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Genetic and population structure of sweet cassava (*Manihot esculenta* Crantz) germplasm collected from Campo Grande, Mato Grosso do Sul, Brazil

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Abstract

Knowledge of germplasm diversity and its genetic characterization is crucial to crop improvement and conservation strategies. This study aimed at characterization of diversity genetic and population structure of traditional sweet cassava germplasm at the molecular level. A total of 60 sweet cassava accessions from Campo Grande city, Mato Grosso do Sul State, Brazil, and a commercial variety, were analyzed using microsatellite markers. Allelic frequencies were used in order to assess the genetic diversity indexes for each marker. Population structure was analyzed using genetic distances and Bayesian model. A total of 88 alleles were identified from the 19 SSR markers, with an average of 4.63 alleles per marker. The mean of polymorphic information content (PIC) was 0.525 and indicated that the markers used were informative and polymorphic, especially the marker SSRY 13. The heterozygosity observed (H_o) ranged from 0.569 to 0.984, with a mean of 0.862, evidencing the existence of high natural heterozygosis in cassava. The genetic diversity mean was of 0.601 and revealed considerable genetic diversity among the genotypes. Population structure analysis resulted in the distribution of the 61 accessions in two distinct sub-populations, by Bayesian analysis, Neighbor-Joining Tree and Principal Coordinates Analysis, indicating the existence of significant genetic diversity between sub-populations. Combination of cassava accessions BGM 570 × BGM 613 and BGM 570 × IAC 576/70 is recommended for obtaining superior segregation in order to improve yield. The results will contribute to the conservation of the Mato Grosso do Sul germplasm and sweet cassava breeding.

Keywords: genetic diversity, *Manihot esculenta* Crantz, microsatellite markers, population's structure, sweet cassava.
Abbreviations: AMOVA_Analysis of molecular variance; PCoA_Principal Coordinates Analysis; PIC_polymorphism information content; SSR_Simple Sequence Repeat.

Introduction

Cassava (*Manihot esculenta* Crantz) is a crop for subsistence considered an important food for about 800 million people in subtropical and tropical countries of Africa, Asia and the Americas (Prochnik et al., 2012; FAO, 2013; Okogbenin et al., 2013). The world production of cassava tuberous roots is about 262 million tones and currently the African countries have the largest area cultivated with cassava, especially Nigeria which is the largest world producer, followed by countries in south-east Asia, mainly Indonesia and Thailand (FAO, 2013). In South America, Brazil is outstanding, with a cultivated area of 1,572,665 ha and production of 23,087,828 tons of storage tubes (IBGE, 2015).

Originated from central region of Brazil (Olsen and Schaal, 1999), cassava belongs to the Euphorbiaceae family, *Manihot* genus, which consists of about 98 species (Olsen and Schaal, 1999), and presents an allopolyploid genome, with 2n=2x=36 chromosomes (Nassar and Ortiz, 2007). The species are traditionally vegetatively propagated for human cultivation, despite producing seeds by sexual reproduction, which allied to an alogamus mating system (Elias et al., 2001) conferring a high level of heterozygosity (Nassar and Ortiz, 2007). The crop can be harvested practically every month of the year, presenting great rusticity and tolerance of drought in low fertility soils (Nassar and Ortiz, 2007).

Sweet cassava is the traditional cultivated type, which main characteristic is the low cyanide content (< 100 mg HCN kg^{-1}) in the storage roots (Kizito et al., 2007). This species is cultivated for centuries by smallholders in traditional agricultural systems known as "backyard cultivations" that provided the enrichment and maintenance of genetic variation that exists in the sweet cassava germplasm (Zuin et al., 2009; Costa et al., 2013).

The "backyard cultivations" includes sweet cassava accessions with unique features and high genetic diversity, which need to be explored and maintained in germplasm banks, because of the risk of genetic erosion as result of the accelerate urban growth of cities such as Campo Grande, Mato Grosso do Sul, Brazil (Zuin et al., 2009; Costa et al., 2013). Furthermore, successful breeding programs depends on the genetic base available and is determined by the collection of useful genetic resources available in accessions collected and properly characterized (Kalia et al., 2011).

Several studies have reported morphological characterization to study the genetic diversity among cassava genotypes (Zuin et al., 2009; Rimoldi et al., 2010; Mezette et

al., 2013). However, molecular markers are also an efficient method of germplasm characterization, considering they detect polymorphisms directly in the genomic DNA without the influence of environmental variation, and in early stages of plant development (Agarwal et al., 2008).

