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ORIGINAL PAPER

Population Structure and Genetic Diversity in Sweet Cassava Cultivars from Paraná, Brazil

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Abstract The objectives of the present study were to assess the population structure and genetic diversity in traditional sweet cassava cultivars collected in "backyard" cultivations in the municipalities of Maringá, Cianorte, and Toledo, State of Paraná, Southern Brazil, using 13 SSR molecular markers. All the loci analyzed were considered polymorphic with a mean of 3.15 alleles per locus; the mean polymorphism information content (PIC) value found was 0.4598, indicating that the primers were reasonably informative and the heterozygosity amplitude observed ranged from 0.0270 (GA134) to 0.8718 (SSRY45), with a mean of 0.4762, while the mean genetic diversity obtained was 0.5407, ranging from 0.3138 (GA21) to 0.6502 (GA140). The most divergent combinations were BGM434T-BGM20M, BGM35C-BGM20M, BGM430T-BGM232M, BGM430T-BGM164M, BGM430T-BGM322M, and BGM35C-BGM164M. The analysis of population structure distributed the traditional cultivars assessed in four different groups. The analysis of molecular variance (AMOVA) estimated that 11, 15, and 74 % of the variance were between groups, between individuals within groups, and between individuals within the population as a whole, respectively. The F_{is} (0.170) and F_{it} (0.259) values indicated a number of heterozygotes present in the population under study lower than that necessary to reach the Hardy-Weinberg equilibrium. The genetic variability found among the traditional sweet cassava cultivars assessed was considered wide, and the groups that were most distant were mostly cultivars from Toledo and Maringá.

Keywords *Manihot esculenta* · Genetic diversity · Polymorphism · SSR markers

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Introduction

Cassava (*Manihot esculenta* Crantz) is cultivated in Africa, Asia, and Latin America in areas situated between latitudes 30°N and 30°S (Nassar and Ortiz 2007), usually in low-fertility soils (El-Sharkawy 2004). This fact is associated to its wide adaptability and productive stability in unfavorable environments (Nassar and Ortiz 2007; Hurtado et al. 2008), along with high photosynthetic efficiency (El-Sharkawy 2006) and drought tolerance (Chen et al. 2012). It is considered the main carbohydrate source for approximately 800,000,000 people in developing countries, representing the third greatest source of calories in the tropical and subtropical regions, after rice and corn (FAO 2013). The *M. esculenta* species originated in the southwest of the Amazon (Olsen and Schaal 1999; Olsen 2004). The states of Goiás, Mato Grosso, and Rondônia in Brazil are the possible domestication sites (Allem 1997), and Goiás is the main diversity center of species of the genus (Allem 2002).

One important characteristic of *M. esculenta* is the presence of cyanogenic glycosides, mainly in the storage roots (Siritunga and Sayre 2004). The cyanogenic glycosides linamarin and lotaustralin, when hydrolyzed, release hydrogen cyanide (HCN), which is toxic when ingested (Siritunga

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Table 1 Names, locations, and collectors of traditional sweet cassava cultivars

Order number	Cultivar	Location	Collector	Order number	Cultivar	Location	Collector
1	BGM 430 T[a]	Toledo	1[e]	62	BGM 16 FLM[c]	Maringá	3
2	BGM 432 T	Toledo	1	63	BGM 17 M	Maringá	3
3	BGM 434 T	Toledo	1	64	BGM 18 M	Maringá	3
4	BGM 439 T	Toledo	1	65	BGM 20 M	Maringá	3
5	BGM 441 T	Toledo	1	66	BGM 25 M	Maringá	3
6	BGM 443 T	Toledo	1	67	BGM 30 M	Maringá	3
7	BGM 444 T	Toledo	1	68	BGM 31 M	Maringá	3
8	BGM 445 T	Toledo	1	69	BGM 33 M	Maringá	3
9	BGM 449 T	Toledo	1	70	BGM 34 M	Maringá	3
10	BGM 454 T	Toledo	1	71	BGM 36 M	Maringá	3
11	BGM 455 T	Toledo	1	72	BGM 37 M	Maringá	3
12	BGM 458 T	Toledo	1	73	BGM 40 M	Maringá	3
13	BGM 459 T	Toledo	1	74	BGM 43 M	Maringá	3
14	BGM 460 T	Toledo	1	75	BGM 50 M	Maringá	3
15	BGM 461 T	Toledo	1	76	BGM 51 M	Maringá	3
16	BGM 467 T	Toledo	1	77	BGM 56 M	Maringá	3
17	BGM 468 T	Toledo	1	78	BGM 58 M	Maringá	3
18	BGM 469 T	Toledo	1	79	BGM 59 M	Maringá	3
19	BGM 470 T	Toledo	1	80	BGM 62 M	Maringá	3
20	BGM 478 T	Toledo	1	81	BGM 77 M	Maringá	3
21	BGM 480 T	Toledo	1	82	BGM 80 M	Maringá	3
22	BGM 482 T	Toledo	1	83	BGM 81 M	Maringá	3
23	BGM 483 T	Toledo	1	84	BGM 82 M	Maringá	3
24	BGM 487 T	Toledo	1	85	BGM 84 M	Maringá	3
25	BGM 488 T	Toledo	1	86	BGM 88 M	Maringá	3
26	BGM 493 T	Toledo	1	87	BGM 89 M	Maringá	3
27	BGM 494 T	Toledo	1	88	BGM 90 M	Maringá	3
28	BGM 495 T	Toledo	1	89	BGM 91 M	Maringá	3
29	BGM 496 T	Toledo	1	90	BGM 92 M	Maringá	3
30	IAC 576/70[d]	–	–	91	BGM 93 M	Maringá	3
31	BGM 35 C	Cianorte	2[f]	92	BGM 95 M	Maringá	3
32	BGM 39 C	Cianorte	2	93	BGM 96 M	Maringá	3
33	BGM 47 C	Cianorte	2	94	BGM 105 M	Maringá	3
34	BGM 48 C	Cianorte	2	95	BGM 112 M	Maringá	3
35	BGM 55 C	Cianorte	2	96	BGM 118 M	Maringá	3
36	BGM 63 C	Cianorte	2	97	BGM 119 M	Maringá	3
37	BGM 64 C	Cianorte	2	98	BGM 121 M	Maringá	3
38	BGM 66 C	Cianorte	2	99	BGM 124 M	Maringá	3
39	BGM 67 C	Cianorte	2	100	BGM 139 M	Maringá	3
40	BGM 70 C	Cianorte	2	101	BGM 161 M	Maringá	3
41	BGM 72 C	Cianorte	2	102	BGM 162 M	Maringá	3
42	BGM 73 C	Cianorte	2	103	BGM 163 M	Maringá	3
43	BGM 74 C	Cianorte	2	104	BGM 164 M	Maringá	3
44	BGM 75 C	Cianorte	2	105	BGM 178 M	Maringá	3
45	BGM 76 C	Cianorte	2	106	BGM 179 M	Maringá	3
46	BGM 78 C	Cianorte	2	107	BGM 198 M	Maringá	3
47	BGM 79 C	Cianorte	2	108	BGM 201 M	Maringá	3
48	BGM 83 C	Cianorte	2	109	BGM 214 M	Maringá	3
49	BGM 86 C	Cianorte	2	110	BGM 218 M	Maringá	3

