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Genetic diversity of potential mother trees of *Myracrodruon urundeuva* Allemão in a remnant population from Brazilian Cerrado using ISSR

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ABSTRACT: *Myracrodruon urundeuva* Allemão is a tree species of the family Anacardiaceae, native from the northeast, southeast and midwest regions of Brazil, locally known as aroeira. Because of its long history of predatory exploitation, this species has been lately classified as vulnerable to extinction. The present work had as objective to evaluate the genetic diversity of potential mother trees of *M. urundeuva* using ISSR molecular markers in order to subsidize the collection of seeds with large genetic diversity. The selected trees are located in the forest reserve of Brita Guia, in the municipality of Cuiabá – MT, Brazil. Of the 18 ISSR primers tested, seven were selected to characterize genetic diversity. The total amplified fragments were 116, with a percentage of 99.15% polymorphism. The individuals presented Nei's genetic diversity (He) of 0.2706 and Shannon's diversity index (I) of 0.4226. The UPGMA grouping method revealed the existence of two large groups among the individuals, which could be considered to perform seed collection among divergent genotypes.

Diversidade genética de potenciais matrizes de *Myracrodruon urundeuva* Allemão em uma população remanescente do Cerrado brasileiro por meio de marcadores ISSR

RESUMO: *Myracrodruon urundeuva* Allemão é uma espécie arbórea da família Anacardiaceae, nativa das regiões nordeste, sudeste e centro do Brasil, conhecida localmente como aroeira. Devido à sua longa história de exploração predatória, essa espécie tem sido classificada ultimamente como vulnerável à extinção. O presente trabalho teve como objetivo avaliar a diversidade genética de potenciais matrizes de *M. urundeuva* utilizando marcadores moleculares ISSR, a fim de subsidiar a coleta de sementes com grande diversidade genética. As árvores selecionadas estão localizadas na reserva florestal de Brita Guia, no município de Cuiabá - MT, Brasil. Dos 18 primers ISSR testados, sete foram selecionados para caracterizar a diversidade genética. O total de fragmentos amplificados foi de 116, com uma porcentagem de 99,15% de polimorfismo. Os indivíduos apresentaram diversidade genética (He) de Nei de 0,2706 e índice de diversidade de Shannon (I) de 0,4226. O método de agrupamento UPGMA revelou a existência de dois grandes grupos entre os indivíduos, que poderiam ser considerados para realizar a coleta de sementes entre genótipos divergentes.

Introduction

Myracrodruon urundeuva Allemão is a tree from the Anacardiaceae family, popularly known as aroeira. It is a heliophilous, deciduous and selective xerophilous species (Lorenzi 2008) that occurs in the northeast, southeast and midwest regions of Brazil and also in the Chaco region of Bolivia, Paraguay and Argentina (Carvalho 2003; Lorenzi 2008; Nogueira 2010).

The wood of *M. urundeuva* is suitable for multiple purposes which makes the species attractable for market demands. The heartwood has a good resistance to the attack of xylophagous organisms and a natural durability due to its density and amount of soluble extractives (Paes et al. 2004). Thus, the aroeira is widely used in external works such as poles, pillars, beams in bridge frames and mills. In the civil construction, it is used as rafters, beams, parquet, slats and turned parts (Lorenzi 2008). The inner bark, roots and leaves of the aroeira have effective pharmacological properties in the gastric treatment of ulcers as for the treatment of rheumatism. The species has also anti-inflammatory, astringent, antiallergic and healing properties (Carlini et al. 2010).

Due to its wide geographical distribution, the aroeira has been widely used in reforestry programs for degraded lands and in the establishment of protected forests in different regions of Brazil. The good quality of its wood has also drawn interests for studies of breeding and genetic improvement (Costa et al. 2011).

During the selection of the mother trees for seed collection for reforestry programs, phenotypic characteristics associated with plant vigor are generally considered, such as good phytosanitary condition and crown size. This is based on the principle that a phenotypically good individual has a good genetic constituition, increasing the chances of originating offspring with similar characteristics, when subjected to the same environmental conditions. However, in addition to these characteristics, knowing the genetic variability of seed suppliers is also essential to ensure that the new forest represents not only the species to be conserved but also its genetic diversity (Kageyama and Gandara 1993). This information is also fundamental to the formation of base populations of forest breeding programs (Pires et al. 2011).

