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Population structure and genetic diversity of traditional sweet cassava accessions in Mato Grosso, Paraná, Santa Catarina and São Paulo States, Brazil

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ABSTRACT. With the increasing demand for more productive cultivars that are tolerant or resistant to diseases and pests, the exploration of genetic resources combined with molecular analyses has proven to be an essential research strategy. Through molecular analysis, it is possible to identify and select desirable traits with high precision, increasing the efficiency of breeding programs. This study aimed to analyze the population structure and genetic diversity of traditional sweet cassava accessions collected from rural and peri-urban areas of municipalities in the states of Mato Grosso, Paraná, Santa Catarina, and São Paulo States, Brazil, via microsatellite markers. A total of 227 traditional sweet cassava accessions were evaluated using 29 microsatellite markers. The analysis revealed polymorphisms across all loci, with an average of 3.21 alleles per locus and 12 rare alleles identified within the population, potentially linked to unique genetic traits. The mean polymorphism information content (PIC) value of 0.52 suggests that the markers were moderately to highly informative. The average observed heterozygosity (H_o) was 0.65, whereas the average genetic diversity was 0.60. The sweet cassava accessions were grouped into four subpopulations on the basis of population structure at K = 4. The PhiPT value was 0.13, indicating low to moderate genetic differentiation among the evaluated subpopulations and highlighting their distinct genetic characteristics. The most divergent combinations were observed between the accessions from Western Paraná and Northern Mato Grosso, as well as between accessions from Northern Mato Grosso and Central-Western Santa Catarina. These results are important for addressing aspects related to germplasm conservation and the demands of genetic improvement programs, which benefit from high genetic variability, for the development of new sweet cassava cultivars.

Keywords: *Manihot esculenta* Crantz; SSR molecular markers; genetic variability.

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Introduction

The genus Manihot belonging to the family Euphorbiaceae comprises approximately 98 species (Rogers & Appan, 1973), among which the dicotyledonous species *Manihot esculenta* Crantz stands out. Commonly known as cassava, manioc, or yuca (Ferraro et al., 2016), this species adapts to various soil and climatic conditions and is cultivated in tropical and subtropical regions between the latitudes 30°N and 30°S (Monteros-Altamirano et al., 2021). Cultivated cassava (*Manihot esculenta* ssp. *esculenta*), derived via domestication of the species *Manihot esculenta* ssp. *flabellifolia* (Pohl) Cif., has been consumed for approximately 9,000 years (Alves-Pereira et al., 2022).

Evolution under different selective pressures has resulted in two main groups of domesticated cassava cultivars: sweet cassava and bitter cassava, also known as industrial cassava (Mühlen et al., 2019; Alves-Pereira et al., 2020). Sweet cassava is characterized by low levels of cyanogenic glycosides in its tuberous roots ($\leqslant$ 100 mg kg^{-1} of fresh weight), which is essential for ensuring its safety for human consumption. In contrast, bitter or industrial cassava has tuberous roots with high concentrations of cyanogenic glycosides ($\geqslant$ 100 mg kg^{-1} of fresh weight), a trait important for its industrial application (Ndubuisi & Chidiebere, 2018).

Cassava is widely cultivated in approximately 100 countries, with global production exceeding 330 million tons (Food and Agriculture Organization of the United Nations [FAO], 2022). The leading producers are in Africa, Asia, and Latin America, with Nigeria as the global leader, producing more than 60 million tons,

followed by the Democratic Republic of the Congo, Thailand, Ghana, and Cambodia (FAO, 2022). Brazil, a traditional producer, ranks sixth, with an annual output of 17.6 million tons, with the states Pará and Paraná being the main producers (Instituto Brasileiro de Geografia e Estatística [IBGE], 2022). In Brazil, sweet cassava is a key component of family farming, representing an important source of income, particularly in the North, South, and Northeast regions, which together account for approximately 70% of the country's cassava production (IBGE, 2017). The versatility of sweet cassava in Brazilian cuisine strengthens cultural traditions, while its production, predominantly by small-scale farmers, contributes to the economic stability of rural communities (Rondon et al., 2023).

Sweet cassava is particularly significant because of its genetic variability, providing essential resources for the development of cultivars that are more productive, resistant to pests and diseases, and adapted to different environmental conditions (Ceballos et al., 2020). Breeding programs have prioritized the study and selection of cultivars with desirable traits, aiming to understand genetic diversity and address the challenges posed by the wide distribution of traditional accessions in Brazil. This genetic variability is preserved by small-scale farmers, whose cultivation practices maintain crop biodiversity and foster innovations in agricultural management, highlighting the strategic role of sweet cassava in human nutrition and the strengthening of family farming (Siqueira et al., 2009; Alves-Pereira et al., 2020; Pierre et al., 2022).

In this context, characterizing cassava germplasms is essential and can be effectively achieved using molecular markers (Alves-Pereira et al., 2020). Among these, microsatellite markers, also known as simple sequence repeats (SSRs), and single-nucleotide polymorphisms (SNPs) stand out as particularly valuable tools. SSRs are highly polymorphic, codominant, and informative, enabling the identification of specific alleles and precise differentiation between genotypes. Conversely, SNPs represent the most abundant form of genetic variation in the genome, allowing high-resolution genotype differentiation.

Numerous studies have employed molecular markers to characterize cassava germplasms, significantly advancing the understanding of the species' evolution, population structure, and genetic diversity (Ortiz et al., 2016, 2019; Mühlen et al., 2019; Adjebeng-Danquah et al., 2020; Costa et al., 2020; Rocha et al., 2020; Alves-Pereira et al., 2022). Notably, Alves-Pereira et al. (2022) demonstrated the effectiveness of SNP markers on a large scale, revealing high genetic diversity among cassava accessions from different Brazilian biomes. While SSRs remain a valuable tool for genetic diversity analyses on a smaller scale and at a lower cost, SNPs allow more comprehensive and detailed assessment of genetic variability.

These studies reveal important alleles and contribute to the identification of agronomically valuable traits, which are essential for the development of superior genotypes (Gepts, 2014; Lopez-Lavalle et al., 2021). Furthermore, by considering genotype × environment interactions, these studies aimed not only to expand scientific knowledge but also to contribute to the conservation of these accessions in germplasm banks (Kizito et al., 2007; Ortiz et al., 2016, 2019; Rocha et al., 2020; Amelework & Bairu, 2022).

The study of traditional cassava accessions is highly important, as it allows the identification of desirable traits and the development of adapted cultivars (Mendonça et al., 2020; Vieira et al., 2022). To date, sweet cassava accessions have received little attention in genetic studies, highlighting the importance of research focused on the population structure and genetic diversity of traditional accessions cultivated by small-scale farmers (Ferreira et al., 2015; Nakabonge et al., 2018; Mutoni et al., 2023). In this context, the present study aimed to analyze the population structure and genetic diversity of traditional sweet cassava accessions collected from rural and peri-urban areas in the municipalities of the states of Mato Grosso, Paraná, Santa Catarina, and São Paulo, Brazil via microsatellite markers.