Table 1 (continued)

Order number	Cultivar	Location	Collector	Order number	Cultivar	Location	Collector
50	BGM 125 C	Cianorte	2	111	BGM 222 M	Maringá	3
51	BGM 126 C	Cianorte	2	112	BGM 223 M	Maringá	3
52	BGM 130 C	Cianorte	2	113	BGM 232 M	Maringá	3
53	BGM 144 C	Cianorte	2	114	BGM 236 M	Maringá	3
54	BGM 154 C	Cianorte	2	115	BGM 252 M	Maringá	3
55	BGM 170 C	Cianorte	2	116	BGM 289 M	Maringá	3
56	BGM 173 C	Cianorte	2	117	BGM 296 M	Maringá	3
57	BGM 5 M	Maringá	3[g]	118	BGM 317 M	Maringá	3
58	BGM 12 M	Maringá	3	119	BGM 322 M	Maringá	3
59	BGM 13 M	Maringá	3	120	BGM 323 M	Maringá	3
60	BGM 15 M	Maringá	3	121	BGM 324 M	Maringá	3
61	BGM 16 FEM[b]	Maringá	3	122	BGM 326 M	Maringá	3

[a] Cassava germplasm bank

[b] FE = narrow leaf

[c] FL = wide leaf

[d] IAC = Instituto Agronômico de Campinas

[e] Silva et al. 2015

[f] Zuin et al. 2009

[g] Costa et al. 2013

and Sayre 2004; Nyirenda et al. 2011). Thus, according to the cyanogenic glycoside content present in the storage roots, *M. esculenta* cultivars can be classified as sweet or bitter cassava. Bitter cassava has cyanogenic glycoside concentrations of over 100 mg kg^{-1} in raw pulp, making it unsuitable for *in natura* human consumption (Kizito et al. 2007; Zuin et al. 2009). However, sweet cassava presents acceptable concentrations (less than 100 mg kg^{-1} in raw pulp) of cyanogenic glycosides (Kizito et al. 2007), so the cultivars with these characteristics are the most important as staple food in developing countries, due to the big importance of reduced amounts of cyanogenic glycosides in food safety.

There are reports of a great genetic diversity in sweet cassava in Brazil (Rimoldi et al. 2010; Costa et al. 2013; Mezette et al. 2013; Oliveira et al. 2014), mainly in small areas where cultivars are grown by family farmers for their own consumption, in a type of cropping called "backyard"(Zuin et al. 2009). These areas of small crops have decreased considerably because of population migration to towns and big cities in the last 50 years (IBGE 2014). Furthermore, intense urbanization and expansion of exploitation areas for more profitable crops such as corn, soybean, and cotton (CONAB 2014) has contributed to the elimination of the small sweet cassava cropping areas, leading to the loss of genetic material of unknown value (Willemen et al. 2007; Zuin et al. 2009).

Thus, germplasm characterization and knowledge of the population structure and genetic divergence among the traditional sweet cassava cultivars have become essential (Kizito et al. 2007; Rimoldi et al. 2010; Kawuki et al. 2009; Siqueira et al. 2009; Costa et al. 2013). In this context, molecular markers are an important tool to characterize germplasm (Collard et al. 2005; Agarwal et al. 2008; Raji et al. Raji et al. 2009a), especially microsatellite markers, which have high informative content (Chavarriaga-Aguirre et al. 1998; Agarwal et al. 2008), allow differentiation between homozygote and heterozygote individuals (Kalia et al. 2011), and are frequently present in the sweet cassava genome (Raji et al. Raji et al. 2009a). Thus, the objective of the present study was to assess the population structure and genetic diversity of 121 traditional sweet cassava cultivars from Paraná state, south Brazil, by means of microsatellite molecular markers (simple sequence repeat (SSR)).

Materials and Methods

Plant Material

A total of 121 traditional cultivars of sweet cassava from State of Paraná, Brazil, were used in the present study. Ninety-two cultivars were collected in the northwestern region of the State of Paraná, 26 in Cianorte (Zuin et al. 2009) and 66 in Maringá (Costa et al. 2013). Twenty-nine cultivars were collected in Toledo, located in the western region of the State of Paraná (Silva et al. 2015; Table 1). Besides, the commercial cultivar IAC 576/70 was used as a standard for comparison

with the other traditional cultivars. It is derived from the cross between SRT 797-Ouro do Vale cultivar and the clone IAC 14–18 (Villela et al. 1985), is widely distributed in the central southern region of Brazil, and has excellent organoleptic characteristics (Mezette et al. 2009) and good agricultural performance in consideration of yield and bacteriosis resistance (*Xanthomonas axonopodis* pv. *manihotis*; Aguiar et al. 2011).

DNA Extraction and Amplification

Stem pieces of approximately 0.20 m long from the 121 traditional cultivars were planted in boxes with washed sand and kept in a greenhouse. After sprouting, young leaves were collected, placed in aluminum foil packets, and the DNA was extracted following the protocol described by Dellaporta et al. (1983). The DNA was quantified using a fluorometer (Qubit® Fluorometer Invitrogen) and standardized at a concentration of 50 ng μL^{-1}.

Each PCR consisted of a total volume of 25 μL containing 50 ng of DNA, 0.25 mM of each dNTP, 1.5 mM $MgCl_2$, 10 mM buffer ×10 (Invitrogen), 0.08 μM of each primer (forward and reverse), 1 U of *Taq* polimerase (Invitrogen), and ultrapure water (q.s.p.; Chavarriaga-Aguirre et al. 1998; Mba et al. 2001).