Molecular markers have been used to estimate genetic diversity in cassava, like RAPD (Random Amplified Polymorphic DNA) (Rimoldi et al., 2010; Silva et al., 2015), AFPL (Amplified Fragment Length Polymorphism) (Benesi et al., 2010) and microsatellites (Turyagyenda et al., 2012; Costa et al., 2013; Fu et al., 2014). Considering all molecular markers available, one of the most efficient on genetic diversity studies is a microsatellite, also known as SSR (Simple Sequence Repeat). The SSRs markers are highly informative due to their multi-allelic nature, co-dominant inheritance and wide distribution along the genome of the species (Kalia et al., 2011; Kantartzi et al., 2013).

Several studies of genetic diversity and population structure of cassava using SSR markers have been successfully exploited in Brazil and other tropical countries (Turyagyenda et al., 2012; Costa et al., 2013). However, genetic characterization of the traditional sweet cassava germplasm originated from Campo Grande, Mato Grosso do Sul, Brazil, are still scarce. The present work aimed to characterize the genetic diversity and population structure of traditional sweet cassava accessions collected in Campo Grande, Mato Grosso do Sul, Brazil, using microsatellites markers, to guide the breeders in the choice of parental for future crosses.

Results and Discussion

Genetic diversity indexes

A total of 88 alleles were amplified with 19 SSR markers, and all molecular markers were polymorphic in the traditional sweet cassava accessions. The number of alleles observed per marker ranged from 3 (SSRY 51, GA 12, GA 21, GA 57 and GA 127) to 8 (SSRY 13 and GA 140) with an average of 4.63 alleles per marker (Table 3). These results were similar to those obtained in other studies of genetic diversity of cassava (Kizito et al., 2007; Siqueira et al., 2009). Kizito et al. (2007) evaluated 288 cultivars from Uganda with 11 SSR markers and obtained average of 4.9 alleles per marker. Similarly, Siqueira et al. (2009) used 9 microsatellites markers for evaluating the genetic diversity in 42 cassava accessions from several regions in Brazil and obtained average of 5 alleles per marker.

The major allelic frequency per microsatellite ranged between 0.39 (GA 140 and SSRY 13) and 0.72 (GA 57), with an average of 0.48 (Table 3). Besides the high allelic frequency on GA 57 marker, its heterozygosity was high (0.57) in the Campo Grande's sweet cassava populations, evidencing high genetic differentiation. Asare et al. (2011) accessed 43 cassava accessions through 20 SSR markers and the average allelic frequency per marker was 0.53 and Elibariki et al. (2013) evaluated the genetic diversity of 21 cultivars from Tanzania with 20 SSR markers and obtained average allelic frequency per marker equal to 0.43.

Rare alleles ($f < 0.05$) (Botstein et al., 1980) were noted in 14 markers (Table 3). The GA 131 and GA 140 markers presented the highest number of rare alleles identified as 68pb, 83pb, 88pb, 95pb in GA 131 and 141pb, 146pb, 154pb, 165pb in GA 140, respectively (Table 3). These results of rare alleles were consistent with several cassava SSR studies (Siqueira et al., 2009; Turyagyenda et al., 2012; Costa et al., 2013). In our study, a rare allele was found in the GA 126 marker and Siqueira et al. (2009) and Costa et al. (2013) observed a rare allele in GA 126 marker when assessing the genetic diversity of Brazilian cassava accessions.

The average heterozygosity was 0.86 and ranged from 0.57 to 0.98. The GA 12, GA 126, GA 131 and GA 140 microsatellites markers had major heterozigosity (Table 3). According to Rimoldi et al. (2010) the various factors that contribute to the existence of a high degree of heterogeneity found in natural populations of sweet cassava include: (1) sweet cassava is a species native to Brazil; (2) the traditional accessions were obtained from smallholders, and (3) producers propagate sweet cassava vegetatively by cuttings from mature plants in their fields. Both markers SSRY 21 and SSRY 13 presented the highest diversity, 0.69 and 0.70, respectively (Table 3), and the lowest one was GA 57 marker with 0.42. The average genetic diversity was 0.60 and similar to the result obtained in previous studies with 66 landraces cassava using 26 microsatellites markers (Turyagyenda et al., 2012). The existence of wide genetic variety found in sweet cassava can be explained by the fact that the species is allogamous (Rimoldi et al., 2010; McKey et al., 2010). So, that new combinations are constantly generated naturally (Ayling et al., 2012). The PIC (Polymorphic Information Content) is an index used to qualify a marker for genetic studies, reflecting the polymorphism detected. Thirteen markers tested in sweet cassava accessions were highly informative ($PIC>0.5$) and six markers were moderately informative ($0.25<PIC<0.5$) (Table 3), according to the classification proposed by Anderson et al. (1993). SSRY 13 marker showed the highest PIC of 0.65, and GA 57 marker showed the lowest value, 0.35 (Table 3). The average PIC for all markers was 0.525. Turyagyenda et al. (2012) obtained average PIC equal to 0.61, analyzing the genetic diversity of 66 cassava landraces from Uganda, Africa, with 26 SSR markers, of which 5 molecular markers were used in this study. Values of PIC obtained in the current study indicate that the markers used were sufficiently informative to be used for genetic studies in cassava, and demonstrated that there is significant genetic diversity among sweet cassava set evaluated.

