PAB
Pesquisa Agropecuária Brasileira

Genetics/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Genetic diversity and structure of *Anacardium humile* (Anacardiaceae) populations

Abstract – The objective of this work was to describe and compare the patterns of genetic variation in 19 populations of *Anacardium humile* from the Cerrado biome of the Brazilian Midwestern region. The ex situ germplasm collection of Universidade Federal de Jataí, in the state of Goiás, Brazil, was used for the study. To quantify the genetic variability of *A. humile*, 529 plants from 17 populations in Goiás from 2 in the state of Mato Grosso were studied, from which nine microsatellite loci were genotyped by capillary electrophoresis. The populations showed high levels of genetic diversity, with an average value of 0.830, and high inbreeding values, which suggests a deficit of heterozygotes. The genetic differentiation value between populations was 0.065. The greatest variability, which is moderately structured, was observed within populations. There is a significant inbreeding within the *A. humile* population in the Cerrado biome of the Brazilian Midwestern region. The *A. humile* populations are divided into two groups.

Index terms: cajuzinho-do-cerrado, gene flow, Mantel's test, microsatellite markers.

Diversidade genética e estrutura de populações de *Anacardium humile* (Anacardiaceae)

Resumo – O objetivo deste trabalho foi descrever e comparar os padrões de variação genética em 19 populações de *Anacardium humile* do bioma Cerrado da região Centro-Oeste brasileira. A coleção ex situ de germoplasma da Universidade Federal de Jataí, no estado de Goiás, Brasil, foi utilizada para o estudo. Para quantificar a variabilidade genética de *A. humile*, 529 plantas oriundas de 17 populações de Goiás e de 2 do estado de Mato Grosso foram estudadas, das quais nove loci microssatélites foram genotipados por eletroforese capilar. As populações apresentaram altos níveis de diversidade genética, com valor médio de 0,830, e altos valores de endogamia, o que sugere um déficit de heterozigotos. O valor de diferenciação genética entre as populações foi de 0,065. Já a maior variabilidade, estruturada de forma moderada, foi observada dentro das populações. Há endogamia significativa na população de *A. humile* no bioma Cerrado da região Centro-Oeste brasileira. As populações de *A. humile* estão divididas em dois grupos.

Termos para indexação: cajuzinho-do-cerrado, fluxo gênico, teste de Mantel, marcadores microssatélites.

Introduction

Intense exploitation of natural resources has caused changes in tropical landscapes, resulting in their fragmentation and leading to a marked

Josimar Morais de Souza[1], Mariana Pires de Campos Telles[2], Jefferson Fernando Naves Pinto[3] and Edésio Fialho dos Reis[3]✉

[1] Universidade Federal de Goiás, Instituto de Ciências Biológicas, Avenida Esperança, s/nº, Câmpus Samambaia, CEP 74690-900 Goiânia, GO, Brazil. E-mail: josimar.moraisdesouza@gmail.com

[2] Pontifícia Universidade Católica de Goiás, Escola de Ciências Médicas e da Vida, Rua 235, nº 722, Setor Leste Universitário, CEP 74605-050 Goiânia, GO, Brazil. E-mail: tellesmpc@ufg.br

[3] Universidade Federal de Jataí, Instituto de Biociências, Rodovia BR 364, Km 195, nº 3.800, CEP 75801-615 Jataí, GO, Brazil. E-mail: jefferson_naves@ufj.edu.br, edesioreis@ufj.edu.br

✉ Corresponding author

Received
March 03, 2023

Accepted
September 29, 2023

How to cite
SOUZA, J.M. de; TELLES, M.P. de C.; PINTO, J.F.N.; REIS, E.F. dos. Genetic diversity and structure of *Anacardium humile* (Anacardiaceae) populations. **Pesquisa Agropecuária Brasileira**, v.58, e03186, 2023. DOI: https://doi.org/10.1590/S1678-3921.pab2023.v58.03186.

loss of genetic diversity, as it reduces the populations (Neiva et al., 2016; Cota et al., 2017). Therefore, according to Cota et al. (2017), the conservation of tropical biomes is a necessity for the future of the species. Cerrado, a Brazilian tropical biome classified as a global biodiversity hotspot, is among the ones that have been suffering from anthropization and urges for conservation measures.