Thirteen pairs of microsatellite primers from the series GA (Chavarriaga-Aguirre et al. 1998) and SSRY (Mba et al. 2001) were used in the present study (Table 2). These primers were successfully used by Costa et al. (2013). The PCRs were conducted using specific programs in a thermocycler Techne Endurance TC-512 (Analítica), and

Table 2 Microsatellite loci analyzed in the 121 traditional sweet cassava cultivars collected in Maringá, Cianorte, and Toledo, Paraná, Brazil

Loci	LG[a]	Motif	Primer (5′-3′)[b]	$MgCl_2$ (μL)	AR[c] (bp)	AT[d] (°C)	Ref[e]
GA 21	nd[f]	NP[g]	F GGCTTCATCATGGAAAAACC R CAATGCTTTACGGAAGAGCC	1.25	104–126	58.0	A
GA 57	nd	NP	F AGCAGAGCATTTACAGCAAGG R TGTGGAGTTAAAGGTGTGAATG	2.00	153–183	59.0	A
GA 126	K	NP	F AGTGGAAATAAGCCATGTGATG R CCCATAATTGATGCCAGGTT	0.75	178–214	58.0	A
GA 127	K	NP	F CTCTAGCTATGGATTAGATCT R GTAGCTTCGAGTCGTGGGAGA	3.00	203–239	57.0	A
GA 134	nd	NP	F ACAATGTCCCAATTGGAGGA R ACCATGGATAGAGCTCACCG	3.00	309–337	59.0	A
GA 136	nd	NP	F CGTTGATAAAGTGGAAAGAGCA R ACTCCACTCCCGATGCTCGC	2.00	145–161	55.0	A
GA 140	nd	NP	F TTCAAAGGAAGCCTTCAGCTC R GAGCCACATCTACTGCACACC	4.00	154–164	55.0	A
SSRY 13	1	$(CT)_{29}$	F GCAAGAATTCCACCAGGAAG R CAATGATGGTAAGATGGTGCAG	0.75	234	55.0	B
SSRY 19	V	$(CT)_8(CA)_{18}$	F TGTAAGGCATTCCAAGAATTATCA R TCTCCTGTGAAAAGTGCATGA	0.75	214	55.0	B
SSRY 21	B	$(GA)_{26}$	F CCTGCCACAATATTGAAATGG R CAACAATTGGACTAAGCAGCA	0.75	192	55.0	B
SSRY 45	2	$(CT)_{27}$	F TGAAACTGTTTGCAAATTACGA R TCCAGTTCACATGTAGTTGGCT	0.75	228	55.0	B
SSRY 101	20	$(GCT)_{13}$	F GGAGAATACCACCGACAGGA R ACAGCAGCAATCACCATTTC	0.75	213	55.0	B
SSRY 135	G	$(CT)_{16}$	F CCAGAAACTGAAATGCATCG R AACATGTGCGACAGTGATTG	0.75	253	45.0	B

[a] Linkage group (Chavarriaga-Aguirre et al. 1998; Mba et al. 2001)

[b] Primers forward (F) and reverse (R) synthesized by Invitrogen

[c] Amplified region (bp)

[d] Annealing temperature

[e] References A: Chavarriaga-Aguirre et al. (1998); B: Mba et al. (2001)

[f] Not determined

[g] Motif not published based on (GA) sequence

the amplification conditions were carried out according to Mba et al. (2001) and Chavarriaga-Aguirre et al. (1998).

The amplified fragments were separated by electrophoresis in a non-denaturing 10 % polyacrylamide gel for approximately 3 h 30 min at 100 V. The gels were stained using

Table 3 Diversity indexes estimated for each microsatellite loci

LG[a]	Loci	Number of alleles	Allele (bp)	Frequency	PIC[b]	H_o[c]	Genetic diversity
nd[d]	GA 21	2	102 108	0.8051 0.1949	0.2646	0.3559	0.3138
nd	GA 57	2	146 171	0.2375 0.7625	0.2966	0.4750	0.3622
K	GA 126	4	183 190 228 242	0.4955 0.0714 0.4018 0.0313[f]	0.5027	0.6786	0.5869
K	GA 127	3	200 227 242	0.2679 0.1071 0.6250	0.4595	0.2679	0.5261
nd	GA 134	2	317 324	0.5631 0.4369	0.3710	0.0270	0.4920
nd	GA 136	2	147 158	0.5880 0.4120	0.3671	0.3611	0.4845
nd	GA 140	4	147 148 158 162	0.0439[f] 0.1535 0.4342 0.3684	0.5824	0.3333	0.6502
1	SSRY 13	3	223 237 252	0.2193 0.2588 0.5219	0.5434	0.6053	0.6125
V	SSRY 19	3	194 218 236	0.1102 0.5042 0.3856	0.4995	0.6695	0.5849
B	SSRY 21	4	150 165 174 190	0.0045[f] 0.1071 0.4866 0.4018	0.5047	0.6250	0.5903
2	SSRY 45	4	185 204 214 218	0.1282 0.4744 0.0128[f] 0.3846	0.5315	0.8718	0.6105
20	SSRY 101	4	174 177 189 195	0.4914 0.2888 0.2155 0.0043[f]	0.5582	0.2931	0.6287
G	SSRY 135	3	249 263 284	0.44.915 0.10.170 0.44.915	0.4964	0.6271	0.5862
Average		3.0769		0.5539[e]	0.4598	0.4762	0.5407

[a] Linkage group (Chavarriaga-Aguirre et al. 1998; Mba et al. 2001)

[b] Polymorphism information content

[c] Observed heterozygosity per locus

[d] Not determined

[e] Mean of high allelic frequencies

[f] Rare alleles, frequency <0.05 (Siqueira et al. 2009)

SYBR® Safe DNA gel stain (Life Technologies™) and digitalized using the L-Pix EX photo documentation system (Loccus Biotechnology). For fragment length assignment, a 100-bp standard molecular marker (DNA Ladder, Invitrogen) was used.

Statistical Analyses

The probabilistic method, established by the Structure 2.3.4 program (Pritchard et al. 2000), was used to analyze the population structure of the sweet cassava cultivars studied. This analysis was carried out with 10,000 replications in burn-in and 100.000 replications in the Markov-Monte Carlo chain (MCMC) (Pritchard et al. 2000; Evanno et al. 2005). With the standard parameters of the program and maintaining the admixture model, 14 clustering simulations were made with the *K* factor (number of groups) ranging from 2 to 15, and the probabilities P (*K*) were assessed on the output file of the individuals belonging to the *k-e*th group in the bar plot generated by the program (Kwak and Gepts 2009). Based on the results of the Structure program, the Structure Harvester application (Earl and von Holdt 2012) was used to obtain the ΔK value and consequently determine the optimum *K* value that represents the quantity of groups that best fits the population structure of the traditional cultivars.

In addition to the probabilistic method (Pritchard et al. 2000), the genetic diversity was analyzed by the neighbor-joining tree (Fig. 3) constructed based on the C.S. Chord distances (Cavalli-Sforza and Edwards 1967) implemented by the PowerMarker 3.25 program (Liu and Muse 2005).