Population structure and cluster analysis

The sub-division of the population was determined by software Structure 2.3.3 and Neighbor Joining Tree based on C.S. Chord genetic distance. (Cavalli-Sforza and Edwards, 1967). The analyses showed a significant diversity between the sub-populations. The Bayesian analysis (Pritchard et al., 2000) indicated that 61 accessions were distributed in two distinct populations (Fig 1), determined by ΔK generated by the software Structure Harvester (Earl and von Holdt, 2012). For value K=2, 26 accessions were allocated in sub-population 1 (BGM 565 to BGM 618) and other 35 accessions were allocated in the sub-population 2 (BGM 572 to BGM 597) (Fig 1).

The genetic dissimilarity matrix and the Neighbor Joining Tree analysis were performed with POWERMARKER 3.25 (Liu and Muse, 2005) and MEGA 5.2 (Tamura et al., 2011), respectively, to estimate the phylogenetic relation between the sub-populations.

The clustering of the accessions by Neighbor-Joining Tree resulted in the formation of two sub-populations. The sub-population 1 allocated 23 accessions (BGM 578 to BGM 573) and the sub-population 2 allocated 38 accessions (BGM 588 to BGM 615) (Fig 2). This clustering showed partial concordance in terms of sub-populations members obtained by Bayesian analysis (Fig 1 and Fig 2).

Table 1. List of the 60 sweet cassava accessions for genetic diversity analysis collected in Campo Grande city, Mato Grosso do Sul State, 2012.

Number of the order	Accession number	Collection neighborhood	Number of the order	Accession number	Collection neighborhood
1	BGM 565	Vilas Boas	31	BGM 599	Residencial Búzios
2	BGM 566	Vilas Boas	32	BGM 600	União 2
3	BGM 567	Carandá Bosque 2	33	BGM 601	Oliveira 3
4	BGM 568	Carandá Bosque 2	34	BGM 603	Oliveira 3
5	BGM 569	Danúbio Azul	35	BGM 605	Cidade Jardim
6	BGM 570	Danúbio Azul	36	BGM 607	Vilas Boas
7	BGM 571	Danúbio Azul	37	BGM 608	Jardim Itamaracá
8	BGM 572	São Benedito	38	BGM 609	Jardim Itamaraty
9	BGM 573	São Benedito	39	BGM 611	Figueiredo
10	BGM 574	Jardim Seminário 1	40	BGM 612	Figueiredo
11	BGM 575	Jardim Seminário 2	41	BGM 613	Jardim Paulista
12	BGM 576	Jardim Seminário 1	42	BGM 614	Jardim Paulista
13	BGM 578	Jardim Seminário 1	43	BGM 615	Jardim das Nações
14	BGM 579	Jardim Seminário 2	44	BGM 616	Jardim das Nações
15	BGM 580	São Caetano	45	BGM 617	Jardim das Nações
16	BGM 581	São Caetano	46	BGM 618	Jardim das Nações
17	BGM 582	São Caetano	47	BGM 619	Jardim das Nações
18	BGM 583	São Sebastião	48	BGM 620	Jardim das Nações
19	BGM 585	São Sebastião	49	BGM 621	Jardim das Nações
20	BGM 586	Nova Campo Grande	50	BGM 622	Vila Ipiranga
21	BGM 587	Carioca	51	BGM 624	Vila Ipiranga
22	BGM 588	Popular	52	BGM 625	Vila Ipiranga
23	BGM 589	Popular	53	BGM 626	Vila Ipiranga
24	BGM 590	Popular	54	BGM 627	Albuquerque
25	BGM 591	Jardim Aeroporto 1	55	BGM 628	Vilas Boas
26	BGM 592	Jardim Aeroporto 1	56	BGM 629	Vilas Boas
27	BGM 593	Jardim Aeroporto 1	57	BGM 630	Vilas Boas
28	BGM 594	Jardim Aeroporto 1	58	BGM 631	Vila Ipiranga
29	BGM 596	Residencial Búzios	59	BGM 632	Residencial Búzios
30	BGM 597	Residencial Búzios	60	BGM 633	Vila Ipiranga