Among the native fruit trees of Cerrado threatened by the effects of anthropic action is cajuzinho-do-cerrado (*Anacardium humile* A. St. Hil), a subshrub of the Anacardiaceae family, that is frequently found in Campo Sujo, a Cerrado phytophysionomy, and Cerrado sensu stricto (Lacchia et al., 2016). This species has a high potential for commercial exploitation and may be consumed fresh or used by the food and pharmaceutical industries (Soares et al., 2013; Ressel et al., 2015; Pereira et al., 2016). Since there has been no commercial planting of the species, it is only explored in an extractive way, which requires a considerable displacement of extractivists to the small remaining areas of *A. humile* to obtain a good amount of the fruit. In addition, collectors always collect the fruits with best appearance and that would have greater chances of leaving competitive descendants, which might lead to the loss of genetic variability.

Due to the high economic and social potential combined with the need for its preservation, several studies in different areas have been conducted, such as on characterization and morphological variability (Santos & Santos Júnior, 2015; Pereira et al., 2016), as well as the ones involving genetic variability using molecular markers, as RAPD (Carvalho et al., 2012), ISSR (Borges et al., 2018; Gomes et al., 2021), and SSR (Cota et al., 2012, 2017; Soares et al., 2013).

Given the current fragmentation of the Cerrado biome, it is important to know the genetic variability within and between populations, how the gene flow occurs and what is the effect of inbreeding. To access this information, microsatellite markers can be used, which, according to Cota et al. (2012), generate data on the genetic diversity and structure of natural populations, patterns of gene dispersion, cross systems, and effective population size.

Cota et al. (2017) studied the genetic variability of *A. humile* populations using microsatellites in populations located in northern Minas Gerais state; however, for populations of Goiás state, there are no reports of studies in literature.

The objective of this work was to describe and compare the patterns of genetic variation in 19 populations of *A. humile* from the Cerrado biome of the Brazilian Midwestern region.

Materials and Methods

The study was carried out using an ex situ biological collection of *Anacardium humile* of Universidade Federal de Jataí, in the state of Goiás, Brazil, which contains 529 plants, from 13 municipalities in Goiás and two municipalities in the state of Mato Grosso, both located in the Brazilian Midwestern region (Table 1), with samples of 19 populations (Figure 1), in which more than one population was collected in the municipalities of Jataí and Mineiros, in Goiás.

DNA was extracted from young leaves from each of the 529 plants collected and stored for the study, and the extraction was performed using the 2% cetyl trimethylammonium bromide (CTAB).

Nine microsatellite loci (SSR) (Table 2), previously developed for *A. occidentale*, were transferred to *A. humile* (Soares et al., 2013) to be used. The primers were labeled with the specific fluorochromes: 6-FAM, HEX or NED. Polymerase chain reaction (PCR) was performed following the recommendations of Soares et al. (2013), using a reaction volume of 15 µL containing 12 ng of template DNA, 3.8 µM of each primer, 1 U Taq DNA polymerase (Phoneutria Biotecnologia e Serviços LTDA, Belo Horizonte, MG, Brazil), 250 µM of each dNTP, 0.25 µg BSA and 1X reaction buffer, composed of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$. Amplifications were performed in a Veriti 96-Well thermal cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA), under the following conditions: one cycle at 94°C for 5 min; 94°C for 1 min, 58 to 62°C (depending on the primer) for 1 min and 30 cycles at 72°C for 1 min; and one cycle at 72°C for 7 min. Amplifications were observed in 1% agarose gels.

The PCR products generated were visualized in the ABI 3500 automatic analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA), aided by the ROX 500 size standard (Thermo Fisher Scientific Inc., Waltham, MA, USA). After electrophoresis in the automatic analyzer, the projects were analyzed by the GeneMapper software version 3.5 program (Thermo

Table 1. Geographic coordinates of the 19 populations of *Anacardium humile* from the Cerrado biome of Brazilian Midwestern region.