The parameters of polymorphism information content (PIC), observed heterozygosity (H_o), genetic diversity, the analysis of molecular variance (AMOVA), and a principal coordinate analysis were performed using the GenAlEx 6.5 (Peakall and Smouse Peakall and Smousse 2006, Peakall and Smousse 2012) and PowerMarker 3.25 software (Liu and Muse 2005). Furthermore, the genetic diversity among the cultivars was assessed by constructing a distance matrix based on the C.S. Chord distance (Cavalli-Sforza and Edwards 1967).

Results and Discussion

Genetic Diversity

All the 13 loci were polymorphic, and a total of 40 alleles were obtained with a mean of 3.07 alleles per locus. The number of alleles per locus ranged from 2 (GA 21, GA 57, GA 134, and GA 136) to 4 (GA 126, GA 140, SSRY 21, SSRY 45, and SSRY 101). The mean of the most frequent alleles was 0.5539, and their frequency values ranged from 0.4342 for GA 140 to 0.8051 for GA 21 (Table 3).

Rare alleles, which are present in a population with frequencies lower than 0.05 (Siqueira et al. 2009), were found in this study in all the microsatellites loci that presented four different alleles. Some examples were the 242 base pairs of the *locus* GA 126, 147 bp in GA 140, 150 bp in SSRY 21, 214 bp in SSRY 45, and 195 bp in SSRY 101 (Table 3).

The 242-bp allele of GA 126 was present in the cultivars BGM 454 T, BGM 488 T, BGM 47C, BGM 55C, BGM 76C, BGM 78C, and BGM 92 M and the 147 bp allele of GA 140 in the cultivars BGM 252 M, BGM 323 M, BGM 17 M, BGM 18 M, BGM 51 M, BGM 56 M, and BGM 62 M, while the 150 bp allele SSRY 21 was exclusive to BGM 73C; the 214-bp allele was found in BGM 73C, BGM 74C, and BGM 75C; and the 195 bp allele was exclusive to BGM 488 M. Rare alleles are useful in molecular marker-assisted selection, when some genes of agronomic interest are found linked to them (Weiser et al. 2013); therefore, the rare alleles found are opportunities for future studies.

The mean heterozygosity value observed (H_o) was 0.4762 (Table 3). These results were comparable to those by Rocha et al. (2008), who studied sweet cassava populations in two indigenous reserves, and commercial cultivars, in Costa Rica. Other studies on sweet cassava have identified higher means for H_o such as that by Turyagyenda et al. (2012), who found H_o of 0.726 in accessions in Uganda. Nevertheless, there are some studies such as those by Siqueira et al. (2009) and Sree Lekha et al. (2010) that reported much lower observed heterozygosities of 0.265 and 0.2255, respectively. High heterozygosity values were found in the present study (Table 3), suggesting that there is wide heterosis among the sweet cassava plants cropped by the farmers. High heterosis is a typical characteristic of plants that have vegetative propagation and a crossed mating system (allogamy) such as *M. esculenta* (Fregene et al. 2003).

Regarding genetic diversity, the *loci* GA 140, SSRY 101, and SSRY 13 presented highest values of 0.6502, 0.6287, and 0.6125, respectively. Excluding loci GA 21 and GA 57 that presented lower diversity values (Table 3), other SSR loci performed similarly for genetic diversity, presenting high values with little variation around the mean, as in most of other studies (Peroni et al. 2007; Siqueira et al. 2009; Turyagyenda et al. 2012; Mezette et al. 2013).

The mean PIC value was 0.4598 (Table 3), indicating that the primers were reasonably informative (Botstein et al. 1980). According to Xia et al. (2005), the PIC values revealed by SSR markers used in the sweet cassava crop range from 0.5 to 0.7; thus, the mean value found was lower for SSR in sweet cassava. The mean PIC value found (Table 3) was low compared to most of the studies reported in the literature (Raghu et al. 2007; Asare et al. 2011; Turyagyenda et al. 2012). However, the PIC values found in the present study were superior to those obtained in the studies by Moyib et al. (2007), who reported a mean value of 0.4206. Furthermore,

studies by Costa et al. (2013), with the same traditional sweet cassava cultivars from Maringá used in the present study, showed a lower value of 0.4040. This fact can be explained because when the populations from Cianorte and Toledo were added to the genetic statistical analyses, there was an increase in the genetic diversity of the set, increasing the number of alleles per primer and consequently the genetic divergence means and the PIC. Thus, it can be stated that larger and less

Table 4 Allelic frequency and polymorphism information content (PIC) for each microsatellite locus per collection site

		Toledo		Cianorte		Maringá	
Loci	Allele (bp)	Frequency	PIC	Frequency	PIC	Frequency	PIC
GA 21	102	1.0000		0.8462		0.6936	
	108	–	0.0000	0.1538	0.2265	0.3064	0.3347
GA 57	146	–		–		0.4453	
	171	1.0000	0.0000	1.0000	0.0000	0.5547	0.3720
GA 126	183	0.4821		0.3600		0.5593	
	190	0.1786		0.1200		–	
	228	0.3036	0.5778	0.4400	0.5922	0.4322	0.3833
	242	0.0357		0.0800		0.0085	
GA 127	200	–		–		0.5263	
	227	0.3667	0.3566	0.0400	0.0739	–	0.3743
	242	0.6333		0.9600		0.4737	
GA 134	317	0.4286	0.3698	0.4231	0.3690	0.4474	0.3722
	324	0.5714		0.5769		0.5526	
GA 136	147	0.7593	0.2987	0.5625	0.3711	0.5175	0.3747
	158	0.2407		0.4375		0.4825	
GA 140	147	–		–		0.0833	
	148	0.3750		0.2692		–	
	158	0.4821	0.5259	0.2500	0.5624	0.4917	0.4775
	162	0.1429		0.4808		0.4250	
SSRY 13	223	0.2222		0.2826		0.1953	
	237	0.2963	0.5587	0.3044	0.5832	0.2266	0.5126
	252	0.4815		0.4130		0.5781	
SSRY 19	194	0.2500		0.0577		0.0703	
	218	0.3929	0.5810	0.8269	0.2767	0.4219	0.4631
	236	0.3571		0.1154		0.5078	
SSRY 21	150	–		0.0217		–	
	165	0.3913		0.1087		0.0076	
	174	0.2173	0.5707	0.6957	0.4316	0.5076	0.3860
	190	0.3914		0.1739		0.4849	
SSRY 45	185	0.1786		0.2000		0.0781	
	204	0.4286	0.5519	0.4600	0.6076	0.5000	0.4717
	214	–		0.0600		–	
	218	0.3928		0.2800		0.4219	
SSRY 101	174	0.1607		0.2400		0.7381	
	177	0.5893	0.5143	0.5600	0.5230	0.0476	0.3543
	189	0.2321		0.2000		0.2143	
	195	0.0179		–		–	
SSRY 135	249	0.3889		0.3800		0.5000	
	263	0.3704	0.5787	0.0800	0.4678	–	0.3750
	284	0.2407		0.5400		0.5000	
		0.5847	0.4219	0.6432	0.3912	0.5559	0.4040

homogeneous populations tend to present high values of informative content per *locus*.

