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Genetic diversity and population structure of traditional sweet cassava accessions from Southern of Minas Gerais State, Brazil, using microsatellite markers

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Sweet cassava is a food culture of great importance because it is a source of nutrition and energy for millions of people in tropical and sub-tropical regions. For that reason, genetic diversity and population structure studies are necessary in order to obtain more information regarding the evaluated genotypes, reassuring their use in future breeding programs. The objective of this study was to evaluate the genetic diversity and population structure of 51 traditional sweet cassava accessions collected in the Southern of Minas Gerais State, Brazil, using 20 microsatellite markers. All markers used to genotype the 51 sweet cassava accessions were polymorphic (PIC = 0.4080). Four sub-populations were identified using different methods (Bayesian analysis and multivariate analysis). The PhiPT (analogous Wright Fst) index of 0.073 indicated a moderated genetic variability among the studied traditional sweet cassava accessions. The dissimilarity index ranged from 0.097 to 0.560. Among the most divergent accessions stands out BGM 690, BGM 655 and BGM 660, which are the most recommended for obtaining a heterosis in order to increase yield production.

Key words: *Manihot esculenta*, genetic diversity, microsatellite markers, heterozygosity.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), originally from the Neotropical region (Olsen and Schaal, 2001) is a perennial plant of the dicotyledonous class (Euphorbiacea family), which plays a considerable role as the fourth most cultivated food culture in the world (Nassar, 2000; Olsen and Schaal, 2001; Perera et al., 2014; Legg et al.,

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2014). The genus of highest importance is *Manihot* which consists of approximately 98 species with a diploid genome of 36 chromosomes (Nassar, 2000; El-Sharkawy, 2004).

According to data recently published by FAO (2014), cassava is pointed out as crop that serves as nutrient source for more than 800 million people from the tropical and sub-tropical regions. African countries are currently leading the sweet cassava production as they possess most of the cultivated areas, such as Nigeria, the biggest world producer, followed by the Asian countries of Thailand and Indonesia (FAO, 2014). Brazil is the fourth biggest producer of sweet cassava in the world with a harvested area of 2,342.619 ha and yield production of 23,087.828 tons (IBGE, 2014). In a regional context, the yield production of cassava tuberous root in the year of 2013 in Minas Gerais State was estimated in 820,000 tons in a harvested area of 59,000 ha, occupying this way, the second place as the largest producer of cassava in the Southeastern Brazilian region, surpassed only by the state of São Paulo**.**

Cassava in Brazil is mainly grown on smallholdings by low-income farmers for their own consumption, a condition that ensures great genetic diversity to this crop (Zuin et al., 2009). This kind of subsistence farming has been extinguished in the past years due to the migration from rural to urban areas, and also to the expansion of large-scale farmers that now dominate areas previously occupied by smallholders, consequently leading to a drastic reduction of genetic diversity (Cleveland et al., 1994). Therefore, the characterization of the available germplasm, elucidation of genetic identity and population structure of sweet cassava accesses is very important (Costa et al., 2013; Ortiz et al., 2016). Genetic diversity can be assessed by using biochemical and morphological markers (Elias at al., 2001; Ortiz et al., 2016), but molecular markers are highly recommended because they provide more detailed information about polymorphism, independently of physiological state of the plant and environmental conditions (Agarwal et al., 2008; Raji et al., 2009). Molecular marker techniques, such as microsatellites (Raji et al., 2009; Monteiro-Rojas et al., 2011; Asare et al., 2011; Turyagyenda et al., 2012; Costa et al., 2013; Mezette et al., 2013; Kawuki et al., 2013; Ndung'u et al., 2014; Fu et al., 2014; Ortiz et al., 2016) have showed satisfactory results for evaluating the genetic diversity and population structure in sweet cassava. Furthermore, microsatellite markers, also known as simple sequence repeats (SSR), are informative due to their multi-allelic nature, codominant inheritance and wide distribution through the genome (Varshney et al., 2005; Raji et al., 2009). These tools require a low cost and are highly reproducible, which make them attractive and applicable for breeding programs (Turyagyenda et al., 2012).

The objective of the present work was to study the genetic diversity and population structure of sweet cassava accessions from South of Minas Gerais State, Southeastern of Brazil, using microsatellite markers.

MATERIALS AND METHODS

Germplasm collection

Fifty traditional sweet cassava accessions from the counties of Poços de Caldas (21°S 47′, 46°W 33′), Botelhos (21°S 38', 46°W 23'), Caldas (21°S 55', 46°W 23') and Alfenas (21°S 25', 45°W 56'), located at the Southern region of Minas Gerais state, Brazil, were collected for further analysis (Figure 1). The climate of this region according to Köppen's classification is Cwa, a humid temperate with dry winters (Mello and Viola, 2013).

At the instance of collection, the traditional sweet cassava accessions were catalogued and phenotypic variability among plants were registered. Plants with different characteristics located at the same site were collected separately. Approximately five to eight branches of 0.8 m from each mature adult plant were collected and individually identified. At the end of the collection a total of 50 accessions were obtained, which were maintained *ex situ* at the Sweet Cassava Germplasm Bank located at the Fazenda Experimental de Iguatemi (Iguatemi Experimental Farm), 23°S 20' 48'', 52°W 04' 17'') of the Universidade Estadual de Maringá (Maringá State University - UEM), PR, Brazil. The list of the evaluated accessions is shown in Table 1.