State	Municipality	Population code	Coordinates	
			S	W
Goiás	Abadiânia	Abadiânia	16°02'56"	48°51'25"
	Aporé	Aporé	18°54'19"	51°54'47"
	Baliza	Baliza	16°07'60"	52°21'29"
	Caiapônia	Caiapônia	16° 53'57"	51°52'21"
	Chapadão do Céu	Chapadão do Céu	18°32'09"	52°38'02"
	Doverlândia	Doverlândia	16º42' 58"	52º16'01"
	Iporá	Iporá	16°23'28"	51°10'06"
	Itarumã	Itarumã	18°45'03"	51°22'53"
	Jataí	Jataí 1	17°40'57"	51°45'45"
		Jataí 2	18°05'58"	51°30'27"
		Jataí 3	17°32'41"	51°51'28"
		Jataí 4	17°54'01"	51°47'01"
	Mineiros	Mineiros 1	17°56'59"	53°00'22"
		Mineiros 2	17°39'31"	52°35'05"
	Piranhas	Piranhas	16°24'35"	51°51'18"
	Portelândia	Portelândia	17°25'39"	52°36'41"
	Serranópolis	Serranópolis	18°20'39"	51°51'07"
Mato Grosso	Barra do Garças	Barra do Garças	15°51'36"	52°16'22"
	Torixoréu	Torixoréu	16°11'28"	52°38'44"

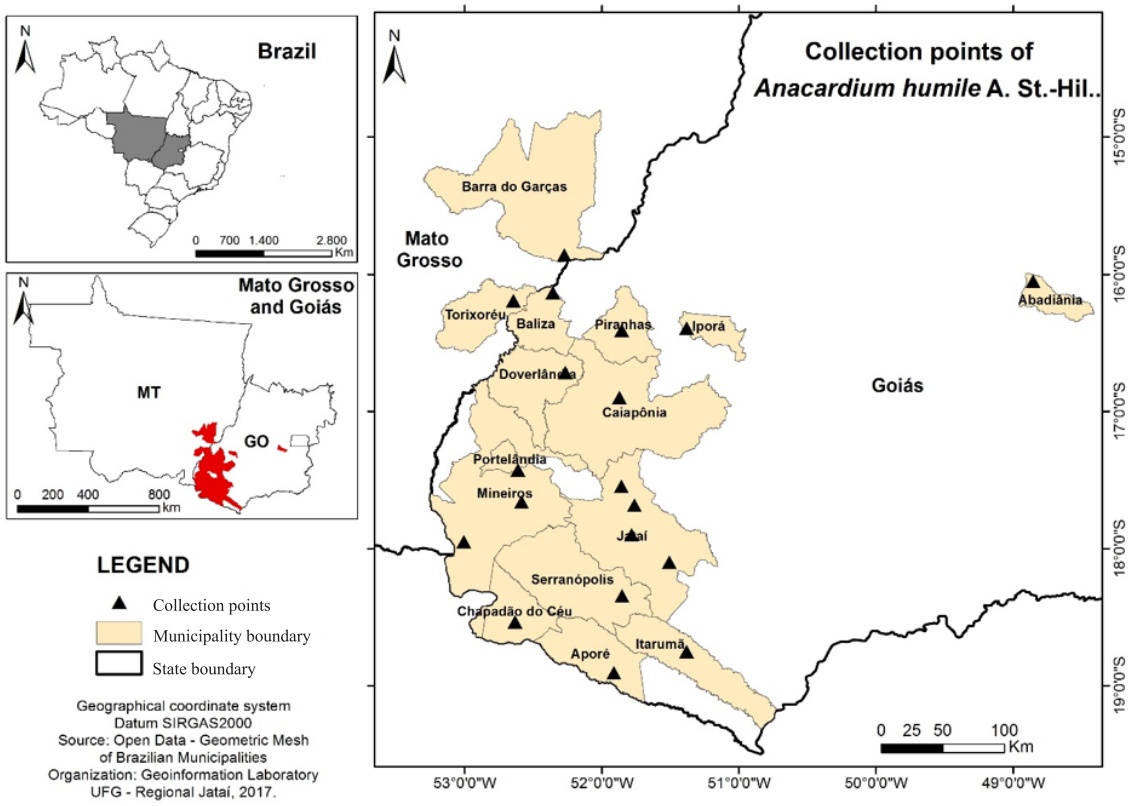

Figure 1. Collection points of *Anacardium humile* accessions from the Cerrado biome of the Brazilian Midwestern region.

Fisher Scientific Inc., Waltham, MA, USA) to visualize the genotypes. An allelic ladder was constructed through a new capillary electrophoresis to confirm the existence of each allele, in a way it contains all alleles from each locus.