Great divergence can be observed in the occurrence rates of the alleles and also some unique alleles in some locations. Some examples are of the 146 bp allele of the *locus* GA 57; 227 bp alleles of GA 127 and 147 bp of GA 140, which occurred only in Maringá; the 150 bp allele of SSRY 21 and 214 bp of SSRY 45 exclusive to Cianorte; and 195 bp allele of SSRY 101, exclusive to Toledo (Table 4).

A distance of 0.7809 was observed between BGM 434 T-BGM 20 M, followed by BGM 35C-BGM 20 M, BGM 430 T-BGM 232 M, and BGM 430 T-BGM 164 M with 0.7773, 0.7669, and 0.7556, respectively. It is pointed out that all the combinations shown, with distances from 0.7022 to 0.7773, can be considered highly divergent and contain the most genetically distant individuals among the possible 7381 cross combinations from the 122 traditional cultivars analyzed (Table 5).

Of the 33 most divergent combinations shown, 58 % were among traditional cultivars from Maringá and Toledo, 30 % between Cianorte and Maringá, 9 % between Toledo and Cianorte, and 3 % exclusively from Toledo. This reflected the general tendency observed among the 122 cultivars of greater dissimilarity between the populations from Toledo and Maringá.

The most divergent traditional cultivars found in the present study can be used in breeding programs as parents for heterotic clones (Gonçalves-Vidigal et al. 1997) as long as the parents are genetically complementary (Nick et al. 2010). For this, it is necessary to determine their agronomic characteristics regarding the storage root dry matter content, yield, organoleptic characteristics, and the presence of flowering.

The possible crosses indicated for the region of Maringá involve the traditional cultivars BGM 20 M, BGM 232 M, BGM 164 M, BGM 322 M, BGM 56 M, BGM 323 M, and BGM 222 M, because those cultivars produce flowers (Kvitschal 2008), which enables pollinations. Furthermore, according to Kvitschal (2008), it was observed that among the cultivars that present flowering, BGM 20 M and BGM 322 M have the most interesting characteristics in a cross, such as above average starch content found (300.96 g kg^{-1}), and tolerance to super elongation and bacteriosis.

As shown in Table 5, the least divergent cultivars found are BGM 460 T and BGM 467 T; BGM 441 T and BGM 444 T; BGM 39C and BGM 47C; BGM 48C, BGM 55C, and BGM 63C; BGM 25 M, BGM 30 M, and BGM 31 M; and BGM 36 M with BGM 37 M.

Population Structure

Four groups were generated among the 122 traditional sweet cassava cultivars (Fig. 1). In addition, it can be observed a coincidence between the population structure found by the Structure 2.3.4 program (Fig. 1; Pritchard et al. 2000) and the divergence among the most and least similar cultivars (Table 5). Thus, the most divergent traditional cultivars, such as BGM 434 T and BGM 20 M, were found in different groups (*K*1 and *K*4). The same was observed for the most similar cultivars that were placed in the same groups with BGM 460 T and BGM 467 T in group *K*3 and BGM 441 T and BGM 444 T in group *K*1.

The traditional cultivars analyzed were divided into a greater number of groups than the number of locations. In addition, some cultivars from Toledo and Cianorte were placed together with traditional cultivars from Maringá that in turn were all distributed between groups *K*2 and *K*4 (Fig. 2).

It is further pointed out that the bred cultivar IAC 576/70, that was used for comparison, was placed in group 1. Thus, it can be said that generally, this cultivar was close to the others belonging to this group, which come mainly from Toledo. It is known that this cultivar was derived from a cross between the clone IAC 14–18 and cultivar SRT 797-Ouro do Vale. According to Valle[1], the parent Ouro do Vale was commonly found in Paraná state. Therefore, its alleles may have been shared among the cultivars from the region of Toledo, leading to greater genetic similarity to the traditional cultivars collected in this location.

The proportions of cultivars in each *K* group were the following: Toledo 63 % (19 traditional cultivars) in group 1, 7 % (2) in group 2, and 30 % (9) in group 3; Cianorte obtained 19 % (5) in group 1, 23 % (6) in group 2, 54 % (14) in group 3, and 4 % (1) in group 4, while Maringá obtained 39 % (26) of the traditional cultivars in group 2 and 61 % (40) in group 4 (Figs. 1 and 2).

The data obtained reinforce the results presented by Costa et al. (2013) in their studies regarding clustering of traditional cultivars from Maringá; which divided these cultivars into two groups, according to the genetic divergence among the individuals. A similar result was observed in the present study, on which the constitution of the two groups kept the same, except for some cultivars that in the study by Costa et al. (2013) were found in different groups but with balanced probabilities of separation between the groups. Thus, as the present study included populations from Toledo and Cianorte, two more groups were formed (1 and 3), causing some cultivars to be included in a different group but maintaining the cultivars from Maringá exclusively in two groups (Fig. 2).

Several studies (Lokko et al. 2006; Kizito et al. 2007; Costa et al. 2013) highlighted the influence of propagule exchanges among producers from different locations on

[1] Personal communication: Valle, Teresa Losada. Researcherat Instituto Agronômico de Campinas, Brazil.