Material and methods

Plant material

In this study, 227 traditional accessions of sweet cassava from small rural areas and peri-urban areas of the municipalities of Northern Mato Grosso, Northern and Western Paraná, Central-West São Paulo and Central-West Santa Catarina were used. In the state Mato Grosso, Brazil, 50 accessions were collected from the following municipalities: Nova Bandeirantes (latitude 9º50'59" S, longitude 57º48'38" W); Nova Monte Verde (latitude 9º58'24" S, longitude 57°32'7" W); Apiacás (latitude 9°32'40" S, longitude 57°27'4" W); Paranaíta (latitude 9º39'53" S, longitude 56º28' 36" W); Alta Floresta (latitude 9°54'0" S, longitude 55°54'0" W); and Carlinda (latitude 9°57'50" S, longitude 55°49'54" W) (Figure 1A).

In Paraná State, Brazil, 42 accessions were collected from the following municipalities: Sarandi (latitude: 23°27'8" S, longitude: 51°51'10" W); Paiçandu (latitude: 23°27'29" S, longitude: 52°2'58" W); Farol (latitude 24°5'25" S, longitude 52°37'25" W); Marialva (latitude 23°29'06" S, longitude 51°47'31" W); Maringá (latitude 23°25'38" S, longitude 51°56'15" W); Toledo (latitude 24°43'12" S, longitude 53°44'36" W); and Santa Fé (latitude 23°1'56" S, longitude 51°49'50" W) (Figure 1B).

In São Paulo State, Brazil, 5 accessions were collected from the municipality of Dois Córregos (latitude 22º21'58" S, longitude 48º22'49" W) (Figure 1C). In turn, 130 accessions were collected in Santa Catarina State, Brazil, in the following municipalities: Caçador (latitude 26°46'31" S, longitude 51°00'24" W); Macieira (latitude 26°51'20" S, longitude 51°22'41" W); Videira (latitude 27°00'30" S, longitude 51°09'06" S); Rio das Antas (latitude 26°53'55" S, longitude 51°04'28" W); Arroio Trinta (latitude 26°55'58" S, longitude 51°20'21" W); Pinheiro Preto (latitude 27°03'02" S, longitude 51°13'51" W;), Iomerê (latitude 27°00'15" S, longitude 51°14'32" W); and Salto Veloso (latitude 26°54'16" S, longitude 51°24'23" W) (Figure 1D).

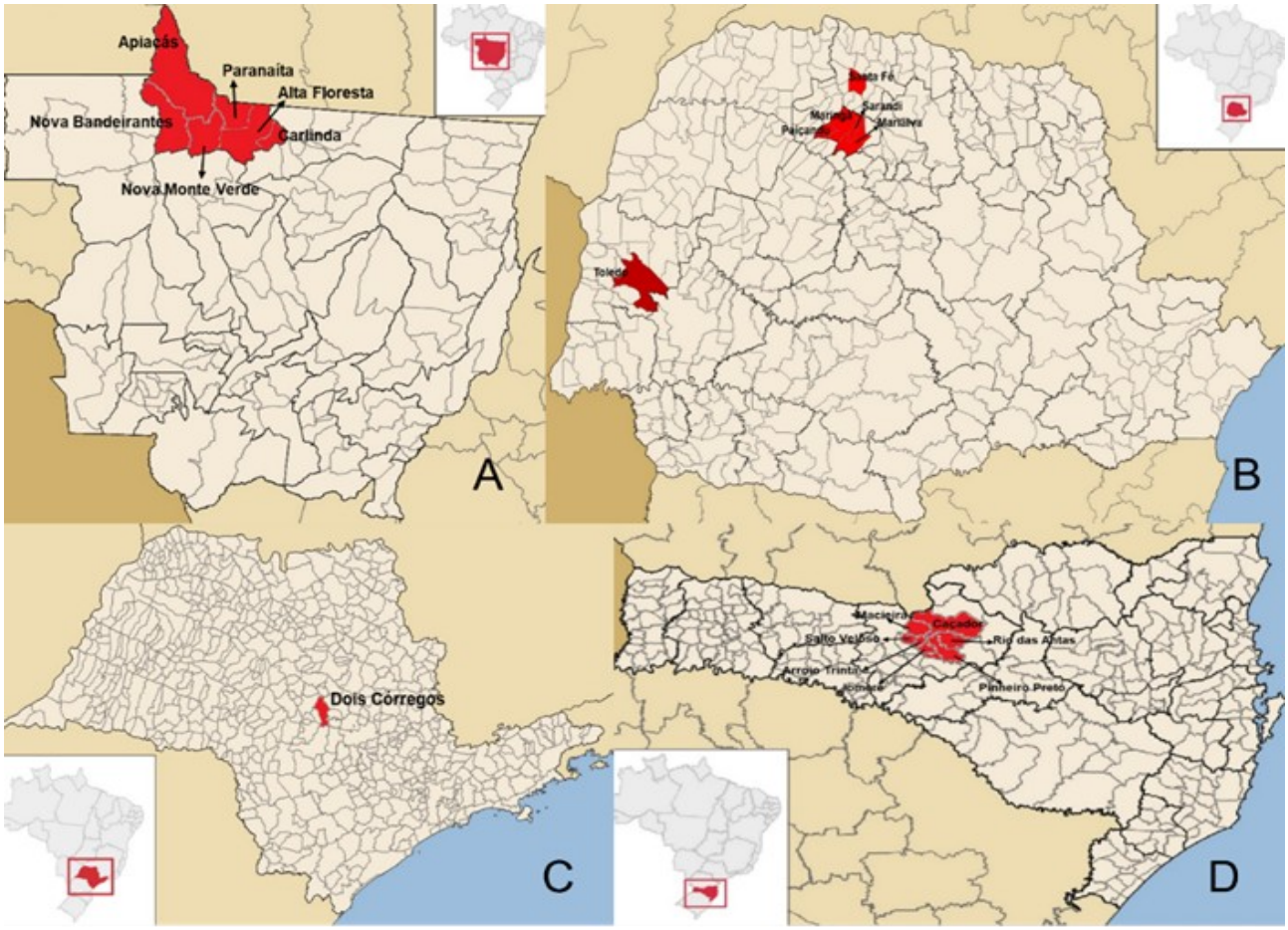

Figure 1. Map showing the locations of the municipalities. A. Northern Region of Mato Grosso: Nova Bandeirantes, Nova Monte Verde, Apiacás, Paranaíta, Alta Floresta and Carlinda. B. Northern and Western Regions of Paraná: Sarandi, Paiçandu, Farol, Marialva, Maringá, Toledo and Santa Fé. C. Central-West Region of São Paulo: Dois Córregos. D. Central-West Region of Santa Catarina: Arroio Trinta, Caçador, Iomerê, Macieira, Pinheiro Preto, Rio das Antas, Salto Veloso and Videira. Source: Wikipedia (2023).