The planting was performed in February 2013. The stems were cut in small fragments of 0.15 to 0.20 m and planted at 0.10 m depth. For comparison purposes, the commercial sweet cassava cultivar IAC 576-70 was added as a control. The cultivar IAC 576/70 has some interesting features, such as resistance to the causal agent of bacteriosis (*Xanthomonas axonopodis* pv. *manihotis*), high yield in relation to other sweet cassava varieties, besides great biochemical and commercial characteristics (external root structure, cooking time, low content of HCN acid, yellowish root pulp), which fulfills the requirements of consumers in Brazilian Southeastern region (Vilella et al., 1985). At the end the total of access evaluated in this study were 51.

DNA extraction

The molecular analysis were conducted at the Laboratório de Melhoramento do Feijão Comum e de Biologia Molecular of the Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) at the UEM (23ºS 26' 8'', 51ºW 53' 42"). Genomic DNA extraction from freshly young leaves of each accession was performed following the protocol described by Dellaporta et al. (1983). The obtained genomic DNA was quantified using FluorometerQubit® (Invitrogen, California, USA). DNA samples were diluted to obtain a final concentration of 50 ng μL^{-1}.

A set of 20 microsatellite markers identified and characterized in previous studies (Chavarriaga-Aguirre et al., 1998; Mba et al., 2001, Kunkeaw et al., 2010; Sraphet et al., 2011) were used to genotype the sweet cassava accessions (Table 2). PCR reactions were performed using 50 ng μL^{-1} of DNA; 0.25 mM of each deoxyribonucleotide (dATP, dCTP, dGTP and dTTP); 1.5 mM of $MgCl_2$; 10 mM of 10x PCR Buffer, 0.08 µM of each primer (forward and reverse), 1 U of *Taq* DNA polymerase enzyme and add ultrapure water to achieve a total volume of 25 µL.

Amplification reactions were carried out in a thermal cycler Endurance TC-412 (Techne Limited, Staffordshire, England) followed by pre-established parameters for each marker (Chavarriaga-Aguirre et al., 1988; Mba et al., 2001).

Thermal parameters for SSRY primers started of 94°C by 5 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing

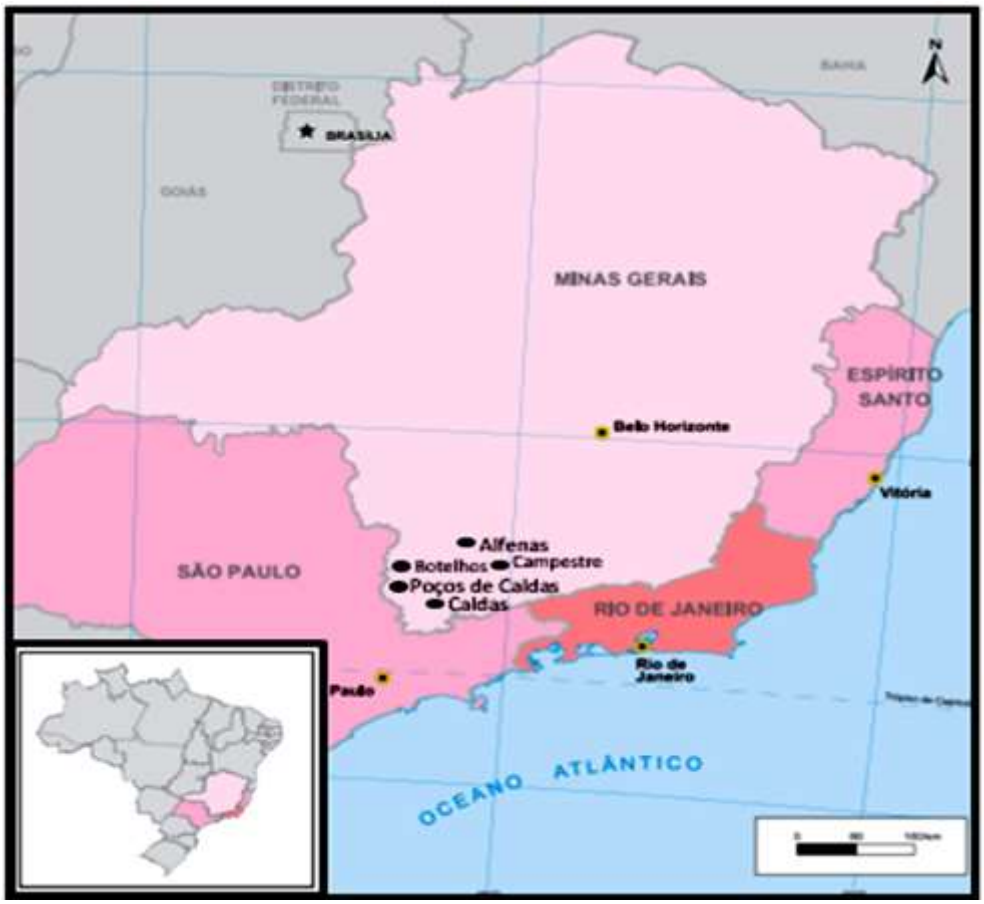

Figure 1. Geographic localization of the counties from Southern of Minas Gerais State, Brazil, where the traditional sweet cassava accessions were collected. Source: IBGE(2016).

temperature established for each primer for 2 min, extension at 72°C for 2 min and final extension for 5 min (Mba et al., 2001).

Further, amplification reaction for GA primers run as follows: initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 1 min, annealing temperature varying between 45 and 59°C, extension at 72°C for 2 min, and final extension at 72°C for 5 min (Chavarriaga-Aguirre et al., 1998). The amplification products were analyzed on 10% polyacrylamide gel stained with 0.02% SYBR Safe (Invitrogen, Oregon, EUA). The DNA bands were visualized under ultraviolet light and images were digitalized using the L-Pix EX photo documentation system (Loccus Biotech, Cotia, Brazil).