Table 5 Divergent and similar combinations among the 122 traditional sweet cassava cultivars collected in Paraná state, according to C.S. Chord distances

Most divergent combinations	Most similar combinations
BGM 434 T[a]-BGM 20 M = 0.7809	BGM 460 T-BGM 467 *T* = 0.0000
BGM 35C-BGM 20 M = 0.7773	BGM 441 T-BGM 444 *T* = 0.0000
BGM 430 T-BGM 232 M = 0.7669	BGM 39C-BGM 47C = 0.0000
BGM 430 T-BGM 164 M = 0.7556	BGM 48C-BGM 55C = 0.0000
BGM 430 T-BGM 322 M = 0.7441	BGM 48C-BGM 63C = 0.0000
BGM 35C-BGM 164 M = 0.7441	BGM 25 M-BGM 30 M = 0.0000
BGM 66C B-GM 322 M = 0.7441	BGM 25 M-BGM 31 M = 0.0000
BGM 432 T-BGM 460 *T* = 0.7386	BGM 36 M-BGM 37 M = 0.0000
BGM 495 T-BGM 20 M = 0.7361	BGM 59 M-BGM 162 M = 0.0375
BGM 434 T-BGM 34 M = 0.7357	BGM 5 M-BGM 16FE M = 0.0375
BGM 434 T-BGM 36 M = 0.7357	BGM 30 M-BGM 33 M = 0.0375
BGM 495 T-BGM 322 M = 0.7351	BGM 31 M-BGM 33 M = 0.0375
BGM 434 T-BGM 56 M = 0.7326	BGM 34 M-BGM 36 M = 0.0375
BGM 449 T-BGM 56 M = 0.7326	BGM 12 M-BGM 139 M = 0.0487
BGM 430 T-BGM 323 M = 0.7311	BGM 25 M-BGM 37 M = 0.0443
BGM 430 T-BGM 326 M = 0.7311	BGM 25 M-BGM 33 M = 0.0406
BGM 63C-BGM 222 M = 0.7267	BGM 34 M-BGM 37 M = 0.0406
BGM 73C-BGM 236 M = 0.7261	BGM 444 T-BGM 445 *T* = 0.0538
BGM 460 T-BGM 35C = 0.7249	BGM 161 M-BGM 214 M = 0.0541
BGM 66C-BGM 164 M = 0.7239	BGM 162 M-BGM 214 M = 0.0541
BGM 495 T-BGM 164 M = 0.7237	BGM 163 M-BGM 214 M = 0.0541
BGM 434 T-BGM 37 M = 0.7220	BGM 59 M-BGM 214 M = 0.0541
BGM 449 T-BGM 50 M = 0.7187	BGM 222 M-BGM 232 M = 0.0541
BGM 35C-BGM 179 M = 0.7187	BGM 121 M-BGM 124 M = 0.0541
BGM 483 T-BGM 214 M = 0.7167	BGM 12 M-BGM 90 M = 0.0541
BGM 430 T-BGM 222 M = 0.7157	BGM 441 T-BGM 445 *T* = 0.0579
BGM 73C-BGM 33 M = 0.7124	BGM 59 M-BGM 161 M = 0.0693
BGM 66C-BGM 323 M = 0.7092	BGM 461 T-BGM 467 *T* = 0.0693
BGM 461 T-BGM 35C = 0.7067	BGM 467 T-BGM 496 *T* = 0.0693
BGM 461 T-BGM 35C = 0.7067	BGM 434 T-BGM 449 *T* = 0.0693
BGM 443 T-BGM 50 M = 0.7062	BGM 67C-BGM 48C = 0.0693
BGM 430 T-BGM 214 M = 0.7040	BGM 67C-BGM 63C = 0.0693
BGM 70C-BGM 236 M = 0.7022	BGM 47C-BGM 63C = 0.0693

Source: Cavalli-Sforza and Edwards (1967)

[a] Traditional cultivars followed by the letter T were collected in Toledo, C in Cianorte and M in Maringá

the population structure of sweet cassava. Because of this, cultivars from nearby regions may have been placed in different groups and cultivars from distant locations may have been placed in the same group. So, based on the data obtained, inference on the population structure based on genetic differentiation can be made and may or may not reflect the geographic location (Jorgensen and Mauricio 2004; Nordborg et al. 2005). This depends mainly on the characteristics of the population compared to the isolation of these accessions that may prevent allele exchange among them, causing separation into different groups (Thomas et al. 2003).

The plants collected in the municipality of Maringá are more distant genetically from those collected in Cianorte and Toledo, which were relatively close. This indicates that some events in the implementation process by the colonists of the sweet cassava crop in the northwestern region of Paraná resulted in fewer exchanges of traditional cultivars from Maringá with the other two locations, contrary to their geographic location, because Maringá is 81 km from Cianorte while Toledo and Cianorte are 215 km apart (DER-PR 2013).

This process may have been linked to the coffee crisis in the 1970s, when in the coffee-producing region,

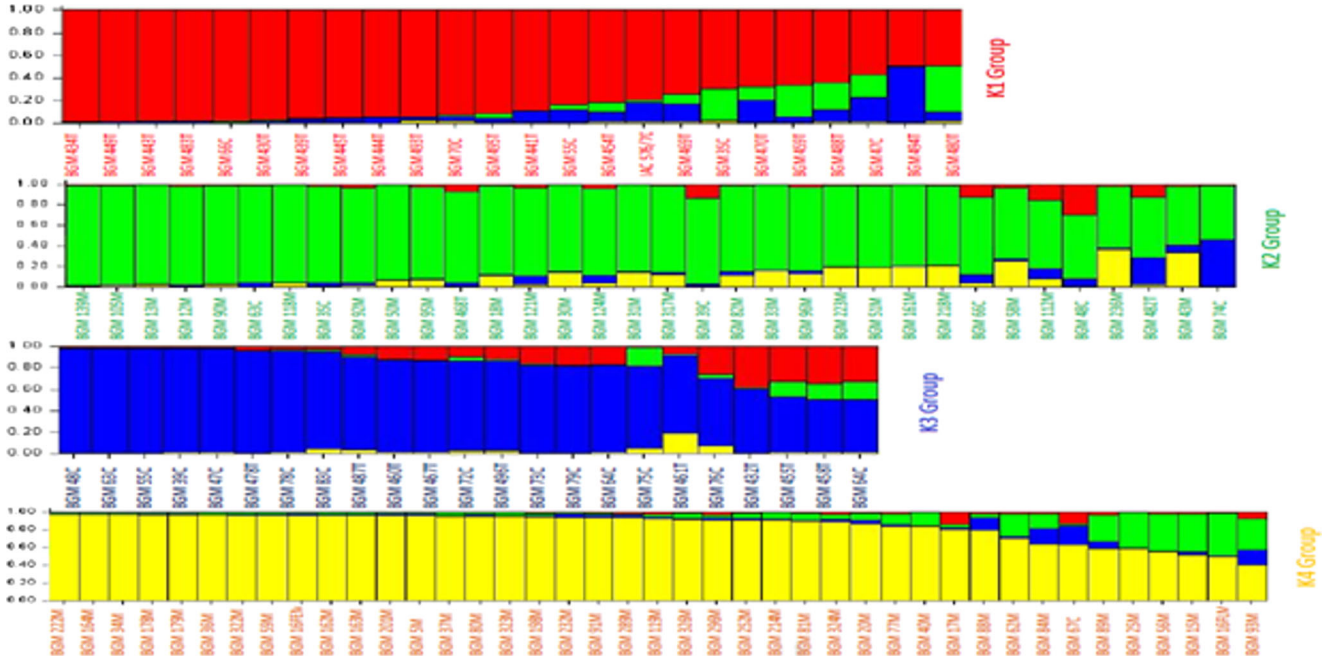

Fig. 1 Population structure analysis of 122 traditional sweet cassava cultivars from Maringá, Cianorte, and Toledo, Paraná, Brazil, for $K = 4$ groups given by the software Structure 2.3.3. (Pritchard et al. 2000) The ordinate axis shows the probabilities of the individuals belonging to the *k*-*e*th group, while the abscissa axis shows every traditional cultivar on its respective bar