After mature branches from each accession were collected, they were sectioned into cuttings measuring approximately 0.20 m in length, and some of them, duly identified, were used to obtain plant material for genomic DNA extraction, with the remainder being deposited (planted) at the Germplasm Bank of the Center for Applied Agricultural Research (Nupagri) at the State University of Maringá (UEM), located in Maringá, Paraná State, Brazil. The plant material for genomic DNA extraction consisted of the cuttings of each accession, which were duly identified, placed in boxes containing washed sand and kept in a greenhouse at Nupagri until they sprouted.

DNA extraction and quantification

After 15 days, genomic DNA was extracted from young leaves using the PureLink® Genomic DNA Minikit from Thermo Fisher Scientific. The extracted genomic DNA was then quantified via a Qubit® fluorimeter

(Invitrogen, California, USA). Each DNA sample was then diluted to a final concentration of 50 ng μL^{-1}, as described by Xia et al. (2005). The experiment was conducted at the Center for Applied Agricultural Research (Nupagri) at the State University of Maringá (UEM).

Molecular analysis

The molecular markers used to genotype the traditional cassava accessions collected are described in Table 1, with 29 pairs of microsatellite markers (SSR) from the GA series (Chavarriaga-Aguirre et al., 1999) and SSRY (Mba et al., 2001).

The use of 29 markers is well-established in genetic diversity studies and falls within the range commonly applied in cassava research. Previous studies have utilized varying numbers of SSR markers, ranging from 11 to 35, including studies by Moura et al. (2016) with 11 markers, Gonçalves et al. (2017) with 20, Ortiz et al. (2019) with 15, Rocha et al. (2020) with 25, Adjebeng-Danquah et al. (2020) with 35, and Wooding and Peña (2023) with 13. Therefore, the selection of 29 markers in this study is consistent with established methodologies and aligns with prior research in the field.

Table 1. SSR markers used for genotyping 227 traditional accessions of sweet cassava from Northern Mato Grosso, Northern and Western Paraná, Central-Western Santa Catarina, and São Paulo States, Brazil.

Loci	Sense	Antisense	RA (pb)[1]	TA (°C)[2]
GA 012	GATTCCTCTAGCAGTTAAGC	CGATGATGCTCTTCGGAGGG	131-157	45
GA 013	TTCCCTCGCTAGAACTTGTC	CTATTTGACCGTCTTCGCCG	137-139	45
GA 016	GTACATCACCACCAACGGGC	AGAGCGGTGGGGCGAAGAGC	89-129	45
GA 021	GGCTTCATCATGGAAAAACC	CAATGCTTTACGGAAGAGCC	104-126	58
GA 057	AGCAGAGCATTTACAGCAAGG	TGTGGAGTTAAAGGTGTGAATG	153–183	59
GA 126	AGTGGAAATAAGCCATGTGATG	CCCATAATTGATGCCAGGTT	178–214	58
GA 127	CTCTAGCTATGGATTAGATCT	GTAGCTTCGAGTCGTGGGAGA	203–239	57
GA 131	TTCCAGAAAGACTTCCGTTCA	CTCAACTACTGCACTGCACTC	75-119	45
GA 134	ACAATGTCCCAATTGGAGGA	ACCATGGATAGAGCTCACCG	309–337	59
GA 136	CGTTGATAAAGTGGAAAGAGCA	ACTCCACTCCCGATGCTCGC	145–161	55
GA 140	TTCAAAGGAAGCCTTCAGCTC	GAGCCACATCTACTGCACACC	154–164	55
GA 161	TGTTCTTGATCTTCTGCTGCA	TGATTGTGGACGTGGGTAGA	64-140	45
SSRY 06	TTTGTTGCGTTTAGAAAGGTGA	ACAAATCATTACGATCCATTTG	298	45
SSRY 13	GCAAGAATTCCACCAGGAAG	CAATGATGGTAAGATGGTGCAG	234	55
SSRY 19	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA	214	55
SSRY 21	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	192	55
SSRY 27	CCATGATTGTTTAAGTGGCG	CCATTGGAGAACTTGGCAAC	277	55
SSRY 28	TTGACATGAGTGATATTTTCTTGAG	GCTGCGTGCAAAACTAAAAT	180	55
SSRY 35	GCAGTAAAACCATTCCTCCAA	CTGATCAGCAGGATGCATGT	282	55
SSRY 45	TGAAACTGTTTGCAAATTACGA	TCCAGTTCACATGTAGTTGGCT	228	55
SSRY47	GGAGCACCTTTTGCTGAGTT	TTGGAACAAAGCAGCATCAC	244	55
SSRY 50	CCGCTTAACTCCTTGCTGTC	CAAGTGGATGAGCTACGCAA	271	55
SSRY 51	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	298	55
SSRY 61	GGCTGCTTTACCTTCTACTCAGA	CAAGAACGCCAATATGCTGA	233	55
SSRY 65	CATCGCCAAATCGTCAAGTA	TGATGCCATGCATTTCACTT	299	55
SSRY 85	AAGGTGGCAGCACTTTTCTG	AAGAATACTATACGGACTACATGCCA	292	55
SSRY 100	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	210	55
SSRY 101	GGAGAATACCACCGACAGGA	ACAGCAGCAATCACCATTTC	213	55
SSRY 135	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	253	45

[1]RA, amplification region, in base pairs (bp); [2]TA, annealing temperature.

DNA amplification of each accession under study via polymerase chain reaction (PCR) was performed by applying DNA samples (50 ng μL^{-1}) in strips of eight tubes with translucent walls and a capacity of 200 µL. In each tube, a homogenized aliquot (5.0 µL) of the working solution containing DNA was added, and the other components for the reaction were subsequently added in the form of a mixture with a volume of 20 µL (Chavarriaga-Aguirre et al., 1999; Mba et al., 2001).

Thus, each reaction (25 µL) was composed of 50 ng of DNA; 0.25 mM of each of the deoxyribonucleotides (dATP, dCTP, dGTP, and dTTP); 1.5 mM $MgCl_2$; 10 mM PCR Buffer 10x, $MgCl_2$ (Invitrogen); 0.08 µM each primer (sense and antisense), one unit of Taq polymerase (Perkin Elmer-Cetus Corp.) and ultrapure water (q.s.p.).

The PCRs were carried out using specific programs in a thermocycler (Techne Endurance TC-512, Analítica). The thermocycling program for the amplification of the SSRY series primers consisted of the following steps: an initial denaturation step at 94°C for 5 minutes; 30 denaturation cycles at 94°C for 1

minute, 2 minutes at the annealing temperature defined for each primer, and polymerization at 72°C for 2 minutes; a final extension cycle at 75°C for 5 minutes; and, finally, maintaining at 4°C (Mba et al., 2001). Amplification with the GA primers was performed as follows: an initial denaturation step at 94°C for 2 minutes; 30 denaturation cycles at 95°C for 4 minutes, 2 minutes at the annealing temperature for each primer, and polymerization at 72°C for 2 minutes; a final extension cycle at 72°C for 5 minutes; and, finally, maintenance at 4°C (Chavarriaga-Aguirre et al., 1999).