Data analysis

The population structure analysis of 51 sweet cassava accessions was performed using the software Structure 2.3.4 (Pritchard et al., 2000) based on a Bayesian algorithm to categorize the accessions into *K* groups. The analysis was conducted by assigning 10,000 interactions as burn-in and performing 100,000 interactions using the Markov Chain Monte Carlo (MCMC) method and 14 grouping simulations with the *K*-factor (number of groups in the population) ranging from 2 to 20. To determine the optimal *K* groups, the "ΔK" parameter was estimated using the Structure Harvester program (Earl and von Holdt, 2012), as proposed by Pritchard et al. (2000) and Evanno et al. (2005).

The software GenAlEx 6.5 (Peakall and Smouse, 2012) was used to estimate the allele frequencies per locus and to perform a principal coordinate (PCoA) analysis and analysis of molecular variance (AMOVA) (Excoffier et al., 1992). The statistical significance of the variances was tested using 999 random permutations at 1%. The genetic diversity of genes and alleles, the allele frequencies and the polymorphism information content (PIC) were calculated for each microsatellite locus using the Power Marker 3.25 software (Liu and Muse, 2005). A matrix based on the CS Chord distances (Cavalli-Sforza and Edwards, 1967) was used to develop a Neighbor-Joining Tree in the software Mega version 6 (Tamura et al., 2011).

RESULTS AND DISCUSSION

Genetic diversity indices

In the present study, 68 different alleles were identified based on the analysis of 20 microsatellite markers, with an average of 3.4 alleles per marker (Table 3). A study developed by Chavarriaga-Aguirre et al. (1998) revealed the presence of 128 alleles in 522 sweet cassava accessions from Colombia (CIAT), showing a range from one to 15 alleles per marker. Later, Fregene et al. (2003)

Table 1. Identification and origin of the sweet cassava accessions collected in the Southern region of Minas Gerais State, Brazil.

Accession	Origin	Accession	Origin
BGM 634	Village, Poços de Caldas	BGM 660	Estrada BR 459, Caldas
BGM 635	Sta Rosália, Poços de Caldas	BGM 661	Santana, Poços de Caldas
BGM 636	Sta Rosália, Poços de Caldas	BGM 662	Santa Cruz, Botelhos
BGM 637	Santana, Poços de Caldas	BGM 663	Rua Tiradentes, Botelhos
BGM 638	Santana, Poços de Caldas	BGM 664	Creboja, Botelhos
BGM 639	Village, Poços de Caldas	BGM 665	Creboja, Botelhos
BGM 640	Campo das Antas, Poços de Caldas	BGM 666	Creboja, Botelhos
BGM 641	Campo das Antas, Poços de Caldas	BGM 667	Santana, Poços de Caldas
BGM 642	Alfenas	BGM 668	Santa Cruz, Botelhos
BGM 643	Alfenas	BGM 669	Santa Cruz, Botelhos
BGM 644	Vila Togni, Poços de Caldas	BGM 670	Pica Pau Amarelo, Botelhos
BGM 645	Jd. Kenedy, Poços de Caldas	BGM 671	Pica Pau Amarelo, Botelhos
BGM 646	Jd. Kenedy, Poços de Caldas	BGM 662	Pica Pau Amarelo, Botelhos
BGM 647	Jd. Kenedy II, Poços de Caldas	BGM 673	Santana, Poços de Caldas
BGM 648	Jd. Kenedy II, Poços de Caldas	BGM 674	Santana, Poços de Caldas
BGM 649	Jd. Kenedy II, Poços de Caldas	BGM 675	Sta. Rosália, Poços de Caldas
BGM 650	Alfenas	BGM 676	Herculano do Lago, Botelhos
BGM 651	Jd. Kenedy II, Poços de Caldas	BGM 677	Sta. Cruz Botelhos
BGM 652	Jd. Kenedy II, Poços de Caldas	BGM 678	Sta. Cruz, Botelhos
BGM 653	Campo das Antas, Poços de Caldas	BGM 680	Casa dona Benedita, Botelhos
BGM 654	São José II, Poços de Caldas	BGM 681	Sta. Rosália, Poços de Caldas
BGM 655	Cond. das Antas, Poços de Caldas	BGM 682	Sta. Rosália, Poços de Caldas
BGM 656	Cond. das Antas, Poços de Caldas	BGM 683	Sta. Rosália, Poços de Caldas
BGM 657	Santana, Poços de Caldas	BGM 690	Santana, Poços de Caldas
BGM 658	Estrada BR 459, Caldas	BGM 686	IAC 576-70*
BGM 659	Estrada BR 459, Caldas		

analyzed the genetic diversity of cassava accessions from Tanzania using 67 microsatellite markers. The authors identified 66.7 alleles per locus with an average of 4.03 alleles per marker. Further, a recent study presented by Turyagyenda et al. (2012) shows the most expressive results. In this case, genetic diversity and population structure were investigated in 51 farmer-preferred cassava landraces and 15 elite accessions grown in Uganda by using 26 SSR markers. A total of 154 alleles with an average of 6.77 alleles per marker was reported.