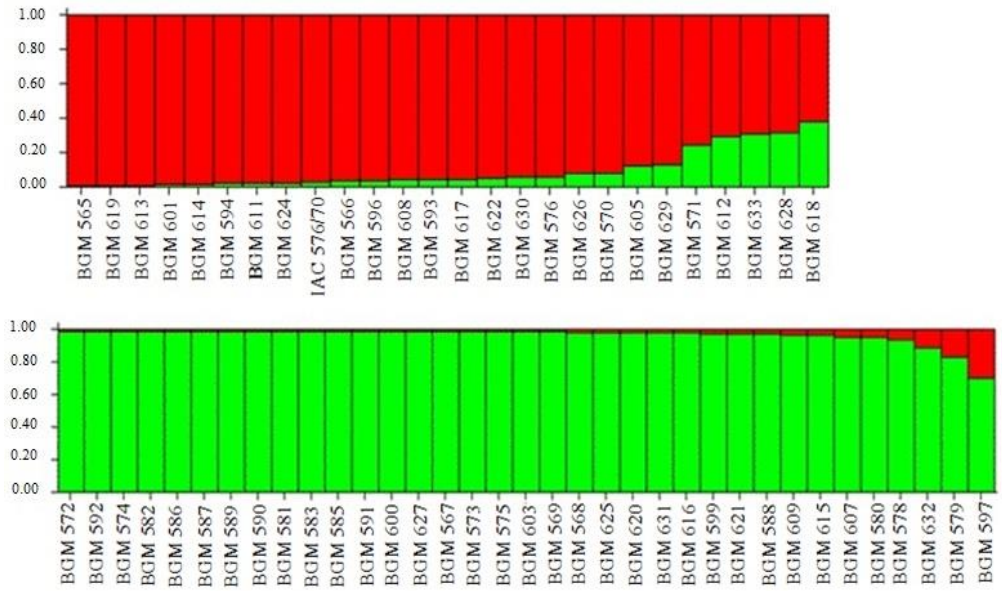

Fig 1. Q-plot showing clustering of 61 sweet cassava accessions based on analysis of genotypic data using Structure 2.3.3 for K=2. Each accession is represented by a vertical bar (colors represent different sub-population). Sub- population 1 (from BGM 565 to BGM 618) and sub-population 2 (from BGM 572 to BGM 597).

Table 2. List of nineteen markers utilized in reactions for amplification of the DNA fragments.

Number of the order	Markers	LG	Motif	Marker (5'-3')	AT(ºC)
1	SSRY 13	nd	$(CT)_{29}$	F´:GCAAGAATTCCACCAGGAAG R´:CAATGATGGTAAGATGGTGCAG	55
2	SSRY 19	V	$(CT)_8(CA)_{18}$	F´:GCAAGAATTCCACCAGGAAG R´:CAATGATGGTAAGATGGTGCAG	55
3	SSRY 21	B	$(GA)_{26}$	F´:GCAAGAATTCCACCAGGAAG R´:CAACAATTGGACTAAGCAGCA	55
4	SSRY 27	nd	$(CA)_{14}$	F´:GCAAGAATTCCACCAGGAAG R´:CCATTGGAGAACTTGGCAAC	55
5	SSRY 28	UMB	$(CT)_{26}(AT)_3AC(AT)_2$	F´:GCAAGAATTCCACCAGGAAG R´:GCTGCGTGCAAAACTAAAAT	55
6	SSRY 35	nd	$(GT)_3GC(GT)_{11}(GA)_{19}$	F´:GCAAGAATTCCACCAGGAAG R´:CTGATCAGCAGGATGCATGT	55
7	SSRY 45	nd	$(CT)_{27}$	F´:GCAAGAATTCCACCAGGAAG R´:TCCAGTTCACATGTAGTTGGCT	55
8	SSRY 51	N	$(CT)_{11}CG(CT)_{11}(CA)_{18}$	F´:GCAAGAATTCCACCAGGAAG R´:GGATGCAGGAGTGCTCAACT	55
9	SSRY 100	L	$(CT)_{17}TT(CT)_7$	F´:GCAAGAATTCCACCAGGAAG R´:TTCGCAGAGTCCAATTGTTG	55
10	SSRY 135	G	$(CT)_{16}$	F´:GCAAGAATTCCACCAGGAAG R´:AACATGTGCGACAGTGATTG	45
11	GA12	nd	NP	F´:GCAAGAATTCCACCAGGAAG R´:CGATGATGCTCTTTCGGAGGG	57
12	GA 21	nd	NP	F´:GCAAGAATTCCACCAGGAAG R´:CAATGCTTTACGGAAGAGCC	58
13	GA 57	nd	NP	F´:AGCAGAGCATTTACAGCAAGG R´:TGTGGAGTTAAAGGTGTGAATG	59
14	GA 126	K	NP	F´:AGTGGAAATAAGCCATGTGATG R´:CCAATAATTGATGCCAGGTT	58
15	GA 127	K	NP	F´:CTCTAGCTATGGATTAGATCT R´:ACAATGTCCCAATTGGAGGA	57
16	GA 131	G	NP	F´:TTCCAGAAAGACTTCCGTTCA R´:CTCAACTACTGCACTGCACTC	54
17	GA 134	nd	NP	F´:ACAATGTCCCAATTGGAGGA R´:ACCATGGATAGAGCTCACCG	59
18	GA 136	nd	NP	F´:CGTTGATAAAGTGGAAAGAGCA R´:ACTCCACTCCCGATGCTCGC	55
19	GA 140	nd	NP	F´:TTCAAAGGAAGCCTTCAGCTC R´:GAGCCACATCTACTGCACACC	55