The amplified fragments were separated by 10% nondenaturing polyacrylamide gel electrophoresis, with a standard 100 bp molecular marker (Ladder – Invitrogen) resolved on the same gel. After electrophoresis, the gels were stained with SYBR® Safe DNA gel stain (Life TechnologiesTM) and digitized (in JPEG format) using the L-Pix EX photographic documentation system (Loccus Biotechnology). Finally, using a standard molecular marker as a reference, the fragment sizes were determined and photographed using the LabImage photographic documentation program version 1.10 (Loccus Biotechnology).

Statistical analysis

The population structure of the 227 cassava accessions was analyzed via a Bayesian clustering approach using Structure 2.3.4 software (Pritchard et al., 2000). This analysis was performed with a burn-in of 50,000 and 250,000 Markov chain Monte Carlo (MCMC) interactions and clustering (K) of 2 to 10 in 20 independent runs. The ΔK was calculated using Structure Harvester (Earl & vonHoldt, 2012), as proposed by Evanno et al. (2005), and the probability of each accession belonging to a specific genetic group was determined by aligning the 20 runs of the best K.

Genetic diversity calculations were subsequently performed at each SSR locus, including estimates such as the major allele frequency, number of alleles, genetic diversity, mean observed heterozygosity, and polymorphism information content (PIC). These analyses were conducted using PowerMarker 3.25 software (Liu & Muse, 2005). Alleles were analyzed according to their frequencies in the populations: a) fixed alleles at higher frequencies in the populations, b) private alleles that occurred exclusively in a given population, and c) rare alleles with frequency $p \leqslant 0.05$ (Siqueira et al., 2009).

GenAlEx 6.5 software (Peakall & Smouse, 2012) was used to perform principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA). Genetic differentiation between populations was determined using the PhiPT value, which is equivalent to Fst for codominant genotypic data (Peakall & Smouse, 2012). A matrix based on C.S. chord distances (Cavalli-Sforza & Edwards, 1967) was used to construct a phylogenetic tree through the neighbor joining method via MEGA7 software (Kumar et al., 2016).

Results and discussion

Genetic diversity indices of traditional sweet cassava accessions

The data presented in Table 2 show the genetic diversity indices of the analyzed cassava accessions. All 29 loci studied were considered polymorphic, indicating that there was detectable genetic variation among the accessions. The frequency of the most common allele at each locus did not exceed 0.95, indicating that no single allele was predominant at any of the loci (Clark et al., 1981).

The amplification of these loci yielded a total of 93 alleles, with allele counts per marker ranging from 2 to 5 and an average of 3.21 alleles per marker. This value aligns with findings from previous studies on cassava genetic diversity, such as those by Rocha et al. (2020), who reported an average of 3.36 alleles per marker in 144 accessions, and Adjebeng-Danquah et al. (2020), who identified an average of 4.77 alleles per marker in 89 accessions from Ghana. The observed allelic diversity is crucial for cassava's adaptation to diverse environments and for the preservation of its genetic variability (Ferreira et al., 2015; Costa et al., 2020).

In the present study, the allele frequency per locus ranged from 0.338 to 0.822, with an average of 0.49 (Table 2), indicating genetic diversity in the analyzed population. Allele frequency values close to 1.0, as observed for marker GA 134 (0.822), suggest that a specific allele is very common in the population. This fact may be the result of selective pressures, gene flow or other evolutionary processes that led to a high frequency of this particular allele (Rocha et al., 2020; Soro et al., 2024).

The distribution of rare alleles in the accessions analyzed (Table 2) included 12 alleles in nine of the 29 microsatellite loci evaluated. Notably, rare alleles are those that occur in populations with frequencies less than 0.05 (Siqueira et al., 2009). These rare alleles were identified in accessions from Mato Grosso, western

Paraná, and central-western Santa Catarina, potentially reflecting local adaptation or associations with key phenotypic traits such as disease resistance, tolerance to adverse environmental conditions, or other agronomically relevant characteristics. Furthermore, rare alleles play a strategic role in marker-assisted selection, especially when linked to traits of agricultural importance (Lopez-Lavalle et al., 2021), presenting valuable opportunities for future research.

The presence of rare alleles has also been reported in previous studies. Moura et al. (2016) identified 20 rare alleles using 11 microsatellite markers. Gonçalves et al. (2017) found 17 rare alleles using 20 SSR markers, whereas Ortiz et al. (2019) identified 31 rare alleles in their study of the genetic diversity of 303 sweet cassava cultivars.

The observed heterozygosity (H_o) at each locus constitutes a measure of genetic variation within a population and is intrinsically related to the number of alleles and their respective frequencies (Hershey, 2020). As shown in Table 2, the H_o values ranged from 0.000 (GA 13) to 0.958 (SSRY 100), with an average of 0.65. The occurrence of H_o values = 0.000 suggests the possibility of isolation of cassava plants, indicating low genetic variability. The same result was observed in the study by Wooding and Peña (2023), who, when analyzing the genetic diversity of 43 cassava cultivars using 13 SSR markers, identified H_o = 0.000 at the GA 13 loci. This finding suggests that the genomic region corresponding to the marker in question may be highly conserved or that a specific allele may even be fixed in all individuals analyzed for this marker (Salim et al., 2017).

On the other hand, the high H_o rate can be explained by the allogamous reproduction system in cassava, combined with the occurrence of protogyny, where male flowers open seven days after female flowers do (Long et al., 2024). Several studies have addressed H_o in cassava populations in different countries: Cuba, H_o = 0.60 (Beovides et al., 2015); Ghana, H_o = 0.43 (Adjebeng-Danquah et al., 2020); and Brazil, H_o = 0.64 (Rocha et al., 2020).

Table 2. Estimated indices of genetic diversity per microsatellite locus.

Locus	Number of alleles	Frequency of the most common allele	Frequency of the rarest allele	H_o	Genetic diversity	PIC
GA 12	3	0.358		0.789	0.666	0.591
GA 13	3	0.506		0.000	0.557	0.460
GA 16	4	0.513	0.050	0.423	0.624	0.558
GA 21	3	0.442	0.008	0.723	0.641	0.566
GA 57	3	0.613		0.707	0.536	0.467
GA 126	2	0.560		0.803	0.493	0.371
GA127	3	0.537	0.033/0.043	0.821	0,575	0.493
GA131	3	0.495		0.436	0.587	0.501
GA 134	2	0.822		0.356	0.293	0.250
GA 136	2	0.538		0.708	0.497	0.373
GA 140	2	0.516		0.235	0.499	0.375
GA 161	4	0.406		0.755	0.702	0.648
SSRY06	3	0.367	0.031/0.026	0.548	0.662	0.588
SSRY 13	3	0.487		0.688	0.615	0.538
SSRY 19	4	0.338		0.741	0.703	0.644
SSRY 21	3	0.565		0.587	0.585	0.520
SSRY 27	4	0.443	0.045	0.599	0.690	0.639
SSRY 28	3	0.467		0.719	0.622	0.544
SSRY35	3	0.471		0.744	0.587	0.498
SSRY 45	3	0.380		0.950	0.658	0.584
SSRY 47	4	0.484		0.860	0.623	0.554
SSRY 50	4	0.442	0.012	0.763	0.683	0.629
SSRY 51	4	0.460	0.031	0.831	0.640	0.571
SSRY 61	3	0.591		0.549	0.565	0.501
SSRY 65	5	0.342		0.749	0.741	0.698
SSRY 85	4	0.497	0.029	0.862	0.636	0.573
SSRY100	4	0.487		0.958	0.614	0.540
SSRY101	2	0.623		0.564	0.470	0.359
SSRY135	3	0.492	0.049/0.009	0.230	0.620	0.546
Mean	3.21	0.49		0.65	0.60	0.52

H_o, observed heterozygosity per locus; PIC, polymorphism information content.