All evaluated molecular markers were polymorphic and the average of the most frequent allele was 0.6056 (Table 3). The marker GA 131 showed the presence of five alleles. On the other hand, the molecular markers GA 136, SSRY 100 and SSRY 51 revealed the presence of two alleles (Table 3). The obtained results were consistent, corroborating to most studies regarding the use of SSR markers to evaluate the genetic diversity in sweet cassava and confirmed that approximately 100% of the tested markers are typically polymorphic (Siqueira et al., 2009; Turyagyenda et al., 2012). Although Chavarriaga-Aguirre et al. (1998) reported that the marker GA 57 was not polymorphic when tested in Colombian sweet cassava accessions, in the present study the GA 57 marker revealed the presence of three alleles.

According to Siqueira et al. (2009), rare alleles are those present in the population with frequencies lower to 0.05. Our results show the presence of 17 rare alleles with frequency values ranging from 0.0096 to 0.0306, corroborating with previously published data (Mkumbira et al., 2003; Peroni et al., 2007; Rocha et al., 2008; Siqueira et al., 2009; Asare et al., 2011; Turyiagienda et al., 2012; Costa et al., 2013; Ferreira et al., 2015; Ortiz et al., 2016).

In Costa Rica, a genetic diversity study including cassava accessions collected in indigenous communities has shown the presence of rare alleles for GA 140 marker (Rocha et al., 2008). A study carried out with 41 sweet cassava accessions from different Brazilian regions has further demonstrated the existence of rare alleles for GA 126 marker (Siqueira et al., 2009). In addition, genetic diversity studies performed with cassava accessions from Uganda, Africa (Turyagyenda et al., 2012) showed the presence of rare alleles for SSRY 19,

Table 2. Features of the microsatellite markers used to genotype the 51 sweet cassava accessions collected in the Southern region of Minas Gerais State, Brazil.

Order	Motif	Markers	Primer (5' – 3')[2]	AR[3](bp)	TA[4](°C)	Ref[5]
1	NP[1]	GA57	F´: AGCAGAGCATTTACAGCAAGG R´:TGTGGAGTTAAAGGTGTGAATG	153-183	59	A
2	NP	GA134	F´: ACAATGTCCCAATTGGAGGA R´: ACCATGGATAGAGCTCACCG	309-337	59	A
3	NP	GA136	F´:CGTTGATAAAGTGGAAAGAGCA R´: ACTCCACTCCCGATGCTCGC	145-165	55	A
4	NP	GA 21	F´: GGCTTCATCATGGAAAAACC R´: CAATGCTTTACGGAAGAGCC	104-126	58	A
5	NP	GA131	F': TTCCAGAAAGACTTCCGTTCA R': CTCAACTACTGCACTGCACTC	75-19	54	A
6	NP	GA12	F': GATTCCTCTAGCAGTTAAGC R': CGATGATGCTCTTCGGAGGG	131-157	57	A
7	NP	GA126	F´:AGTGGAAATAAGCCATGTGATG R´: CCCATAATTGATGCCAGGTT	176-214	58	A
8	NP	GA127	F´: CTCTAGCTATGGATTAGATCT R´: GTAGCTTCGAGTCGTGGGAGA	203-239	57	A
9	NP	GA140	F´: TTCAAAGGAAGCCTTCAGCTC R´: GAGCCACATCTACTGCACACC	154-164	55	A
10	$(CT)_{29}$	SSRY13	F´: GCAAGAATTCCACCAGGAAG R´:CAATGATGGTAAGATGGTGCG	234	55	B
11	$(CT)_8(CA)_{18}$	SSRY19	F´:TGTAAGGCATTCCAAGAATTAA R´: TCTCCTGTGAAAAGTGCATGA	214	55	B
12	$(GA)_{26}$	SSRY21	F´: CCTGCCACAATATTGAAATGG R´: CAACAATTGGACTAAGCAGCA	192	55	B
13	$(CT)_27$	SSRY45	F´: TGAAACTGTTTGCAAATTACGA R´: TCCAGTTCACATGTAGTTGGCT	228	55	B
14	$(GCT)_{13}$	SSRY101	F´: GGAGAATACCACCGACAGGA R´: ACAGCAGCAATCACCATTTC	213	55	B
15	$(CT)_{16}$	SSRY135	F´: CCAGAAACTGAAATGCATCG R´: AACATGTGCGACAGTGATTG	253	45	B
16	NP	SSRY35	F': GCAGTAAAACCATTCCTCCAA R': CTGATCAGCAGGATGCATGT	277-285	55	B
17	NP	SSRY100	F': ATCCTTGCCTGACATTTTGC R': GGAGAATACCACCGACAGGA	209-273	55	B
18	NP	SSRY51	F': AGGTTGGATGCTTGAAGGAA R': GGATGCAGGAGTGCTCAACT	298	55	B
19	NP	SSRY28	F': TTCCAGACCTGTTCCACCAT R': ATTGCAGGGATTATTGCTCG	100-120	55	B
20	NP	SSRY27	F':ACAATTCATCATGAGTCATCAAC R': CCGTTATTGTTCCTGGTCCT	265-280	55	B

NP[1] Motif not published; [2]*Primer*, *Forward* (F´) and *Reverse* (R´); [3]AR, amplified region in base pairs (bp); [4]TA, Annealing temperature used; [5]Ref, Reference: A, Chavarriaga-Aguirre et al. (1998); B, Mba et al. (2001).

SSRY 135 and SSRY 21 markers. A recent study that investigated the genetic diversity of traditional sweet cassava accesses from South region of Brazil revealed that GA 126 and GA 140 markers exhibited the occurrence of rare alleles (Costa et al., 2013).

