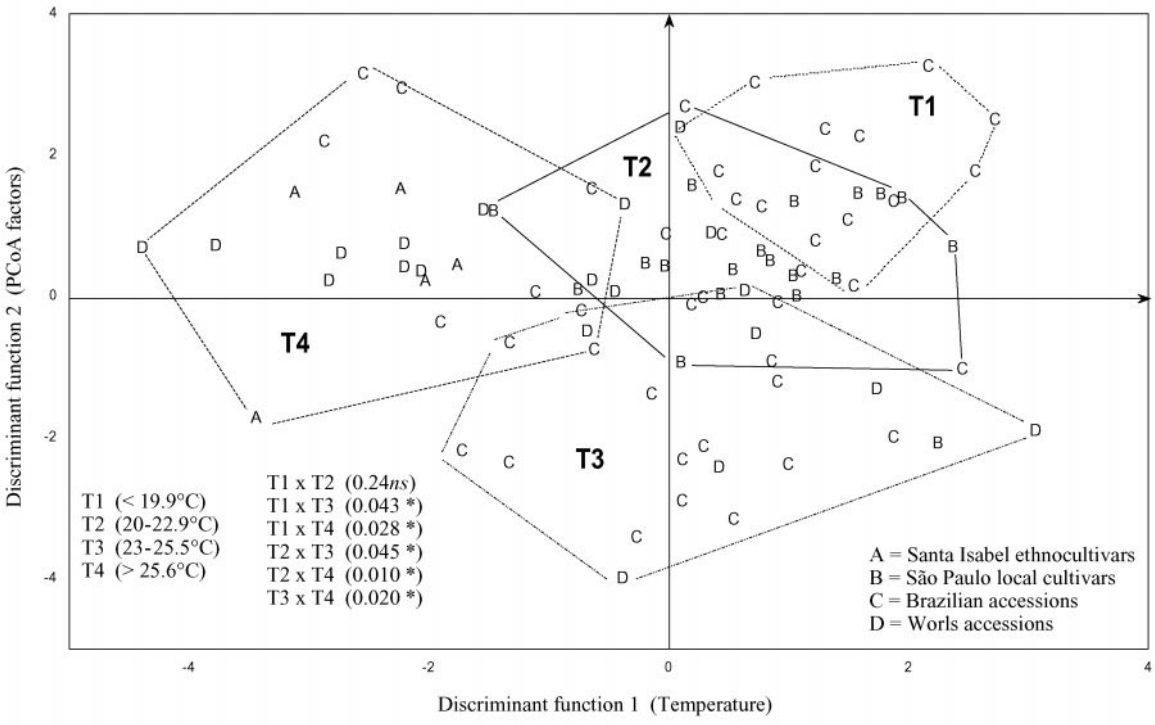

Figure 6 - Scattering of 90 cassava cultivars on a plot defined by discriminant function 1, based on temperature (T1 to T3) of cultivar's place of origin, and discriminant function 2, based on the first 40 principal coordinate analysis (PcoA) factors. ns, Nonsignificant. *Statistically significant at 5%.

tural practice of mixing several cultivars in the same field (Chernela, 1987; Kerr, 1987), possible recombinations between the different cultivars planted, and crosses with wild species.

Jennings (1963) showed that in the traditional African cassava culture system plants are sexually reproduced by spontaneous germination of seeds in parcels and cloned to generate new genotypes. This phenomenon has been reported in Brazil (Boster, 1984; Kerr, 1987, Martins, 1994). In plants like cassava, the ease of obtaining plants from seed and the ease of vegetative propagation probably accelerated the process of domestication and culture, which consequently encouraged ample variation of morphological features, root quality, etc. This has also been observed in grapes (Alleweldt *et al.*, 1990). Nevertheless, the absence of strong structuring among cultivars from the four geographical groups of this study, with the exception of the Santa Isabel ethnocultivar group, may be explained by transplanting of cultivars as well as genetic recombinations at different sites.

Other factors, such as human migration, can explain the abundance of genetic diversity in other continents. For example, we estimated that half of the 33 million inhabitants of the State of São Paulo immigrated, mostly from the northeastern and central regions of Brazil. These regions are known as the two main centers of genetic diversity of cassava (Nassar, 1978). Part of these migrants were farmers. Cassava was their basic food, and was taken with them when they moved. The diversity revealed by the Santa Isabel genotypes suggests a certain geographical isolation and limitation of crossing with other cassava cultivars.