One approach that can be used to characterize the genetic diversity of species is through molecular markers, such as the ISSR markers (inter-simple sequence repeat). The ISSR markers have the advantage of generating a large number of bands through the polymerase chain reaction (PCR) and are very useful for population evaluation in genetic studies, diversity detection and genetic mapping studies (Caixeta et al. 2013). In addition, they do not require prior DNA sequence information, have low cost of laboratory procedures and can be used for various plant species (Dias et al. 2015).

Thus, the objective of this study is to characterize the genetic diversity among potential mother trees of *M. urundeuva* (Allemão) in a remnant population from Brazilian Cerrado in Cuiabá, MT, using ISSR markers.

Material and Methods

Study area

The trees studied are located in a remnant forest of approximately 100 hectares of Deciduous Seasonal Forest, Brazilian Cerrado, located in the District of Nossa Senhora da Guia city of Cuiabá, Mato Grosso, midwest Brazil, at the coordinates S 15 °22'17" and W 56 °12'14" (Figure 1) .

The climate of the region is Aw (warm tropical, with dry season in winter), according to the Köppen classification. In this region, the climate has the following average characteristics: temperature of 25.7 °C, relative humidity of 74%, annual precipitation of 1450 mm, and potential evapotranspiration of 1530 mm year^{-1}. The soil is classified as lithic alic and presents very gravelly texture, undulating relief and rocky outcrop (BRASIL, 1997).

Collection of leaf material

Aroeira mother trees were phenotypically selected based on size and vigor, and then georeferenced (Figure 1). The leaf material was collected with the aid of a pruner. The samples were placed in identified paper bags and transported to the Forest Improvement and Biotechnology laboratory of the Faculty of Forest Engineering of UFMT in Cuiabá Campus - MT, where they were stored at -20 °C until DNA extraction.

DNA extraction

The extraction method used was based on the 2% CTAB protocol (Doyle and Doyle 1990). About 50 mg of leaf material was macerated with liquid nitrogen in a crucible with 700 µL of 2% CTAB extraction buffer (20 mM EDTA; 0.1 M Tris-HCl pH 8.0; 1.4 M NaCl, 2% CTAB and 0.4% ß-Mercaptoethanol). The samples were homogenized and incubated at 60 °C for 45 min. After this time 500 µl of chloroform-isoamyl alcohol (24:1) was added. The material was homogenized and centrifugation was performed for 10 min at 12,000 rpm. The supernatant was then transferred to a new tube containing 500 µL chloroform-isoamyl alcohol (24:1) and the samples were again homogenized and centrifuged for 10 min at 12,000 rpm. Then the supernatant was transferred to a new tube containing 600 µL of ice cold isopropanol and incubated at -20 °C for at least two hours. After this time, the material was centrifuged for 10 min at 12,000 rpm and the supernatant discarded. The precipitate was washed

twice with 70% ethanol and once with 100% etanol, followed by centrifuging at 12,000 rpm for 3 min and discarding the supernatant in each wash. Subsequently, the DNA was kept at room temperature for 1 h for drying and then resuspended in 50 µL of TE buffer (10 mM Tris HCl and 1 mM EDTA, pH 8.0). In each sample 1 µL of RNase solution (10 mg $^{-1}$) was added with subsequent incubation at 37 °C overnight.