On the other hand, the markers GA 136, SSRY 100, SSRY 101, SSRY 27, SSRY 35 and GA 134 showed the presence of high allele frequency which were respectively: 0.9489, 0.8653, 0.8600, 0.7708, 0.7446 and 0.7282 (Table 3). These high values could be directly related to the tendency of these alleles to fix in the population through increasing the homozygosis, since the

Table 3. Genetic diversity among 51 sweet cassava accessions from Southern region of Minas Gerais State, Brazil, using 20 SSR markers.

Marker	N° of alleles	Fragment size (bp)	Frequency	PIC[1]	*Ho*[2]	Genetic diversity
GA 12	3	150 160 180	0.4313 0.5588 0.0098*	0.3852	0.8824	0.5015
GA 21	3	108 110 120	0.0800 0.5200 0.4000	0.4712	0.8000	0.5632
GA 57	3	180 190 200	0.2441 0.5581 0.1976	0.5236	0.6977	0.5898
GA 126	4	180 190 200 230	0.4615 0.0096* 0.5192 0.0096*	0.4022	0.9423	0.5172
GA 127	4	214 229 232 242	0.3085 0.2234 0.3297 0.1382	0.6763	0.9362	0.7270
GA 131	5	100 108 116 124 130	0.4600 0.0098* 0.4900 0.0098* 0.0280*	0.4433	1.0000	0.5463
GA 134	3	310 320 340	0.7282 0.2173 0.0540	0.3659	0.2609	0.4194
GA 136	2	160 152	0.9489 0.0510	0.0921	0.1020	0.0968
GA 140	4	152 163 170 180	0.4803 0.4803 0.0294* 0.0098*	0.4301	1.0000	0.5375
SSRY 13	4	200 210 220 230	0.6538 0.2980 0.0288* 0.0192	0.4052	0.5962	0.4824
SSRY 19	4	178 191 209 218	0.0306* 0.4693 0.0306* 0.4693	0.4587	1.000	0.5575
SSRY21	4	163 183 186 205	0.2873 0.4361 0.0744 0.2021	0.6237	0.8936	0.6809
SSRY27	3	265 267 275	0.7708 0.0208* 0.2083	0.3098	0.4167	0.3620

Table 3. Contd.

Marker	N° of alleles	Fragment size (bp)	Frequency	PIC[1]	*Ho*[2]	Genetic diversity
SSRY28	4	140 150 160 170	0.1442 0.4903 0.0192* 0.3461	0.5456	0.6731	0.6185
SSRY35	4	240 250 280 290	0.1382 0.7446 0.0106* 0.1060	0.3806	0.2340	0.4149
SSRY45	4	172 186 189 199	0.0652 0.5434 0.0108* 0.3804	0.4662	0.5217	0.5555
SSRY51	2	257 270	0.5000 0.5000	0.3750	1.0000	0.5000
SSRY 100	2	190 200	0.8653 0.1346	0.2058	0.2692	0.2330
SSRY 101	3	214 218 224	0.8600 0.1300 0.0100*	0.2183	0.2600	0.2434
SSRY 135	3	263 269 280	0.3333 0.0222* 0.6444	0.3803	0.4889	0.4731
Mean	3.4	-	0.6056	**0.4080**	**0.6487**	**0.4810**

[1]PIC, Polymorphism information content; [2] *Ho,* heterozygosity per *locus;**Rare alleles.

Table 4. Molecular analysis (AMOVA) of the sweet cassava accessions from Southern of Minas Gerais State, Brazil based on SSR markers.

Source of variation	DF[1]	SQ[2]	MS[3]	EV[4]	(%)	PhiPT*	Pvalue[5]
Intra groups	3	50.780	16.927	0.690	7	0.073	0.001
Inter groups	48	418.277	8.714	8.714	93		
Total	51	469.058		9.404	100		

[1]DF, Degrees of freedom; [2]SQ, sum of squares; [3]MS, mean square; [4]EV, estimate variance; [5]P value, significance probability; ** Statistically significance at 1%; *PhiPT, analog of Wright's Fst.

heterozygosity values for these alleles were low (Table 4). According to Nei (1987) these values are important to emphasize the genetic diversity among accessions and essential for evolutionary studies, because genetic change in the allele composition of the population could be evaluated by its allele frequencies.

The lowest allele frequency in this study was 0.0096, which was observed by the marker GA 126. However, this marker showed high heterozygosis value of 0.9423, which emphasizes the existence of allelic variation and, consequently, genetic diversity among the sweet cassava accessions (Costa et al., 2013).

The average of heterozygosity observed for the 20 molecular markers used in this study was estimated in 0.6487, which was considered high due to the fact that cassava plants are able to perform natural crossed fertilization, open pollination and protogynous flowering (Fregene et al., 2003). The mean heterozygosis value reported in this work is similar to previous population structure studies of cassava, which involved the use of microsatellite markers (Monteiro-Rojas et al., 2011; Asare et al., 2011; Turyagyenda et al., 2012; Kawuki et al.,