The reduced genetic structure observed in relation to the geographical origin of cassava cultivars corroborates the major effects of migration and recombination. As for other vegetatively multiplied species (yam, sweet potato, sugarcane), exchanges of botanical material among producers, which are proportional to the extension of the culture, makes it possible for the same cultivar to be found at several different geographical sites. This is the case with cassava. No one knows the place of origin of this cultivar. A survey of cassava diversity in Africa (Léfèvre, 1993) also revealed little genetic structure in this continent. Léfèvre believed structuring to be associated with the origin of its introduction to the continent, in other words, founder effects. The most recent extension of the cassava culture (beginning about 150 years ago) decreased isolation between parcels and therefore encouraged the flow of genetic material among the different regions.

Nevertheless, cassava structure is associated with its original climate. In this study, cassava groups were differentiated by the precipitation and temperature of their places of origin. Using the variable precipitation, one can see that RAPD markers can separate genotypes based on their adaptability to the availability of water. This tool should help breeders select genotypes appropriate for this type of climate, as suggested by Fukuda *et al.* (1993) for the semi-arid northeastern region of Brazil.

For the variable temperature, cassava cultures struc-

tured between 30°N and 30°S. Cordeiro *et al.* (1995) also observed a climatic adaptation of clones, while forming a core collection as well as assessing the botanical data of 300 cassava clones. According to Carter *et al.* (1992), African cassava diffused after its introduction to this vast humid tropical zone in the 16th century, because cultivars were well adapted to these specific climatic conditions. Fukuda (1996) stated that the adaptability of the same cassava genotype to a range of different climatic conditions was not frequent, which would partly explain the wide range of genetic diversity of this species.

This study found through RAPD markers that cassava's genetic diversity is great, weakly structured, and tends to structure according to ecological conditions. The manner in which this diversity is distributed in the American continent could be useful for collections and/or conservation of the genetic resources of cassava as well as for breeders. The surprising genetic diversity observed among ethnocultivars from Santa Isabel suggests that the importance of analyzing other peasant communities should be studied.

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RESUMO

Este trabalho enfoca a diversidade genética de mandiocas americanas através de marcadores moleculares do tipo RAPD. Os 126 genótipos estudados estão distribuídos em quatro escalas geográficas, indo do local ao continental, ou seja, etnocultivares de uma comunidade chamada Santa Isabel, na Amazônia brasileira, cultivares locais coletados no Estado de São Paulo, um grupo representado por acessos oriundos das mais diversas regiões brasileiras e acessos representantes da "core collection" do CIAT. Oitenta e oito bandas RAPD polimórficas foram retidas para as análises. A estrutura genética das mandiocas deste estudo revelou-se fraca. O plano formado pelos dois primeiros eixos da análise de coordenadas principais (PCoA) revelou sobreposição dos genótipos do Estado de São Paulo, do grupo Brasil e dos acessos da "core collection". Por outro lado, os etnocultivares de Santa Isabel mostraram-se estruturados num grupo a parte com relação aos demais genótipos. Além disso, os etnocultivares de Santa Isabel apresentaram importante diversidade genética em relação aos genótipo dos outros três grupos. A fraca estrutura genética das mandiocas cassava pode ser explicada pelas trocas de material botânico entre pequenos produtores, prática normalmente empregada, assim como recombinação entre genótipos, visto que a mistura de diferentes cultivares em uma mesma parcela de cultura é também uma prática usada por pequenos produtores. Entretanto, quando os genótipos foram analisados em função de dados climáticos das localidades de origem dos mesmos, pudemos evidenciar uma estruturação em função da temperatura e da precipitação destes locais. Os marcadores RAPD mostraram-se informativos para o estudo da diversidade genética de mandiocas, oferecendo indicações importantes para a coleta de germoplasma de mandioca, assim como para o seu melhoramento genético. Com relação à importante diversidade genética encontrada na comunidade de Santa Isabel, outros estudos, através de outros marcadores e com genótipos de outras localidades, precisariam ser realizados para se tirarem conclusões mais diretas a respeito desta variabilidade.

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