The genetic diversity and Wright's F statistics: F_{IS}, inbreeding coefficient of an individual (I) relative to the subpopulation (S); F_{ST}, effect of subpopulations (S) compared to the total population (T) and F_{IT}, inbreeding coefficient of an individual (I) relative to the total (T) were estimated under random model according to Weir (1996), in which the sampled populations are representing the species with a common evolutionary history. Allelic frequencies, number of alleles per population (Na), observed heterozygosity (H_o), expected heterozygosity under Hardy-Weinberg's equilibrium (H_e), and intrapopulation fixation index (f) statistics were estimated using Genetic Data Analysis (GDA) software (Lewis & Zaykin, 2001).

Genetic dissimilarity among the 19 populations studied was used as a basis for the construction of the dendrogram using the UPGMA method. The cutoff point at each stage in the dendrogram was established according to the method of Mojena (Mojena, 1977), using the value of k = 1.25 as a stopping rule in the definition of the number of clusters, then the cophenetic correlation coefficient (CCC) was calculated between the genetic dissimilarity matrix and the matrix of cophenetic values, in order to check the consistency of the clustering.

To test the significance of the hypothesis of genetic isolation caused by geographical distance, Mantel's statistical test was performed, using the genetic dissimilarity matrix and the geographical distance in kilometers between the pairs of populations of *A. humile*, with a thousand random permutations. Statistical analyses were performed using the GENES program, version 1990.2019.91 (Cruz, 2016).

The assignment of genotypes to clusters and the relationship among clusters were evaluated using Structure software (2023), which uses a Bayesian approach and indicates the number of genetic clusters (K value) that best fits the data. After a burn-in period of 100,000 iterations, 19 independent runs were performed for each number of clusters (K, 1 to 19), each with 100,000 iterations. The choice of the most likely K was performed by calculating the ΔK statistic, which is based on the rate of change in the log-likelihood of the data between the successive values of K. Selection was made using Structure Harvester (Earl

Table 2. Primers used to obtain microsatellite molecular markers in *Anacardium humile* accessions from the Cerrado Biome of the Brazilian Midwestern region.

Locus	Sequence (5`- 3`)	Repetition	pb	Ta (°C)
mAoR3	CAGAACCGTCACTCCACTCC ATCCAGACGAAGAAGCGATG	$(AC)_{12}(AAAAT)_2$	236–256	60
mAoR11	ATCCAACAGCCACAATCCTC CTTACAGCCCCAAACTCTCG	$(AT)_3(AC)_{16}$	226–242	62
mAoR12	TCACCAAGATTGTGCTCCTG AAACTACGTCCGGTCACACA	$(AC)_{12}ARAC(AT)_4$	318–340	60
mAoR16	GGAGAAAGCAGTGGAGTTGC CAAGTGAGTCCTCTCACTCTCA	$(GT)_8(TA)_{17}(GT)_3$	222–260	60
mAoR17	GCAATGTGCAGACATGGTTC GGTTTCGCATGGAAGAAGAG	$(GA)_{24}$	138–156	58
mAoR29	GGAGAAGAAAAGTTAGGTTTGAC CGTCTTCTTCCACATGCTTC	$(TG)_{10}$	304–322	58
mAoR41	GCTTAGCCGGCACGATATTA AGCTCACCTCGTTTCGTTTC	$(GGT)_8$	151–157	60
mAoR42	ACTGTCACGTCAATGGCATC CGAAGGTCAAAGAGCAGTC	$(CAT)_9TAT(CTT)_7$	187–208	62
mAoR52	GCTATGACCCTTGGGAACTC GTGACACAACCAAAACCACA	$(GT)_{16}(TA)_2$	186–204	60

Source: Adapted from Soares et al., (2013).

& VonHoldt, 2012). Among the 19 runs per K value, the run with the highest maximum likelihood was used to assign the individual genotypes to the clusters.

Results and Discussion

The number of alleles varied from 61 for the population of the municipality of Serranópolis to 107 for the municipality of Baliza, both located in Goiás state, with an average of 89.21 alleles per population (Table 3). These results indicate a high informative content associated with microsatellite markers, which was also found by Moura et al. (2009), reinforcing their usefulness in studies of collections. The nine microsatellite loci (SSR) showed high levels of polymorphism within and among the 19 populations.