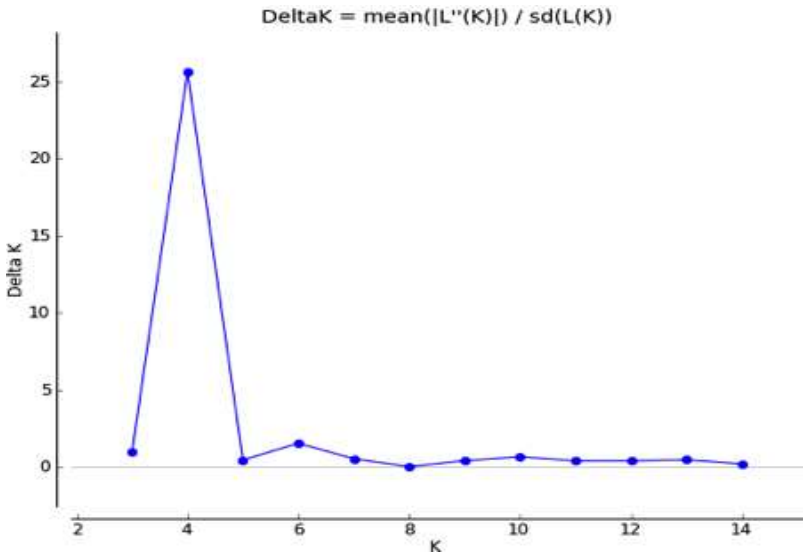

Figure 2. Inference of the number of K groups for the 51 sweet cassava accessions from Southern region of Minas Gerais State, Brazil, according to Pritchard et al. (2000), as obtained using the program Structure Harvester (Earl and von Holdt, 2012).

2013; Costa et al., 2013). In Costa Rica, Monteiro-Rojas (2011) evaluated 185 cassava accessions, demonstrating a mean heterozygosis of 0.6705. Further, Kawuki et al. (2013) reported heterozygosis of 0.57 and genetic diversity of 0.58, when cassava accessions from different African countries were investigated with 26 microsatellite markers.

The microsatellites that detected higher genetic diversity among the cassava accessions were GA 127, SSRY 21 and SSRY 28, with values of 0.7270, 0.6809 and 0.6185, respectively (Table 3). On the other hand, the markers GA 136, SSRY 100 and SSRY 101 exhibited the lowest values for genetic diversity, which were 0.0968, 0.2330 and 0.2434, respectively (Table 3).

Regarding the polymorphic information content (PIC), our results showed a mean value of 0.4080 (Table 3), which is moderately informative according to the methodology proposed by Botstein et al. (1980). The mean PIC value found was low compared to others studies related to cassava reported in the literature (Xia et al., 2005; Asare et al., 2011; Turyagyenda et al., 2012). On the other hand, studies developed by Costa et al. (2013) and Ortiz et al. (2016) with traditional sweet cassava cultivars from Paraná State, Brazil, showed PIC values such as 0.4040 and 0.4598. This fact can be explained because smaller and more homogeneous populations tend to present lower values of informative content per *locus*.

The markers with higher PIC values in this study were GA 127 (0.6763), SSRY 21 (0.6237), SSRY 28 (0.5456) and GA 57 (0.5236), whereas the markers with the lowest were GA 136 (0.0921), SSRY 101 (0.2183); SSRY 100 (0.2058). The other evaluated loci were considered moderately informative with values ranging from 0.3098 to 0.4662.

Population structure

The population structure analysis of the 51 traditional sweet cassava accessions according to Pritchard et al. (2000) revealed the formation of four distinct groups, according to Delta K value (Figure 2). The presence of admixture among the four sub-populations was observed.

The Figure 3 shows the distribution of the 51 sweet cassava accessions into the four sub-populations. The Group 1 allocated seven accessions, while the Group 2 was the most expressive containing 24 accessions, followed by the Group 3 and Group 4, which each one allocated 10 accessions.

The distribution dynamics was heterogeneous, as it was observed that the accessions from the same county or locality, such as BGM 643, BGM 642 and BGM 650 (from Alfenas, MG), were allocated in distinct groups through the probabilistic methodology (Pritchard et al., 2000). Similar results were obtained by Costa et al. (2013) and Ortiz et al. (2016) when these authors analyzed traditional sweet cassava accessions from Paraná State. This fact emphasizes the influence of exchanging cassava germplasm among cassava producers, upon population structure: accesses that were grown in different and distant regions were clustered in the same group (Lokko et al., 2006; Kizito et al., 2007, Ortiz et al., 2016).

The Neighbor-joining Tree (Figure 4) was constructed based on the similarity or dissimilarity among accessions through the genetic distance matrix of C.S. Chord (Cavalli-