LG, Linkage group (Chavarriaga-Aguirre et al., 1998; Mba et al., 2001); NP, motif not published; Primer: forward (F´) and reverse (R´); AT, annealing temperature.

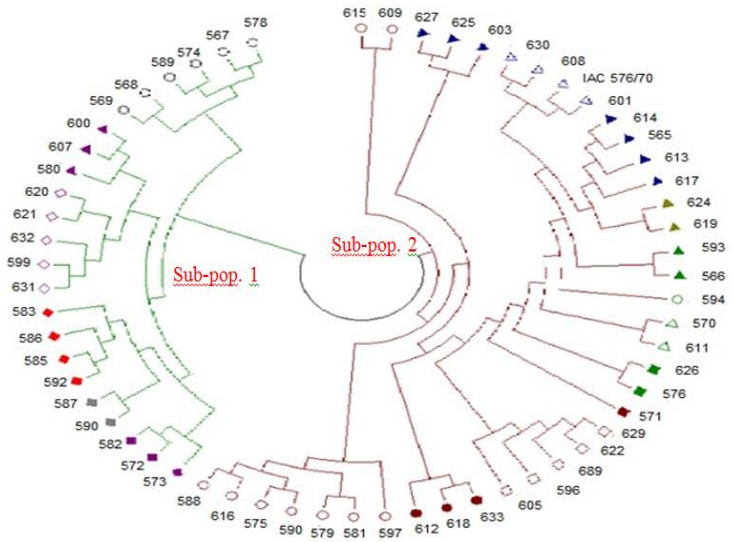

Fig 2. Distribution of 61 sweet cassava accessions, according to Neighbor-Joining Tree based on C.S.Chord distance. Sub-population 1 (from BGM 578 to BGM 573) and sub-population 2 (from BGM 588 to BGM 615).

The genetic distance among accessions was estimated using C.S Chord distance (Cavalli-Sforza and Edwards, 1967), which ranged from 0.0 to 0.67 (Table 4). The accessions BGM 587 and BGM 590, collected in near neighborhood, presented no genetic divergence and so are duplicates. The exchange of vegetative propagation material between smallholders is a common behavior between cassava producers in Brazil (Siqueira et al., 2009; Costa et al., 2013). This explains the occurrence of the duplicates collected in different locations. It suggests that one of these accessions can be eliminated from the UEM's Cassava Germplasm Bank. Turyagyenda et al. (2012) found two Ugandan cassava landraces with 100 % association through 26 SSR markers, suggesting that they might be duplicates. This fact emphasizes the importance and necessity of Germplasm Banks characterization using molecular markers, allowing the discrimination of accessions and identifying duplicates that often receive different identifications (Kalia et al., 2011).

Accessions BGM 572 × BGM 582, BGM 573 × BGM 583, BGM 574 × BGM 589, BGM 585 × BGM 586, BGM 586 × BGM 590 and BGM 587 × BGM 590 were the most similar (Table 4), belonging to the same sub-population (Fig 1 and Fig 2). These accessions were collected in the same or nearby neighborhoods, suggesting that there is an intense flow of varieties among residents and local losses are offset by replacements of existing varieties near each smallholder (Siqueira et al., 2010).

On the other hand, the most divergent combination was between BGM 570 and BGM 613 accessions, belonging to different neighborhoods, with a genetic distance of 0.67, followed by BGM 570 × BGM 619 and BGM 593 × BGM 624, both presenting genetic distance of 0.63, and BGM 605 × BGM 614 and BGM 611 × BGM 619, both with a distance of 0.62 (Table 4).

Understanding the genetic distance among these accessions is important for the selection of diverse parental combinations and these can proportionate heterotic effect in cassava breeding programs, which involves hybridizations (Falconer and Mackay, 1996).