The observed heterozygosities (H_o) were lower than the expected heterozygosities (H_e) in all localities (Table 3), with small differences from one population to another. The observed heterozygosity ranged from 0.572 for the population of the municipality of Piranhas to 0.740 for the population of Jataí 2, while expected heterozygosity ranged from 0.716 for the population of Piranhas to 0.863 for the population of Jataí 2, with averages of 0.673 and 0.830, respectively. The intrapopulation fixation index ranged from 0.094 to 0.275, with an average of 0.193. The evidence of inbreeding within populations makes it possible to infer that the allelic frequencies in the population are not following the expected proportions for the Hardy-Weinberg's equilibrium, suggesting a deficit of heterozygotes within the subpopulations.

Table 3. Characterization of the 19 populations of *Anacardium humile* from the Cerrado biome of the Brazilian Midwestern region based on nine microsatellite loci.

Population	N[1]	Na	H_e	H_o	*f*[2]
Abadiânia	57	106	0.843	0.654	0.226
Aporé	46	82	0.802	0.727	0.094
Baliza	34	107	0.859	0.684	0.207
Barra do Garças	22	87	0.829	0.675	0.190
Caiapônia	17	78	0.811	0.680	0.166
Chapadão do Céu	17	81	0.832	0.682	0.185
Doverlândia	18	82	0.816	0.722	0.118
Iporá	45	97	0.855	0.648	0.244
Itarumã	23	102	0.845	0.670	0.210
Jataí 1	20	97	0.838	0.700	0.168
Jataí 2	15	90	0.863	0.741	0.146
Jataí 3	15	92	0.849	0.652	0.238
Jataí 4	26	94	0.856	0.658	0.235
Mineiros 1	40	92	0.812	0.686	0.156
Mineiros 2	18	69	0.813	0.679	0.169
Piranhas	27	61	0.717	0.572	0.205
Portelândia	25	84	0.843	0.667	0.2120
Serranópolis	34	102	0.856	0.679	0.209
Torixoréu	30	92	0.844	0.615	0.275
Mean	27.84	89.21	0.831	0.673	0.193

[1]N, number of accessions per population; Na, number of alleles per population; H_e, expected heterozygosity; H_o, observed heterozygosity; *f*, intrapopulation fixation index. [2]Significant at 1% probability, by Hardy-Weinberg's equilibrium test.

The H_o and H_e found in this study and the intrapopulation fixation index show higher values than those reported by Cota et al. (2017), who evaluated the structure and genetic diversity of *A. humile* from Cerrado located in the northern region of Minas Gerais state. It can also be considered that the levels of diversity found are high when compared to other studies that used SSR in native species of Cerrado such as *Solanum* spp. (Moura et al., 2009), *Caryocar brasiliense* (Collevatti et al., 2010) and *Acrocomia emensis* (Neiva et al., 2016).

This variation in the values of average expected heterozygosity demonstrates the high genetic diversity observed for *A. humile*. The high polymorphism of the SSR of *A. humile* is explained by the geographical amplitude of the collection areas, by the number of locations sampled, and by the low level of domestication and improvement of the species. This behavior is verified and described for other Cerrado species, such as *Solanum* spp. (Moura et al., 2009).

In the dendrogram, the formation of four clusters can be observed, and that the population of the municipality of Aporé is the most divergent, being allocated separately in one cluster (Figure 2). It can also be noted that the populations were not grouped in their entirety according to their geographical region, which is justified by the Mantel's test that did not reveal significant correlation between genetic distance and geographic distance among the populations. This result corroborates those reported by Cota et al. (2017), who also found no significant correlation for the Mantel's test among eight populations of *A. humile* in the northern region of Minas Gerais state.

The average F_{ST} value found in the present study was 0.065 (Table 4), which is an indicative of moderate genetic structure among populations. The F_{IT} of 0.246 and the F_{IS} of 0.192 also indicate that genetic variability is moderately structured in *A. humile* populations.

The values of F_{ST} and F_{IT} found for the loci mAoR17, mAoR29, and mAoR52 were higher than those reported by Cota et al. (2017). This difference is justified by the greater number of populations studied and their better spatial distribution within Cerrado. This result shows that the differentiation between them occurs mainly by non-random mating within and between populations, and not only by genetic drift. According to Hu et al. (2010), in allogamous plants, the variation is expected to be greater within populations. The greater diversity estimated within populations agrees with studies conducted in natural populations of *Ziziphus joazeiro* Mart. (Gois et al., 2014).