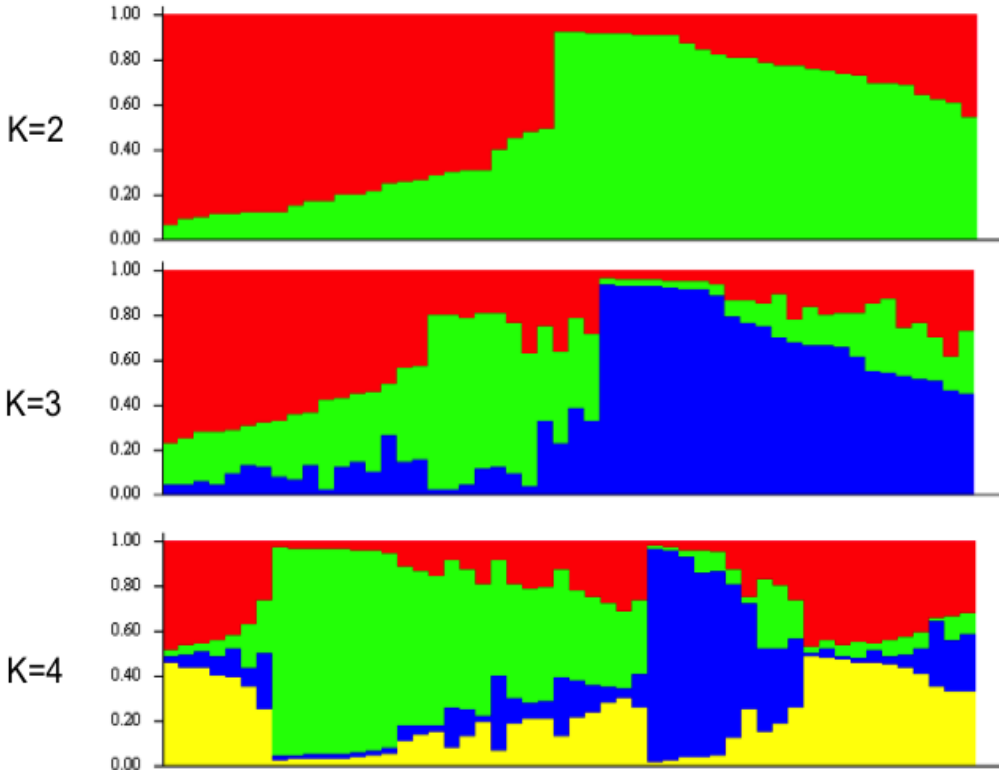

Figure 3. Population structure of 51 accessions of sweet cassava collected in Southern of Minas Gerais State, Brazil, assuming K = 4, according to Structure 2.3.4 (Pritchard et al., 2000).

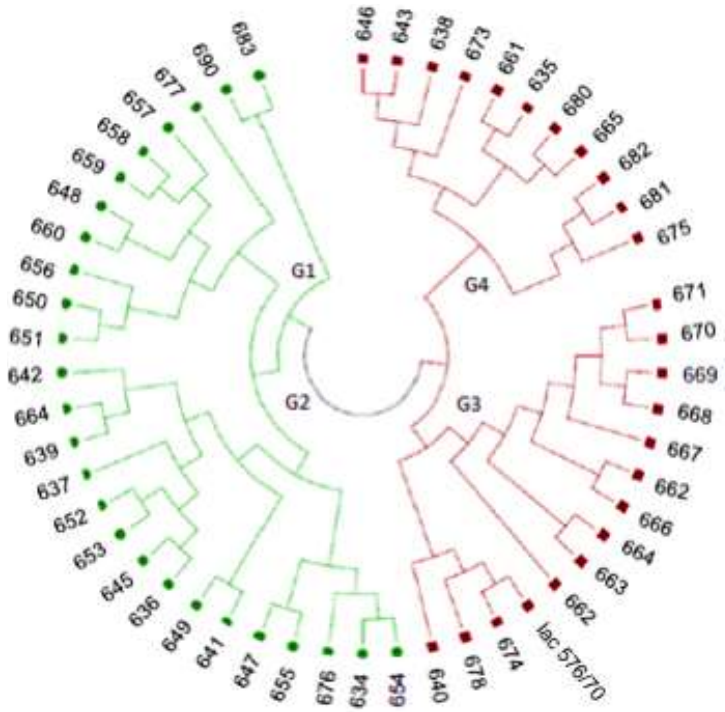

Figure 4. Distribution of 51 sweet cassava accessions from Southern region of Minas Gerais State, Brazil, based on 20 SSR loci obtained by the Neighbor Joining Tree from the genetic distance matrix of CS Chord (Cavalli-Sforza and Edwards, 1967), assuming K = 4 groups (Pritchard et al., 2000).

Sforza and Edwards, 1967). This matrix revealed that the most divergent accessions combination were BGM 690×BGM 655 (D_{ij} = 0.5696), BGM 647×BGM 660, (D_{ij} = 0.5212), BGM 659×BGM 675 (D_{ij} = 0.4854), BGM 674×BGM 655 (D_{ij} = 0.4759). Based on our results, the accessions considered most divergent could be used in breeding programs as parents in order to produce heterotic clones (Gonçalves-Vidigal et al., 1997).

These combinations are interesting because in addition to the divergence of the plants, most of these accessions are highly resistant to bacteriosis, caused by *X. campestris* pv *manihotis*, which is one of the most important cassava´s disease in Brazil, mainly in the Southern of Minas Gerais State where the accessions were collected. On the other hand, accessions BGM 669×BGM 668, BGM 670×BGM 671, BGM 652×BGM 653 were the most similar combination. This genetic similarity could be a reflection of the intense flow of material among the smallholders, generating duplicates (Kizito et al., 2007; Ortiz et al, 2016).

There was a discrepancy in the results when compared to the distribution of the accession inside of the formed groups by both methodologies proposed. In Group 1, the accession BGM 683 was the only one that clustered to the same group according to both methodologies.

According to the probabilistic methodology (Pritchard et al., 2000), the cultivar IAC 576/70 (BGM 686) was allocated in the group K = 2 (Figure 3), among two accessions from Poços de Caldas and Botelhos, MG. This results revealed genetic similarity between the cultivar IAC 576/70 and the accessions of the Group 2 (K=2) from Botelhos and Poços de Caldas. It is known that the IAC 576-70 comes from the cross between the clone IAC 14-18 and the traditional farming SRT 797-Ouro do Vale (Golden Valley), which was commonly found in small crops of cassava from Southeastern region of Brazil (Vilella et al., 1985). This fact probably contributed to the similarity of IAC 576-70 with Botelhos and Poços de Caldas accessions, which are very close to the border of Minas Gerais and São Paulo states.

Data regarding the analysis of molecular variance (AMOVA) is shown in Table 4. Our results revealed that a variance intra-groups was very expressive with a value of 93%, confirming the presence of a significant genetic diversity among the evaluated traditional cassava accessions.