The genetic diversity analyzed among the 29 microsatellite markers highlighted the markers GA 161, SSRY 19 and SSRY 65, which presented values greater than 0.70, with an overall average of 0.60 (Table 2). In a study carried out by Gonçalves et al. (2017), 51 traditional accessions of sweet cassava were analyzed using 20 microsatellite markers, and the authors reported an average genetic diversity of 0.481. Agre et al. (2018) analyzed 96 cassava

cultivars with 12 SSR markers, resulting in an average genetic diversity of 0.510. Ortiz et al. (2019) reported an average diversity of 0.658 when 15 SSR markers were used.

The PIC is an important measure in population genetics and is closely linked to the number and frequency of alleles of a genetic marker. According to Botstein et al. (1980), values greater than 0.5 indicate that the locus is highly informative, values between 0.25 and 0.5 indicate that the locus is moderately informative, and values less than 0.25 indicate that the locus is not very informative. In this context, the mean PIC was 0.52, which was categorized as highly informative, ranging from 0.250 (GA 134) to 0.698 (SSRY 65). Similar results were obtained by Ferreira et al. (2015), with a mean of 0.525; Sousa et al. (2017), who identified a mean PIC of 0.652; and Ortiz et al. (2019), with a mean of 0.605.

With respect to the number of alleles (Table 3), 75 alleles were identified in 50 of the evaluated accessions in Region 1 (Northern Mato Grosso State, Brazil). In Regions 2 and 3, in Western Paraná and Central-West Santa Catarina States, Brazil, which represent 93 and 57 accessions, respectively, 80 and 78 alleles were found. In Region 4, in Northern Paraná and Central-West São Paulo States, Brazil, 74 alleles were detected in 27 accessions. With respect to rare alleles (Table 3), 3, 5, and 4 were identified in the regions of Northern Mato Grosso, Western Paraná, and Central-West Santa Catarina States, Brazil, respectively. The presence of rare alleles (38 alleles) was also identified by Rocha et al. (2020), who analyzed 144 accessions of sweet cassava from seven municipalities in Paraná and Santa Catarina States, Brazil.

In Regions 1 and 4, private alleles (3 and 2) were identified, which are extremely important for assessing the degree of isolation between populations (Table 3). Private alleles are genetic variants found exclusively in a given population and generally arise due to recent mutations or geographic isolation, resulting in distinct genetic characteristics within the population (Carmo et al., 2015).

Table 3. Allele frequency, observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F) per region of the 227 accessions of sweet cassava genotyped with 29 loci.

Loci by Region	Region 1	Region 2	Region 3	Region 4
Number of accessions	50	93	57	27
Number of alleles	75	80	78	74
Number of rare alleles (p ⩽ 0,05)	3	5	4	0
Number allele frequency (0.051–0.249)	25	18	15	18
Number allele frequency (0.25–0.49)	24	37	41	38
Number allele frequency (0.50–1.00)	23	20	18	18
Number private alleles	3	0	0	2
H_o mean	0.471	0.721	0.718	0.569
H_e mean	0.477	0.544	0.551	0.492
F	0.008	-0.314	-0.294	-0.165

Region 1: North Mato Grosso; Regions 2 and 3: West Paraná and Central-West Santa Catarina; Region 4: North Paraná and Central-West São Paulo.

In a study carried out by Moura et al. (2016), who used 11 microsatellite loci to quantify the genetic diversity and structure of traditional cassava accessions in two Brazilian states (Amazonas and Pará), the authors reported 20 private alleles (those that appeared in only one genotype), with the GA12 locus presenting the largest number of private alleles. In another study, Carrasco et al. (2016), using 14 microsatellite loci to characterize 211 traditional cassava accessions from three municipalities in Mato Grosso, Brazil (Santo Antônio do Leverger, Cáceres and Porto Estrela), identified 6 private alleles. These results show that the presence of private alleles is a relevant characteristic of the genetic diversity of cassava populations and may be influenced by local adaptations and the action of specific environmental factors (Alves-Pereira et al., 2020).

The mean H_o among the populations (Table 3) ranged from 0.471 (Region 1) to 0.721 (Region 2), indicating that the mean H_o was relatively high across the different regions where the accessions were collected. This variation may be influenced by factors such as evolutionary history, population size, gene flow, and selection pressure (Kizito et al., 2007). Regions 2, 3, and 4 presented higher than expected heterozygosity frequencies, suggesting an excess of heterozygotes (Table 3). This conclusion is supported by the fixation index (F), which was negative in these regions and positive in Region 1. These findings indicate that the evaluated sweet cassava accessions, which originated from small-scale farmer fields, maintained significant genetic variability, demonstrating their adaptive capacity to different environments. Similar results were reported by Moura et al. (2016) and Rocha et al. (2020), who, when analyzing the genetic diversity of traditional sweet cassava accessions, also identified H_o values higher than the H_e values. This information highlights the relevance of these genetic indices, providing strategies for the conservation and use of cassava genetic diversity.

Population structure of traditional sweet cassava accessions

The determination of the most representative number for the population structure was performed using the program Structure Harvester (Earl & vonHoldt, 2012). On the basis of the value of K = 4, the 227 accessions were classified into four distinct subpopulations (Figure 2).

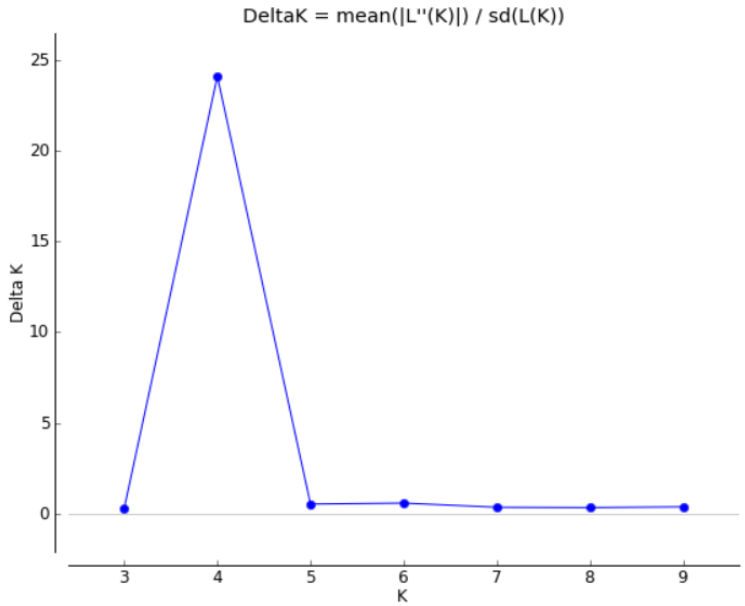

Figure 2. Plot of ΔK for the value of K, generated using Structure Harvester (Earl & vonHoldt, 2012).