BGM 601 accession was the most similar to commercial variety IAC 576/70, with a genetic distance equal to 0.28. The accessions BGM 565 and BGM 570 were the most divergent at the same comparison, with genetic distances of 0.55 and 0.57, respectively (Table 4). The commercial variety IAC 576/70 present high yield and resistance to bacterial blight (*Xanthomonas axonopodis* pv *manihotis*) (Aguiar et al., 2011), while BGM 570 accession presents superelongation resistance (unpublished data)**,** adisease caused by the fungus *Sphaceloma manihoticola* which is considered one of the most important diseases of cassava in Brazil (Alvarez and Mejia, 2003), and also presents resistance to bacterial blight. In this context, the accession BGM 570 and the cultivar IAC 576/70 may be used as parents in crosses to obtain high genetic segregation with high probability to concentrate high yield and disease resistance in descendent populations.

The genetic data acquired in this study can give a guidance tool in the selection of parents in breeding programs of sweet cassava, when combined with the data from morpho-agronomic characterization of these sweet cassava cultivars. Furthermore, according to Falconer and Mackay (1996), cultivars with the greatest genetic distances are candidates for the use as a source of variability in breeding programs in the sweet cassava crop, because greater segregation should occur from crosses among the most divergent parental.

Genetic diversity parameters

Genetic relationships among accessions were further studied using Principal Coordinates Analysis (PCoA) (Fig 3). This analysis confirmed the existence of two sub-populations with total concordance, in terms of sub-population members, with the Bayesian analysis (Fig 1 and Fig 3).

The $PCoA_1$ and $PCoA_2$ justified 36.18% and 17.16% of the variability, respectively, and thus explain 53.34% of the total variation (Fig 3), which reinforces the existence of significant genetic diversity between the sub-populations of this study. Similarly, Costa et al. (2013) observed that the Principal Coordinates 1 and 2 explained 53.25% of the total genetic variation, analyzing 66 traditional sweet cassava accessions from Maringá, Paraná, Brazil.

All genetic diversity analysis methods evidenced that even some accessions collected from distant neighborhoods were allocated in one sub-population, indicating that they share the same alleles and are genetically little divergent. This result may have been determined by selection and exchange of vegetative propagation material from sweet cassava accessions by smallholders (Siqueira et al., 2009; Rimoldi et al., 2010; Costa et al., 2013). On the other hand, some accessions collected in the same neighborhoods were clustered in different sub-populations, evidencing the natural heterozygosity of this species (McKey et al., 2010).

The results obtained by the analysis of Molecular Variance (AMOVA) for the two sub-populations showed that the most of the genetic diversity occurred within the groups (77%) (Table 5), as expected in alogamus species. It confirms the significant genetic diversity among all sweet cassava accessions evaluated. Only 23% of the variation occurred between the two sub-populations (Table 5). These results were similar to those obtained by Costa et al. (2013), who also estimated 77% of variation within sub-populations in the analysis of genetic diversity among 66 sweet cassava accessions collected in Maringá, Paraná, Brazil.

In addition, the value of coefficient F Wright (F_{st}) was 0.07, confirming a moderate genetic diversity (Wright, 1978) between the two sub-populations (Table 5). Therefore, although the sampling of germplasm has been performed only in the urban area of the municipality of Campo Grande, Mato Grosso do Sul, Brazil, there was significant genetic variability in the genotypic set in question.

The results obtained in this study can be related with the genetic origins of the analyzed material. In the eighteenth century, the Mato Grosso do Sul state received indigenous migrations from the Rio Paraguay and, more recently (twentieth century), migrations of Brazilians from other regions and of Paraguayans people. So, those people may have brought cassava varieties from their lands of origin (Siqueira et al., 2010). Furthermore, this region represents one of the main origin centers of cassava diversity (Olsen and Schaal, 1999). All these facts are associated with the management of smallholders and the habit of sweet cassava accessions exchange which have contribute to a wide genetic diversity in cassava (Siqueira et al., 2009; Costa et al., 2013).

Materials and Methods

Plant materials and collection

A total of 60 sweet cassava accessions were collected in "backyard cultivations" of Campo Grande city (latitude: 20 S26' 34"; longitude 54 W38' 47" and altitude: 592m), Mato Grosso do Sul State, Brazil (Table 1). Accessions were

Table 3. Genetic diversity´s index estimates by 19 microsatellites markers assessed.