which included the municipality of Cianorte; there was a rural exodus and subsequent reoccupation of the agricultural areas. Consequently, people migrated from various regions of the state, including those from western Paraná, to the region of Cianorte (Camarano and Abramovay 1999). During this migration, traditional sweet cassava cultivars from the region of Toledo may have been introduced, resulting in allele exchanges with sweet cassava populations from the region of Cianorte, thus reducing the genetic distances around the populations of both locations. Furthermore, there is a constant migratory flow between the western and northern regions of Paraná (Kleinke et al. 1999) that culminates in the exchange of traditional cultivars among the regions.

The results obtained can be used as indicators of the real population structure of the traditional sweet cassava cultivars in the regions where they are native, as these are not known, based on the fact that there is no information on the origin of the materials in the pre-collection periods. Furthermore, the population structure is directly influenced by gene flow, caused by material traffic and exchange among small producers, added to crossed fertilization typical of allogamous plants such as *M. esculenta* (Lokko et al. 2006; Kizito et al. 2007).

In the neighbor-joining tree, two main clusters were generated (Fig. 3), one including predominantly the traditional cultivars from Maringá (*K*2 and *K*4) and the other the cultivars from Cianorte and Toledo (*K*1 and *K*3). The occurrence of similar individuals according to their proximity on the tree can also be observed. As expected, the similarity agrees with the C.S. Chord methodology (Cavalli-Sforza and Edwards 1967) and the traditional cultivars taken as similar belong to the same groups indicated by the Structure 2.3.4 program (Pritchard et al. 2000). The following individuals can be highlighted: BGM 460 T and BGM 467 T; BGM 441 T and BGM 444 T; BGM 39C and BGM 47C; and BGM 48C, BGM 55C, and BGM 63C,

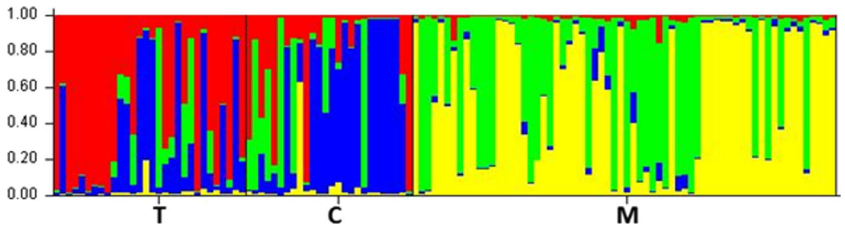

Fig. 2 Population structure of the traditional sweet cassava cultivars from Maringá (M), Cianorte (C), and Toledo (T), Paraná, Brazil, ordered according to their places of origin for $K = 4$ groups (group 1 in red, group 2 in green, group 3 in blue, and group 4 in yellow)

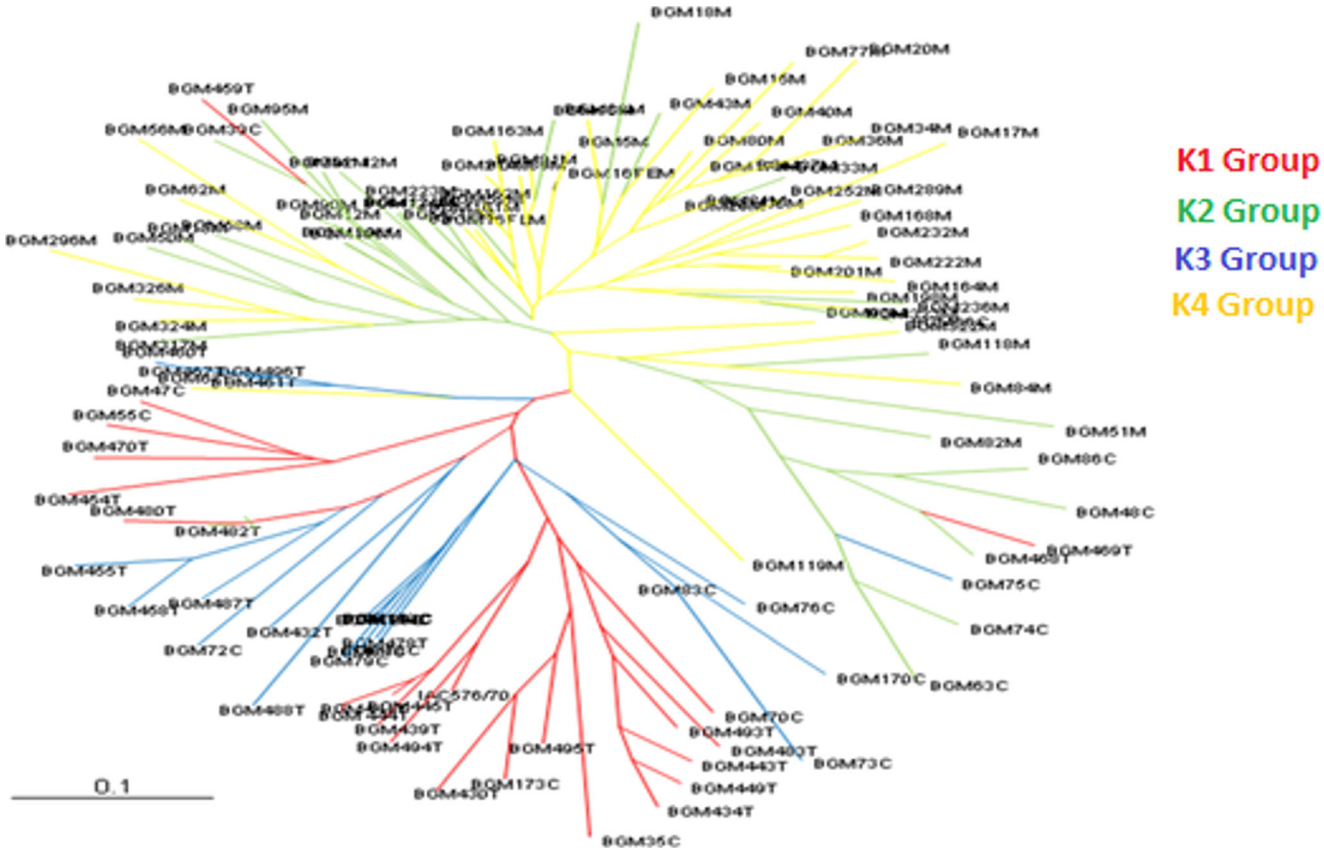

Fig. 3 Distribution of the traditional sweet cassava cultivars collected in Maringá, Cianorte, and Toledo, Paraná, Brazil, represented by their names presented in Table 1, according to the neighbor-joining tree

considered as possible duplicates by the probabilistic methodology (Pritchard et al. 2000) and by the neighbor-joining analysis. Individuals which presented greater divergences belonged mainly to groups *K*1 and *K*4 (Fig. 3).