The Bayesian analysis identified two distinct clusters (K=2). Analyzing the contribution of genotypes to each population, it was possible to note the sharing of many alleles in all populations, which indicates the existence of some continuity in the collection space and that individuals are not totally isolated, with the possible occurrence of migrants. According to Cota et al. (2017), the structure of gene flow between populations of *A. humile* over time caused the allelic frequencies to become similar to each other, since the stochastic processes resulting from the isolation due to geographical distance, the high gene flow between geographically close populations and the low gene flow between geographically distant populations, do not apply to these populations.

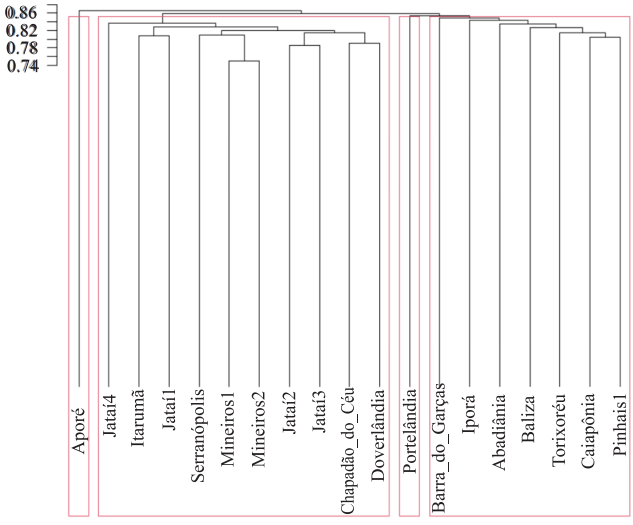

Figure 2. UPGMA dendrogram among the 19 populations of *Anacardium humile* from the Cerrado biome of the Brazilian Midwestern region. Cophenetic correlation: 0.74. Dotted vertical line indicates the cutoff point in the dendrogram.

Table 4. Estimates of statistics analogous to Wright's F statistics, considering 19 populations of *Anacardium humile* from the Cerrado biome of the Brazilian Midwestern region.

Locus	F statistics		
	F_{IS}[1]	F_{IT}	F_{ST}
Maor 03	0.124[3]	0.187[3]	0.072[3]
Maor 11	0.225[3]	0.270[3]	0.058[3]
Maor 12	0.265[3]	0.320[3]	0.051[3]
Maor 16	0.177[3]	0.223[3]	0.056[3]
Maor 17	0.039	0.114	0.078[3]
Maor 29	0.243[3]	0.286[3]	0.056[3]
Maor 41	0.236[3]	0.279[3]	0.056[3]
Maor 42	0.309[3]	0.370[3]	0.089[3]
Maor 52	0.155	0.215[3]	0.071[3]
Mean	0.196[3]	0.249[3]	0.065[3]
Upper CI[2] (95%)	0.241	0.291	0.073
Lower CI (95%)	0.143	0.201	0.057

[1]F_{IS}, intrapopulation fixation index; F_{IT}, inbreeding coefficient for the set of populations; F_{ST}, divergence between populations. [2]CI, confidence interval obtained from 10,000 bootstraps. [3]Significant at 1% probability, by Hardy-Weinberg's equilibrium test.