The expressive value for genetic variance in these accessions reinforces what others mentioned about the cassava genetic nature being heterotic (Fregene et al., 2003). In Africa, using 98 cassava accessions from Uganda, Abaca et al. (2013) found an intra-group genetic variation (98%). Whereas in Brazil, Costa et al. (2013) analyzed the genetic diversity among 66 sweet traditional accessions from the Paraná region (Maringá, PR) and reported a value for the variation intra-groups of 77%. Another study of genetic diversity involving traditional sweet cassava accessions collected in the North of Paraná State showed 83% variation intra-groups (Ortiz et al., 2016). All these values of the variation intra-groups were similar to our results.

The low variation within the groups indicates that the accessions in each group are highly similar to each other but differ from the accessions included in the other groups. The differentiation among the four corresponding groups can also be compared by the Fst index, which estimates the genetic differentiation among groups.

According to Wright (1978), the PhiPT (analogous Wright Fst) coefficient is an indicative of genetic variability level among groups. The present study revealed a FST value of 0.073 that according to Wright's classification is considered of low genetic diversity (Table 4). Similar results of Fst index (0.07) were found by Ferreira et al. (2015), when the authors analyzed the genetic diversity of landraces of sweet cassava from the Mato Grosso do Sul State. Lokko et al. (2006) conducted studies of the genetic diversity in cassava accessions resistant to CMV (cassava mosaic virus). The authors found a values of Fst index that were considered low, while Ortiz et al. (2016) accessions of cassava from Maringá, Cianorte and Toledo, Parana state, verified a moderate diversity between the two groups of cassava accessions originally from Maringá, Paraná State (Fst index = 0.107).

The literature also shows other studies that exhibited low values for the Fst index, indicating low genetic variation among accessions formed in the population. For instance, Turyagyenda et al. (2012) obtained a value of 0.025 for genetic diversity between the two groups formed by the cassava accessions from Uganda. In addition, Mezette et al. (2013) reported that traditional accessions of cassava from different Brazilian regions revealed a low value of Fst equivalent to 0.041. It is plausible that a very low Fst index for cassava accession indicates a reduced overall genetic differentiation due to the random mating between groups or at least among their ancestors (Turyagyenda et al., 2012).

The Principal Coordinate Analysis (PCoA) contributed to the interpretation of the results for genetic diversity and population structure (Figure 5). The First Principal Coordinate (PCoA1) explains 26.33% of the variation among the accessions and is responsible for separating the accessions in four groups. The Second Principal Coordinate (PCoA2) explains 21.88% of the total variation. Together, these results are able to explain 48.21% of the genetic variation.

Principal coordinates analysis

Even though there is a certain divergence considering de grouping of the accessions among de different methods used, the individuals considered most divergent such as BGM 690 and BGM 655 were located in different regions of Figure 4. The same can be observed with the most

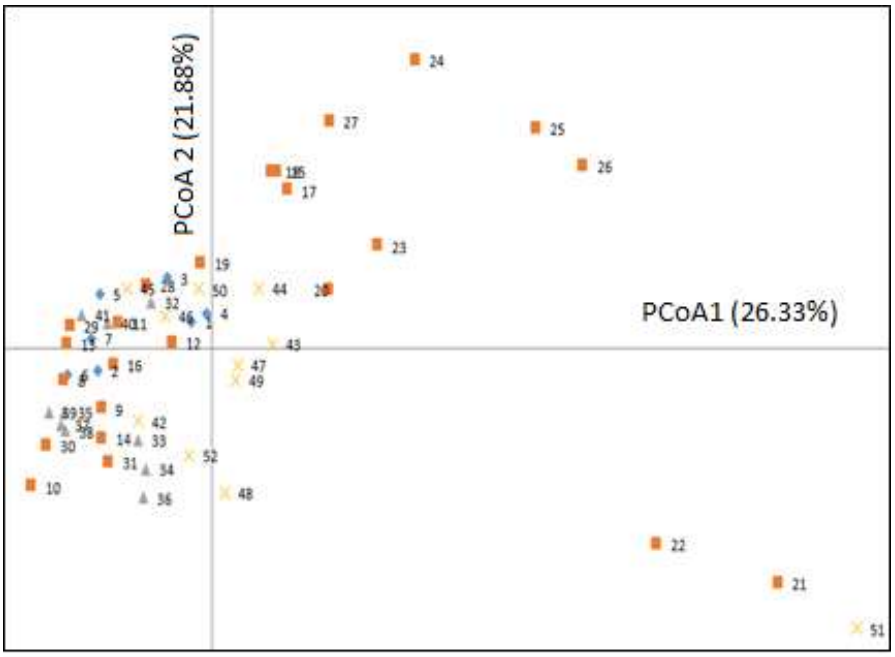

Figure 5. Principal coordinates analysis (PCoA1, 26.33% vs PCoA2, 21.88%) of SSR data indicating 48.21% genetic diversity among 51 traditional sweet cassava accessions from Southern region of Minas Gerais State, Brazil.

similar accessions that are grouped together. It shows that the methods used had a certain convergence of results.

Conclusions

Our results showed that there was a moderate genetic diversity among the traditional sweet cassava accessions collected in the Southern of Minas Gerais State, Brazil, based on the SSR molecular markers. The PhiPT index was 0.073. The collected accessions were distributed in four distinguished sub-populations based on Bayesian analysis and multivariate analysis. The results revealed that the most dissimilar genotypes were the accessions BGM 690, BGM 655 and BGM 660, and the combination of such genotypes could provide a heterotic effect and transgressive segregation, ideally suited for cassava breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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