Figure 3 shows the distribution of the 227 cassava accessions among the four identified subpopulations. Group 1, representing North Mato Grosso State, had 50 accessions. Groups 2 and 3, corresponding to West Paraná and Central-West Santa Catarina States, included 93 and 57 accessions, respectively. Meanwhile, Group 4, associated with North Paraná and Central-West São Paulo States, Brazil, had 27 accessions.

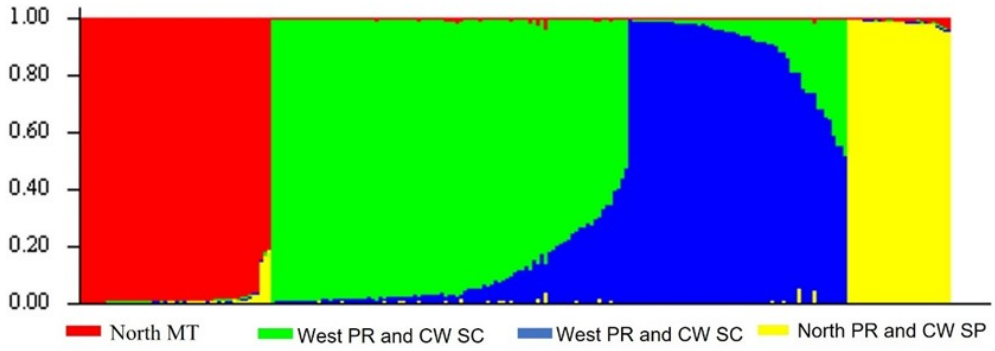

Figure 3. Analysis of the population structure of the 227 traditional accessions of sweet cassava, assuming K = 4 groups. Group 1, North MT (North Mato Grosso); Group 2, West PR and CW SC (West Paraná and Central-West Santa Catarina); Group 3, West PR and CW SC (West Paraná and Central-West Santa Catarina); Group 4, North PR and CW SP (North Paraná and Central-West São Paulo).

The analysis of the subpopulations revealed a genetic mixture, as shown in Figure 3. This mixture occurs when an accession from a subpopulation shares alleles with another, indicating the presence of gene flow. Previous studies on the genetic diversity of cassava, such as those conducted by Siqueira et al. (2009); Ferreira et al. (2015); Ortiz et al. (2016); Pedri et al. (2019) and Rocha et al. (2020), also highlighted the occurrence of gene flow in cassava populations.

Gene flow among cassava accessions refers to the transfer of genetic material, including alleles, between populations. This process is driven by factors, such as pollination, seed dispersal, and the movement of individuals, contributing to increased genetic diversity and enhancing the crop's adaptability to diverse environmental conditions (Alves-Pereira et al., 2020). A key driver of this genetic exchange is the widespread practice of exchanging vegetative planting material, which is common among cassava producers in tropical

and subtropical regions. While this practice plays a crucial role in gene flow, quantifying its impact remains challenging due to its decentralized and difficult-to-monitor nature (Hershey, 2020).

Analysis of genetic similarity and dissimilarity among traditional sweet cassava accessions

The genetic distances ranged from D_{ij} 0.017 to 0.788 among the 25,651 possible combinations obtained among the 227 accessions analyzed (Table 4). The combinations between the most divergent accessions presented distances ranging from D_{ij} 0.723 to 0.788, demonstrating that the combination between relatively genetically distant individuals. For the less divergent (more similar) combinations, the genetic distances D_{ij} ranged from 0.017 to 0.054 (Table 4).

Among the combinations of accessions, Accession 227 × Accession 8, Accession 137 × Accession 16 and Accession 144 × Accession 9, were the most divergent, presenting genetic distances D_{ij} = 0.788, 0.779 and 0.775, respectively (Table 4). Among the 18 most divergent combinations of accessions presented, 44.44% were from Northern Mato Grosso and Western Paraná States, 44.44% originated from Central-West Santa Catarina and Northern Mato Grosso States, and 11.11% were from Central-West Santa Catarina and Northern Paraná States, Brazil (Table 4). These highly divergent traditional accessions present significant potential for use as parents in the development of heterotic clones in genetic improvement programs. For this purpose, it is essential to perform prior evaluation of their agronomic characteristics, including dry mass content in tuberous roots, productivity, organoleptic characteristics, and flowering. This will allow the identification of the most promising combinations in controlled crosses (Lopez-Lavalle et al., 2021).

Table 4. More and less divergent combinations among the 227 traditional accessions of sweet cassava from Northern Mato Grosso, Northern and Western Paraná, Central-Western Santa Catarina, and São Paulo States, Brazil, as determined by the C.S. chord distance analysis (Cavalli-Sforza & Edwards 1967).

Most divergent combinations	Distance	Less divergent combinations	Distance
Acesso 227(K3) x Acesso 8(K1)	0.788	Acesso 4 (K1) x Acesso 6(K1)	0.017
Acesso 137(K3) x Acesso 16(K1)	0.779	Acesso 4(K1) x Acesso 5(K1)	0.018
Acesso 144(K2) x Acesso 9(K1)	0.775	Acesso 12(K1) x Acesso 13(K1)	0.019
Acesso 226(K2) x Acesso 72(K4)	0.768	Acesso 75(K4) x Acesso 77(K4)	0.021
Acesso 10 (K1) x Acesso 227(K3)	0.764	Acesso 58(K4) x Acesso 60(K4)	0.024
Acesso 137(K3) x Acesso 9(K1)	0.764	Acesso 45(K1) x Acesso 46(K1)	0.033
Acesso 13(K1) x Acesso 227(K3)	0.763	Acesso 35(K1) x Acesso 37(K1)	0.036
Acesso 11(K1) x Acesso 227(K3)	0.753	Acesso 71(K4) x Acesso 72(K4)	0.039
Acesso 12(K1) x Acesso 227(K3)	0.753	Acesso 103(K3) x Acesso 105(K3)	0.041
Acesso 137(K3) x Acesso 8(K1)	0.750	Acesso 51(K4) x Acesso 52(K4)	0.042
Acesso 10(K1) x Acesso 137(K3)	0.745	Acesso 131(K3) x Acesso 133(K3)	0.042
Acesso 13(K1) x Acesso 155(K2)	0.740	Acesso 29(K1) x Acesso 30(K1)	0.045
Acesso 226(K2) x Acesso 39(K1)	0.734	Acesso 10(K1) x Acesso 9(K1)	0.046
Acesso 137(K3) x Acesso 23(K1)	0.733	Acesso 11 (K1) x Acesso 12(K1)	0.049
Acesso 115(K3) x Acesso 14(K1)	0.732	Acesso 56(K4) x Acesso 59(K4)	0.050
Acesso 110(K2) x Acesso 16(K1)	0.727	Acesso 21(K1) x Acesso 22(K1)	0.051
Acesso 216(K2) x Acesso 63(K4)	0.725	Acesso 34(K1) x Acesso 35(K1)	0.054
Acesso 14(K1) x Acesso 227(K3)	0.723	Acesso 107(K3) x Acesso 137(K3)	0.054

[1]K = group determined by ΔK, calculated through the probabilistic method of Evanno et al. (2005).