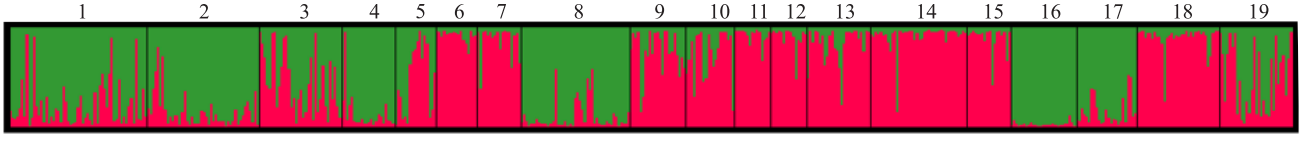

Figure 3. Analysis of the genetic structure of the 529 individuals from the 19 populations of *Anacardium humile* from the Cerrado biome of the Brazilian Midwestern region, analyzed by Structure program using the Bayesian method with K=2. Each individual is represented by a vertical line divided into color, based on genotypic similarity relative to the populations inferred by the program. Identification of populations: 1, Abadiânia; 2, Aporé; 3, Baliza; 4, Barra do Garças; 5, Caiapônia; 6, Chapadão do Céu; 7, Doverlândia; 8, Iporá; 9, Itarumã; 10, Jataí 1; 11, Jataí 2; 12, Jataí 3; 13, Jataí 4; 14, Mineiros 1; 15, Mineiros 2; 16, Piranhas; 17, Portelândia; 18, Serranópolis; and 19, Torixoréu.

Therefore, *A. humile* polulations from Cerrado of the Brazilian Midwestern region is not completely isolated from each other.

Conclusions

1. There is significant inbreeding within *Anacardium humile* population in the Cerrado biome of Brazilian Midwestern region.

2. The *Anacardium humile* populations are divided into two groups.

References

BORGES, A.N.C.; LOPES, A.C.A.; BRITTO, F.B.; VASCONCELOS, L.F.L.; LIMA, P.S.C. Genetic diversity in a cajuí (Anacardium spp.) germplasm bank as determined by ISSR markers. **Genetics and Molecular Research**, v.17, gmr18212, 2018. DOI: https://doi.org/10.4238/gmr18212.

CARVALHO, R. dos S.; PINTO, J.F.N.; REIS, E.F. dos; SANTOS, S.C.; DIAS, L.A. dos S. Variabilidade genética de cajuzinho-do-cerrado (*Anacardium humile* ST. HILL.) por meio de marcadores RAPD. **Revista Brasileira de Fruticultura**, v.34, p.227-233, 2012. DOI: https://doi.org/10.1590/S0100-29452012000100030.

COLLEVATTI, R.G.; LIMA, J.S.; SOARES, T.N.; TELLES, M.P. de C. Spatial genetic structure and life history traits in Cerrado tree species: inferences for conservation. **Natureza & Conservação**, v.8, p.54-59, 2010. DOI: https://doi.org/10.4322/natcon.00801008.

COTA, L.G.; MOREIRA, P.A.; BRANDÃO, M.M.; ROYO, V.A.; MELO JUNIOR, A.F. MENEZES, E.V.; OLIVEIRA, D.A. Structure and genetic diversity of *Anacardium humile* (Anacardiaceae): a tropical shrub. **Genetic Molecular Research**, v.16, gmr16039778, 2017. DOI: https://doi.org/10.4238/gmr16039778.

COTA, L.G.; MOREIRA, P.A.; MENEZES, E.V.; GOMES, A.S.; ERICSSON, A.R.O.; OLIVEIRA, D.A.; MELO JR, A.F. Transferability and characterization of simple sequence repeat markers from *Anacardium occidentale* to *A. humile* (Anacardiaceae). **Genetics and Molecular Research**, v.11, p.4609-4616, 2012. DOI: https://doi.org/10.4238/2012.October.17.7.

CRUZ, C.D. Genes software: extended and integrated with the R, Matlab and Selegen. **Acta Scientiarum. Agronomy**, v.38, p.547-552, 2016. DOI: https://doi.org/10.4025/actasciagron.v38i3.32629.

EARL, D.A.; VONHOLDT, B.M. Structure Harvester: a website and program for visualizing structure output and implementing the Evanno method. **Conservation Genetics Resources**, v.4, p.359-361, 2012. DOI: https://doi.org/10.1007/s12686-011-9548-7.

GOIS, I.B.; FERREIRA R.A.; SILVA-MANN, R.; PLANTALEÃO, S.M.; GOIS, C.B.; OLIVEIRA, R.S.C. Variabilidade genética em populações naturais de *Ziziphus joazeiro* Mart., por meio de marcadores moleculares RAPD. **Revista *Árvore***, v.38, p.621-630, 2014. DOI: https://doi.org/10.1590/S0100-67622014000400005.