The principal coordinate analysis (PCoA; Fig. 4) shows the groups formed in this analysis, compared to the location of each traditional cultivar. There is an approximation of the points in the case of the traditional cultivars that

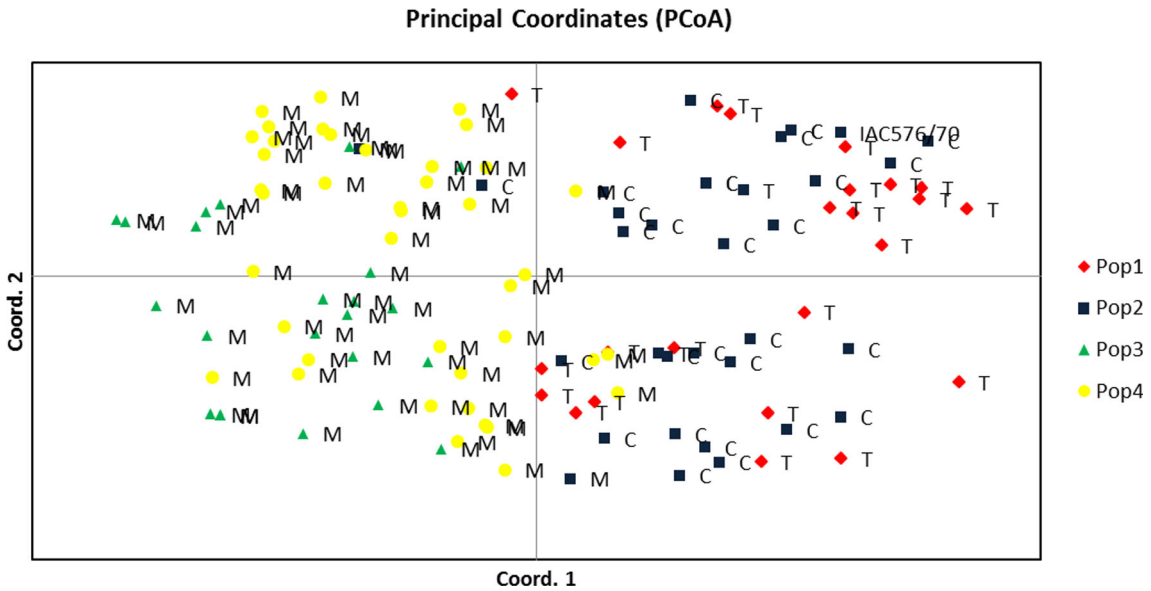

Fig. 4 Principal coordinate analysis of 122 traditional sweet cassava cultivars from Paraná Brazil based on microsatellite data

Table 6 Analysis of the molecular variance (AMOVA) of the 122 traditional sweet cassava cultivars from Paraná, Brazil, considering the four *K* groups generated by the structure analysis

Variation source	DF	SS	MS	EV	Percent	*F* value	MPS
Among groups	3	88.280	29.427	0.423	11	F_{st} = 0.107*	0.001
Within groups	118	487.200	4129	0.599	15	F_{is} = 0.170*	
Within the whole population	122	357.500	2930	2930	74	F_{it} = 0.259*	
Total	243	932.980		9950	100 %		

[a] *df* degrees of freedom, *SS* square sums, *MS* mean square, *EV* estimated variance, *MPS* minimum probabilistic of significance

*Significant at 1 % of probability

were considered similar or even duplicates such as BGM 460 T-BGM 467 T, which were fairly close graphically and were considered similar in the analysis based on the C.S. Chord distance (Cavalli-Sforza and Edwards 1967). As a consequence of the great dispersion of the points on the Cartesian plane, it can be inferred that there was great variation among the traditional cultivars collected, especially between those from Maringá and Toledo, but specifically among those within groups 4 and 1, located in quadrants on opposite diagonals. The two main coordinates shown graphically explained 52.84 % of the total variation observed.

According to the AMOVA, using the Wright fixation indexes (Wright 1951; Table 6), similar levels of among- and within-group variabilities were observed considering the four *K* groups generated by the structure analysis. Most of the variation (74 %) remained among all the individuals analyzed, regardless of the division of the population into groups. Another 15 % of the variation were allocated in the differentiation of individuals inside the subpopulations (groups), showing that even among individuals clustered by genetic proximity, there is still high variability. The remaining 11 % of the variability found were caused by division of the population into groups, which tended to be more homogeneous than the population as a whole (Maringá, Cianorte, and Toledo added) but heterogeneous when compared to each other.

The quantity of differentiation among the groups can be compared by the F_{st} index. The 0.107 value obtained or approximately 11 % indicates a moderate differentiation among the groups. This can be observed in the present study, where the groups present considerable differentiation (Fig. 3), with small divergences in terms of composition of individuals in the different methodologies used.

The F_{is} and F_{it} values obtained (Table 6) indicate, respectively, that there are deficiencies of 17 and 26 % in the expected heterozygote rate within the groups and also in the population generally, considering the Hardy-Weinberg equilibrium (Raji et al. 2009b). This may indicate a narrow genetic base of the population (Turyagyenda et al. 2012).

Conclusions

A high value of genetic diversity was found in the population analyzed. The 122 traditional sweet cassava cultivars analyzed were divided into four groups and individuals from Cianorte and Toledo clustered in the same groups with some individual clustering together with the traditional cultivars from Maringá. Most of the cultivars from Maringá were grouped in groups 2 and 4 by the probabilistic method, while those from Toledo mostly occupied group 1 and those from Cianorte were predominated in group 3.

Based on the high divergence found among some cultivars, they can be recommended as parents in breeding programs, as in the case of the most divergent crosses involving the traditional cultivars BGM 20 M or BGM 322 M, which in addition to flowering present desirable agronomic characteristics such as disease tolerance and high dry matter content in the storage roots.

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