Among combinations of accessions, Accession 4 × Accession 6, Accession 4 × Accession 5 and Accession 12 × Accession 13, stood out as the least divergent, presenting genetic distances D_{ij} = 0.017, 0.018 and 0.019, respectively (Table 4). These results indicate genetic proximity between the combinations of accessions that were collected in the same regions. These observations show that the exchange of plant material between farmers is a common practice within these specific regions since, in cultivated areas, the species *M. esculenta* Crantz is propagated vegetatively (Albuquerque et al., 2019). In this context, the exchange of plant material for planting between farmers can certainly enable the exchange of alleles between materials originating from different regions, thus contributing to the increase in local diversity (Albuquerque et al., 2019).

Genetic diversity among traditional sweet cassava accessions

A phylogenetic analysis using the neighbor joining tree method (Cavalli-Sforza & Edwards, 1967) was conducted to assess genetic diversity (Figure 4). In this tree, four groups can be observed, defined according to the corresponding colors identified in the population structure analysis. The distribution dynamics were

heterogeneous, particularly in Groups 2 and 3, corresponding to the Western Paraná and Central-Western Santa Catarina regions, highlighting that accessions from the same region were allocated to different groups.

The considerable proportion of admixture among the accessions confirms the heterogeneous nature of sweet cassava. This is attributed to cassava being an allogamous species, which allows cross-pollination between plants from different locations, resulting in increased genetic variability within the crop (McKey & Delêtre, 2017). Studies conducted by Gonçalves et al. (2017) and Adjebeng-Danquah et al. (2020) reported similar findings when analyzing traditional sweet cassava accessions using microsatellite markers.

A clear regional trend was observed in the distribution of the analyzed sweet cassava accessions. Group 1 was composed exclusively of accessions originating from Northern Mato Grosso, located in the Central-West region of Brazil. The regional distribution of Group 1 suggests a possible correlation with the origin of the accessions, indicating that sweet cassava accessions from Northern Mato Grosso State may originate from indigenous communities, given the significant presence of ethnic groups in this region (Carrasco et al., 2016).

Groups 2 and 3 were composed of accessions from Western Paraná and Central-Western Santa Catarina, both of which are located in the Southern region of Brazil. In contrast, Group 4 included accessions collected from Northern Paraná and Central-Western São Paulo, areas situated in the Southern and Southeastern regions of Brazil, respectively. The clustering dynamics of accessions from different regions highlight the exchange of planting materials among farmers. These practices occur both locally and across distant regions, promoting the maintenance of genetic variability (Ortiz et al., 2016).

This process is particularly evident in areas where cassava is cultivated in polyculture systems or small "backyard" plots, alongside other food species (McKey & Delêtre, 2017). Such patterns of exchange and genetic diversity are observed in various regions, including parts of Africa, Europe, and Brazil (Parmar et al., 2017). In Brazil, Ortiz et al. (2019) reported significant genetic diversity in the sweet cassava population studied, with this diversity being divided into four distinct groups correlated with different geographical regions.

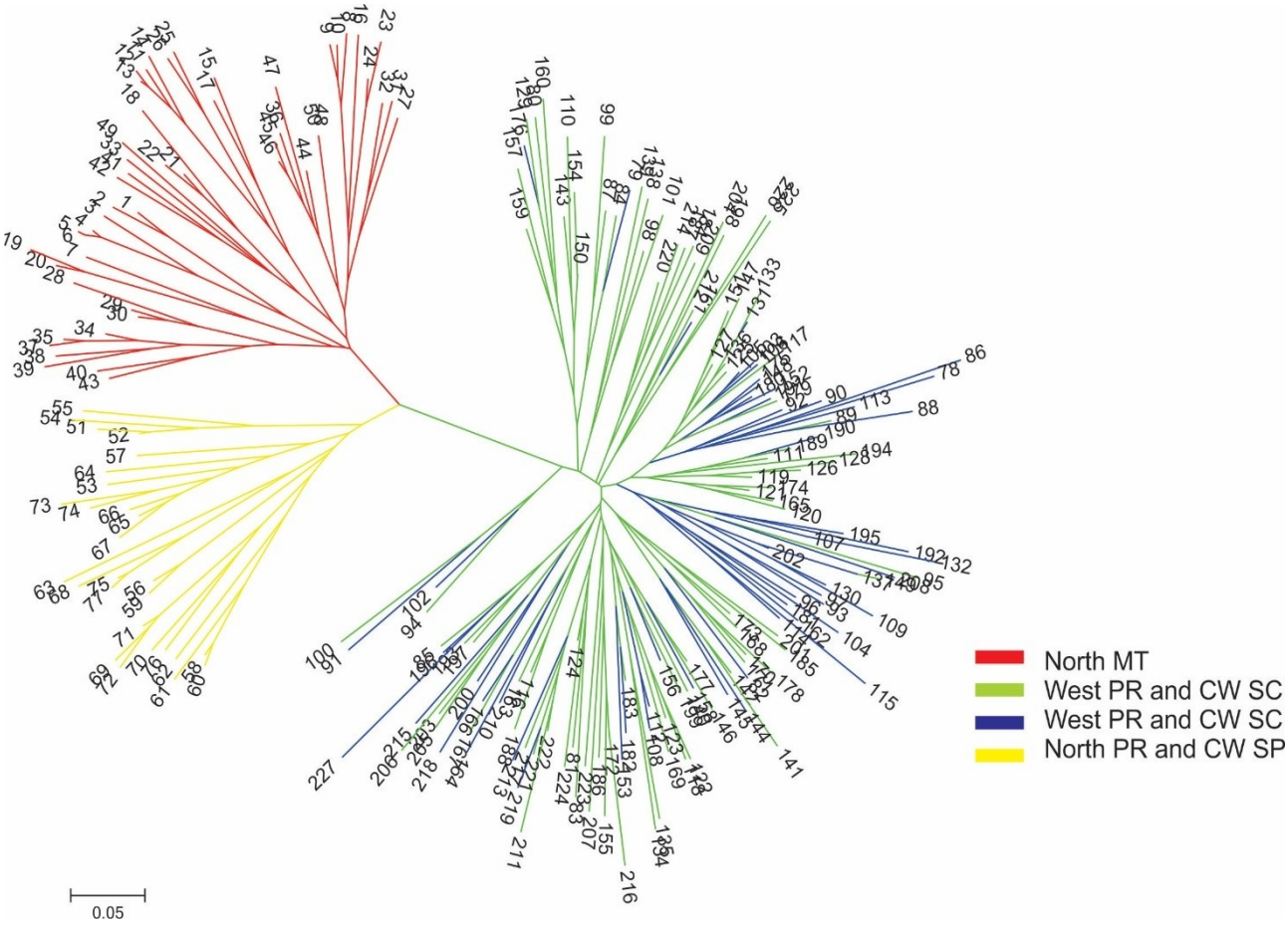

Figure 4. Distribution of the 227 traditional accessions of sweet cassava based on 29 microsatellite markers (neighbor joining tree) from the CS chord genetic distance matrix (Cavalli-Sforza & Edwards, 1967), assuming K = 4 groups (Pritchard et al., 2000). Group 1, North MT (North Mato Grosso); Group 2, West PR and CW SC (West Paraná and West-Central Santa Catarina); Group 3, West PR and CW SC (West Paraná and West-Central Santa Catarina); Group 4, North PR and CW SP (North Paraná and West-Central São Paulo).