GOMES, M.F. da C.; BORGES, A.N.C.; BATISTA, G.S.S.; LUZ, G. de A.; OLIVEIRA, M.E.A.; LOPES, Â.C. de A.; ARAÚJO, A.S.F. de; GOMES, R.L.F.; BRITTO, F.B.; LIMA, P.S. da C.; VALENTE, S.E. dos S. Genetic diversity and structure in natural populations of Cajui from Brazilian Cerrado. **Bioscience Journal**, v.37, e37080, 2021. DOI: https://doi.org/10.14393/BJ-v37n0a2021-53974.

HU, Y.; WANG, L.; XIE, X.; YANG, J.; LI, Y.; ZHANG, H. Genetic diversity of wild populations of *Rheum tanguticum* endemic to China as revealed by ISSR analysis. **Biochemical Systematics Ecology**, v.38, p.264-274, 2010. DOI: https://doi.org/10.1016/j.bse.2010.01.006.

LACCHIA, A.P.S.; TÖLKE, E.E.A.D.; DEMARCO, D.; CARMELLO-GUERREIRO, S.M. Presumed domatia are actually extrafloral nectaries on leaves of *Anacardium humile* (Anacardiaceae). **Rodriguesia**, v.67, p.19-28, 2016. DOI: https://doi.org/10.1590/2175-7860201667102.

LEWIS, P.O.; ZAYKIN, D. **GDA (Genetic Data Analysis)**: Computer Program for the Analysis of Allelic Data. version 1.1. Storrs: University of Connecticut, 2001. Available at: <http://phylogeny.uconn.edu/software/>. Accessed on: Oct. 1 2023.

MOJENA, R. Hierarchical grouping methods and stopping rules: an evaluation. **The Computer Journal**, v.20, p.359-363, 1977. DOI: https://doi.org/10.1093/comjnl/20.4.359.

MOURA, T.M. de; SEBBENN, A.M.; CHAVES, L.J.; COELHO, A.S.G.; OLIVEIRA, G.C.X.; KAGEYAMA, P.Y. Diversidade e estrutura genética espacial em populações fragmentadas de *Solanum* spp. do Cerrado, estimadas por meio de locos microssatélites. **Scientia Forestalis**, v.37, p.143-150, 2009.

NEIVA, D.S.; MELO JÚNIOR, A.F.; OLIVEIRA, D.A.; ROYO, V.A.; BRANDÃO, M.M.; MENEZES, E.V. *Acrocomia emensis* (Arecaceae) genetic structure and diversity using SSR molecular markers. **Genetics and Molecular Research**, v.15, gmr.15017785, 2016. DOI: https://doi.org/10.4238/gmr.15017785.

PEREIRA, L.D.; COSTA, M.L.; PINTO, J.F.N.; ASSUNÇÃO, H.F. da; REIS, E.F. dos; SILVA, D.F.P. da. Diversidade genética do florescimento de *Anacardium humile* A. St. Hill no Sudoeste Goiano. **Revista Brasileira de Agropecuária Sustentável**, v.6, p.19-25, 2016. DOI: https://doi.org/10.21206/rbas.v6i4.373.

RESSEL, K. de A.; LIMA-RIBEIRO, M. de S.; REIS, E.F. dos. Desempenho de progênies de diferentes matrizes de cajuzinho-do-cerrado mediante o armazenamento e o peso das núculas. **Ciência Rural**, v.45, p.1782-1787, 2015. DOI: https://doi.org/10.1590/0103-8478cr20141143.

SANTOS, R. da C. dos; SANTOS JÚNIOR, J.E. dos. Divergência genética por análise multivariada de caracteres fenotípicos de Anacardium humile (St. Hilaire). **Revista Ceres**, v.62, p.553-560, 2015. DOI: https://doi.org/10.1590/0034-737X201562060007.

SOARES, T.N.; SANT'ANA, L.L.; OLIVEIRA, L.K. de; TELLES, M.P.C.; COLLEVATTI, R.G. Transferability and characterization of microsatellite loci in *Anacardium humile* A. St. Hil. (Anacardiaceae). **Genetics and Molecular Research**, v.12, p.3146-3149, 2013. DOI: https://doi.org/10.4238/2013.January.4.24.

STRUCTURE SOFTWARE. Available at: <https://web.stanford.edu/group/pritchardlab/structure.html>. Accessed on: Oct. 1 2023.

WEIR, B.S. **Genetic data analysis**: methods for discretion population genetic data. Sunderland: Sinauer, 1996. 377p.