Markers	Allele number	Allele (pb)	Frequency	Genetic diversity	Ho	PIC
SSRY 13	8	187 195 197 200* 223 230 240* 260*	0.393	0.699	0.885	0.648
SSRY 19	5	180 200 206 215* 235	0.467	0.620	0.833	0.546
SSRY 21	7	150* 163* 165 173 183 186* 190	0.418	0.694	0.885	0.644
SSRY 27	4	280 285 287* 290	0.500	0.611	0.607	0.539
SSRY 28	4	140* 150 170 180	0.549	0.584	0.836	0.513
SSRY 35	4	255 275 295 298*	0.484	0.560	0.934	0.463
SSRY 45	4	190 207 215 228	0.418	0.645	0.869	0.577
SSRY 51	3	277 311 322*	0.508	0.508	0.951	0.387
SSRY 100	4	180 196 206* 208	0.549	0.592	0.803	0.523
SSRY 135	4	275 290 295 301	0.418	0.636	0.787	0.566
GA 12	3	111 120 153	0.508	0.567	0.984	0.475
GA 21	3	85 90 104	0.484	0.552	0.967	0.451

GA 57	3	158* 180 192	0.715	0.420	0.569	0.351
GA 126	4	160 178 206 220*	0.458	0.591	0.983	0.505
GA 127	3	240 244 250	0.534	0.577	0.724	0.495
GA 131	7	68* 76 83* 88* 95* 98 118	0.484	0.595	0.984	0.514
GA 134	5	304 314 329 351 360*	0.418	0.667	0.934	0.607
GA 136	5	130 135 140* 163 170*	0.484	0.609	0.866	0.531
GA 140	8	125 136 141* 142 146* 154* 165* 172	0.393	0.689	0.984	0.635
Mean	4.63		0.483	0.601	0.862	0.525

*rare alleles: frequency < 0.05 according to Botstein et al. (1980).

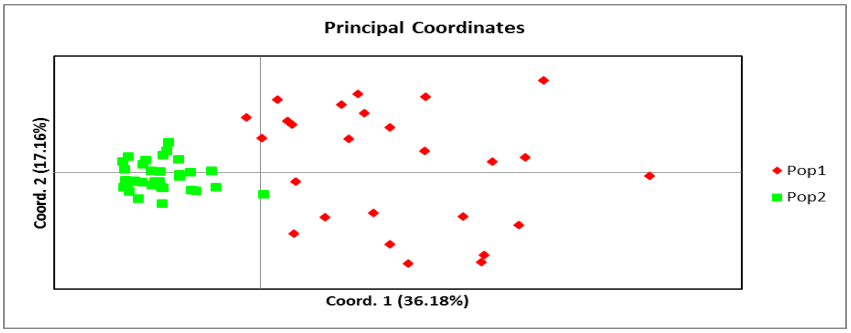

Fig 3. Principal Coordinates Analysis ($PCoA_1$, 36.18% vs $PCoA_2$, 17.16%) of SSR data indicating 53.34 % genetic diversity among 61 traditional sweet cassava accessions. Sub-population 1 (red color) allocated 26 cassava accessions and sub-population 2 (green color) allocated 35 cassava accessions.

Table 4. More divergent and more similar combinations obtained by matrix of Dissimilarity, C.S. Chord distance, generated through SSR markers analysis between 61 sweet cassava accessions.

Divergent combinations	Similar combinations
BGM 565 X IAC 576/70= 0.55	BGM 587 x BGM 590= 0.00
BGM 570 x IAC 576/70= 0.57	BGM 572 x BGM 582= 0.02
BGM 605 x BGM 614= 0.62	BGM 573 x BGM 583= 0.02
BGM 611 x BGM 619= 0.62	BGM 574 x BGM 589= 0.02
BGM 570 x BGM 619= 0.63	BGM 585 x BGM 586= 0.02
BGM 593 x BGM 624= 0.63	BGM 586 x BGM 590= 0.02
BGM 570 x BGM 613= 0.67	BGM 601 x BGM IAC 576/70= 0.28

Table 5. Analysis of molecular variance of two clusters (K=2) of 61 sweet cassava accessions detected by Structure analysis.

Source	DF	SS	MS	% of variation	F de Wright
Between Sub- population	1	58.517	58.517	23	0.07**
Within Sub- population	59	357.122	6.053	77	
Total	60	415.639		100	

DF=Degree freedom, SS=Sum Square, MS=Mean Square, **=significance by 1% de probability.

chosen initially based on the observation of phenotypic traits in order to achieve large variation between plants. The commercial variety IAC 567/70 was included on analysis of traditional sweet cassava accessions collected in Campo Grande. This commercial cultivar presents great performance in terms of productivity, resistance to bacterial blight (*Xanthomonas axonopodis* pv. *manihotis*) (Aguiar et al., 2011), uniformity of roots, postharvest quality, excellent organoleptic characteristics (Mezette et al., 2009) and may be a reference in future work on genome wide selection in this population.

All accessions were maintained in Cassava Germplasm Bank *ex situ* of Universidade Estadual de Maringá (UEM) located in Fazenda Experimental de Iguatemi- FEI (latitude: 23S21'10.08; longitude: 52W 4'6.53" and altitude: 550 m), aiming their use in future breeding programs.