In the principal coordinate analysis (PCoA, Figure 5), the dispersion of points on the Cartesian plane revealed considerable genetic diversity among the evaluated accessions. The first principal coordinate explained 14.40% of the total variation, differentiating traditional table cassava accessions from Western Paraná and Central-Western Santa Catarina from those in Northern Mato Grosso, Northern Paraná, and Central-Western São Paulo States, Brazil. The second principal coordinate accounted for 7.39% of the variation. Together, the two main components explained 21.79% of the total variation.

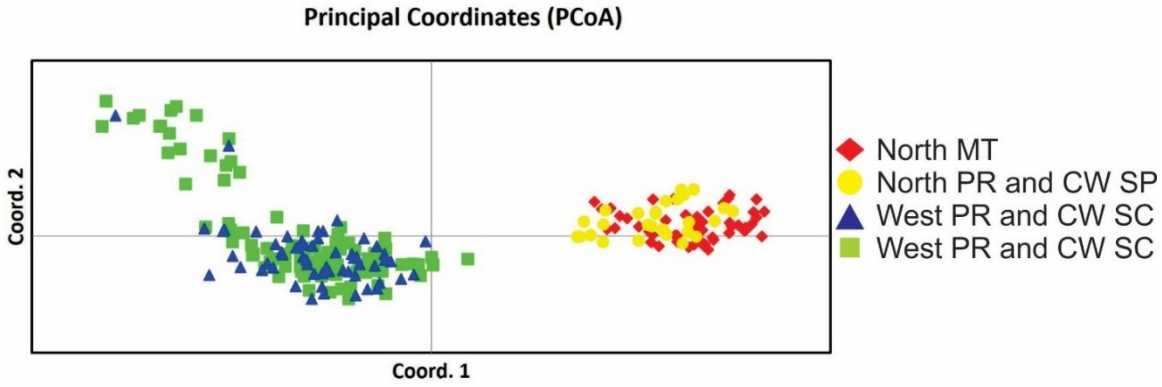

Figure 5. Principal coordinate analysis (PCoA) of 227 traditional accessions of sweet cassava based on microsatellite marker data. Group 1, North MT (North Mato do Grosso); Group 2, West PR and CW SC (West Paraná and Central-West Santa Catarina); Group 3, West PR and CW SC (West Paraná and Central-West Santa Catarina); Group 4, North PR and CW SP (North Paraná and Central-West São Paulo).

Moreover, the large dispersion of points on the Cartesian plane demonstrates a marked variation among the traditional accessions collected, especially between subpopulations 1 and 4, which are located in one quadrant, and subpopulations 2 and 3, which are located in quadrants on opposite diagonals (Figure 5).

Molecular variance analysis (AMOVA) (Table 5) was performed to quantify the variation between and within the four populations (K = 4). The results of the analysis revealed that 14% of the variation was between the four groups (subpopulations), whereas 86% of the observed variability was within the populations, suggesting high genetic variability of the accessions within the total population.

The differentiation between the four subpopulations can be assessed by the Fst index, which estimates the genetic differentiation between populations. According to Wright (1978), the PhiPT coefficient (analogous to Wright's Fst) provides an indication of the level of genetic variability between populations. The Fst values were classified according to Wright's (1978) approach, where intervals of 0.00 to 0.05, 0.05 to 0.15, 0.15 to 0.25, and > 0.25 indicate low, moderate, high, and very high genetic differentiation, respectively.

Table 5. Analysis of molecular variance (AMOVA) of the 227 traditional sweet cassava cultivars from Brazil, considering the ten K groups generated by the structure analysis.

Source	DF	SS	MS	EV	%	PhiPT	*P* value
Among pops	3	479.037	159.679	1.410	14%	0.136	0.001*
Within pops	450	4021.393	8.936	8.936	86%		
Total	453	4500.430		10.346	100%		

DF, degrees of freedom; SS, square sums; MS, mean square; EV, estimated variance; PhiPT, analog of Wright's Fst; p value, significance probability. *Statistically significant at 1%.

This study reported an Fst value of 0.136 (Table 5), which, based on Wright's classification, signifies low to moderate genetic differentiation. Similar results were reported in previous studies: Pedri et al. (2019) obtained an Fst index of 0.082; Yao et al. (2019) reported an Fst of 0.19; Ortiz et al. (2019) reported an Fst of 0.44; and Rocha et al. (2020) identified an Fst of 0.106. The possible occurrence of gene flow between populations may explain this result, suggesting the occurrence of genetic material exchange and the maintenance of relative genetic homogeneity. Other factors, such as evolutionary processes, selective pressure, and occasional crossbreeding, may also influence this genetic dynamic (Hershey, 2020).

The low to moderate differentiation observed is of utmost importance for research related to the conservation and genetic improvement of sweet cassava, as it allows cassava to adapt to different environments and climatic conditions. Understanding this differentiation is essential for developing effective strategies for conserving genetic diversity and identifying potential parents in genetic improvement programs (Gonçalves et al., 2017; Ferguson et al., 2019).

Conclusion

Microsatellite molecular markers (SSRs) were effectively used to characterize the genetic diversity and population structure of 227 cassava accessions collected in the states Mato Grosso, Paraná, Santa Catarina and São Paulo, Brazil. Some of the most divergent combinations were observed between accessions from Western Paraná and Northern Mato Grosso and accessions from Central-Western Santa Catarina and Northern Mato Grosso States, such as Accession 227 × Accession 8, Accession 137 × Accession 16 and Accession 144 × Accession 9. In the present study, low to moderate gene flow of alleles was observed between accessions from different locations, which can be attributed to the exchange of materials by farmers. The presence of admixture was observed between accessions from Western Paraná and Central-Western Santa Catarina States and, to a lesser extent, between accessions from Northern Mato Grosso, Northern Paraná and Central-Western São Paulo States, Brazil. The cultivation areas known as "backyards" are important for the conservation of the genetic diversity of the species. Therefore, strategies that aim to support this germplasm should be prioritized to meet requirements for conservation and the demands of genetic breeding programs, which benefit from genetic variability, for the development of new sweet cassava cultivars.

Data availability

Data generated during this study is available from the corresponding author upon request.

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