DNA extraction and quantification

Genomic DNA extraction was performed at the laboratory of molecular and biotechnology-NUPAGRI (Núcleo de Pesquisa Aplicada a Agricultura), Universidade Estadual de Maringá, PR (latitude: 23S25'31" and longitude: 51W56'19"). Freshly harvested young leaves of a plant of each accession were maintained in liquid nitrogen to preserve the DNA. The extraction was performed using a protocol described by Dellaporta et al. (1983).

Genomic DNA concentration was measured using Fluorometer Qubit® (*Qubit Fluorometer Invitrogen*) and samples were diluted with buffer TE 0.1X to the concentration of 50 ng μL^{-1} and stored at -20ºC.

SSR genotyping

Nineteen SSR markers (Table 2) identified and characterized in previous studies (Chavarriaga-Aguirre et al., 1998; Mba et al., 2001) were obtained from *Invitrogen-Induslab* to genotype all the DNA samples. According to Kunkeaw et al. (2010) and Sraphet et al. (2011) these SSR markers are widely distributed in cassava genome.

Amplification reactions (PCR) were performed in a total volume of 20 µL, which contained: 50 ng of genomic DNA; 0.25 mM of each deoxyribonucleotide (dATP, dCTP, dGTP and dTTP); 1.5 mM of $MgCl_2$; 10 mM of Tris-HCl PCR buffer; 0.08 µM of forward and reverse markers (Invitrogen), and 1.0 unit of *Taq* polymerase enzyme (Invitrogen) and ultra-pure water (q.s.p.).

The PCR amplifications were performed with a thermal cycler Techne (Model TC-512) and followed pre-established sequences for each type of markers (Chavarriaga-Aguirre et al., 1988; Mba et al., 2001) (Table 2). The markers GA were programmed with a hot start of 94°C for 10 min followed by 30 cycles of denaturation at 95°C for 4 min. Annealing varied from 54 to 59°C for 2 min depending upon the melting temperature for the each marker and extension at 72°C by 2 min followed by final extension at 72°C by 10 min (Chavarriaga-Aguirre et al., 1988). The markers SSRY were programmed with a hot start of 94°C by 5 min followed by 30 cycles of denaturation at 94°C by 1 min. Annealing varied from 45 to 55°Cby 2 min depending upon the melting temperature for the each marker and extension at 72°C by 2 min followed by final extension at 72°C by 5 min (Mba et al., 2001).

The amplified products were separated by electrophoresis in 10% non-denaturing polyacrylamide gels with a running time of 3 h at 100 V (Kantartzi et al., 2013).

A 50 bp DNA ladder (Invitrogen) was used as molecular size marker. Gels were stained with SYBR®Safe DNA gel stain (Life Technologies™) and the DNA bands visualized under ultraviolet light; digital images were recorded using the L-Pix EX photo documentation system (Loccus Biotecnologia™).

Statistical analysis

The software LAB IMAGE 1D, version 1.10, Loccus Biotecnologia™ was used to genotype the samples. The major allelic frequency by locus, number of alleles by locus, mean of alleles by locus, heterozygosity, genetic diversity by locus and polymorphism information content by locus (PIC) were obtained utilizing POWERMARKER 3.25 (Liu and Muse, 2005). The loci were classified in relation to their degree of information (PIC) according to Anderson et al. (1993).

The set of molecular data was analyzed using software STRUCTURE 2.3.3 (Pritchard et al., 2000) according the admixture model to infer the population structure of sweet cassava accessions. Initially, the analysis was performed using a period of burn-in with 10.000, run length of 100.000. The number of K was set from 2 to 20. A 20 independent structure runs were made for each K and the average likelihood value across 20 runs was calculated in the Structure Harvester (Pritchard et al., 2000; Earl and vonHoldt, 2012). Additionally, GenAlEx 6.3. (Peakall and Smouse, 2006) software provided a genetic distance matrix used for Principal Coordinates analysis and Analysis of

Molecular Variance (AMOVA), considering the *K* groups of the Bayesian analysis. Furthermore, genetic diversity among accessions was measured through C.S. Chord's distance (Cavalli-Sforza and Edwards, 1967). This data was used to generate the Neighbor-Joining Tree by software Molecular Evolutionary Genetics Analysis- MEGA version 5.2 (Tamura et al., 2011).

Conclusion

There is a significant genetic diversity among the traditional sweet cassava accessions collected in Campo Grande, Mato Grosso do Sul, Brazil, based on microsatellites markers. The inbreeding coefficient, F_{st} was 0.07. The accessions were distributed in two divergent sub-populations according with Bayesiananalysis, Neighbor-Joining Tree and Principal Coordinates Analysis. For Cassava Breeding Programs in Brazil, the hybrid combinations between BGM 570 × BGM 613 and BGM 570 × IAC 576/70 could be used to obtain heterotic effect and transgressive segregation.

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