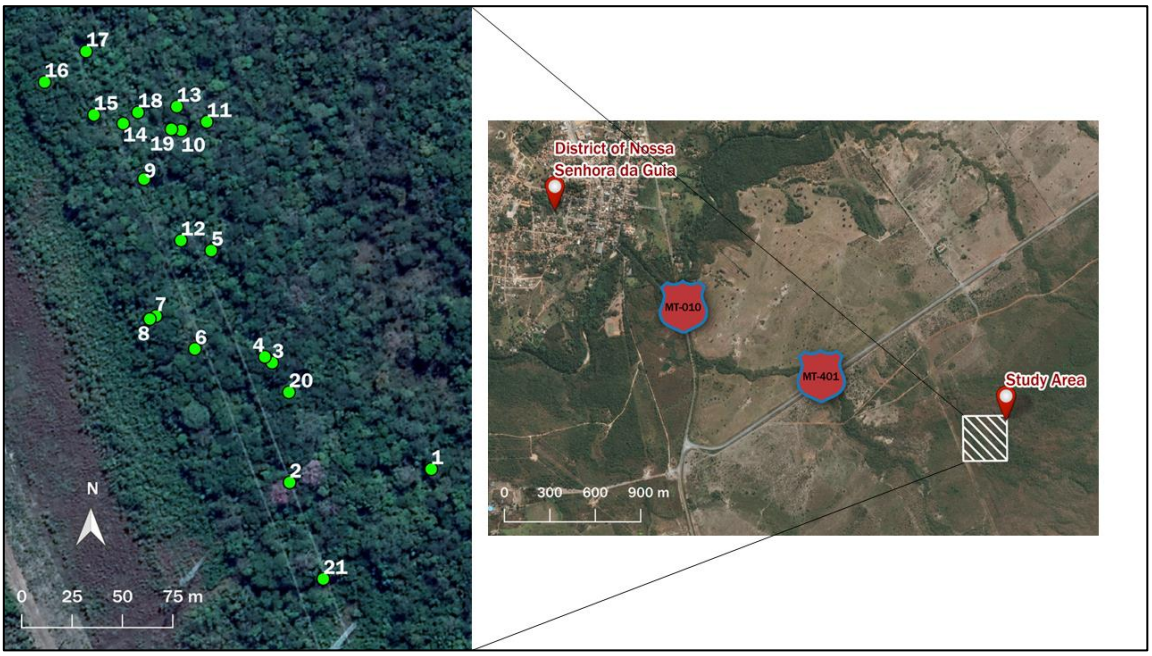

Figure 1. Trees of *Myracrodruon urundeuva* Allemão studied (green circles). The trees are located in a Deciduous Seasonal Forest, Brazilian Cerrado, in the District of Nossa Senhora da Guia city of Cuiabá, Mato Grosso, midwest Brazil, at the coordinates S 15 °22'17" and W 56 °12'14".

DNA quality assessment

The quality evaluation of the extracted DNA was performed on a 1% agarose gel, in which a mix with 4 µL of concentrated DNA, 5 µL of ultrapure water and 1 µL of loading buffer were placed. The gel was submitted to electric currents at 90 V on PowerPac Basic™ Electrophoresis Device - BioRad for 40 minutes. Afterwards, the gel was submerged in a solution with ethidium bromide (0.5 µg/mL) for 15 minutes and subsequently photocumented.

ISSR Primer Selection

The selection of the ISSR markers was performed through PCR reactions, in which 18 primers were tested (BECK, JHON, MANNY, OMAR, UBC807, UBC826, UBC828, UBC830, UBC834, UBC835, UBC840, UBC848, UBC855, UBC857, UBC860, UBC861, UBC864, and UBC889). Subsequently, the ISSR markers with the highest number of clear and polymorphic fragments were selected.

ISSR reactions

Reactions were prepared in PCR microtubes in a solution containing 10 ng DNA, 1 mM primer, 1X Go Taq® Master Mix 2X (Promega) and ultrapure water to complete a final volume of 10 µL. PCR reactions were performed on a thermoCycler T100™ BioRad, in which the samples were initially denatured at 94 °C for 2 min followed by 37 cycles of amplification at 94 °C for 20 sec, 47 °C for 20 sec and 72 °C for 20 sec and a final elongation at 72 °C for 7 min.

Electrophoresis

Electrophoresis was performed in a horizontal tank (Bio-Pac ™ RadPower). PCR products were submitted on a 2% (w/v) agarose gel in 1X TBE buffer (Tris-Borate EDTA) at a voltage of 90 V for two hours. After the electrophoresis run time, the gel was removed from the tank and placed in a solution of ethidium bromide (0.5 µg/mL) for 20 min and then it was photodocumented.

Data analysis

Genotyping

Genotyping was performed by means of the photographs of the gels, whereby a binary matrix (0 and 1) was generated, in which (1) corresponds to the presence and (0) the absence of the fragments for each loci.

Genetic Diversity

Characterization of intrapopulation genetic diversity was performed using the PopGene software, version 1.32 (Yeh et al. 1997). Given the dominant nature of the marker, the software assumes for allelic frequency estimates that loci are at Hardy-Weinberg equilibrium.

Estimated genetic diversity indices were: percentage of polymorphic loci (P %); Shannon's diversity index (I), and Nei's gene diversity (He), where:

a) percentage of polymorphic loci (P %): obtained by the arithmetic mean of the number of polymorphic loci by the total number of loci. Loci were considered polymorphic when the frequency of the most common allele did not exceed 0.95, as suggested by Nei (1973).

b) Shannon's diversity index (I):

$$I = -\sum p_i \, . \, log_2 p_i$$

Where *pi* is the frequency of the presence or absence of a given band

c) Nei's genetic diversity (expected heterozygosity) (He)

$$\hat{H}_e = (1-\sum p_i^2)$$

Where pi is the frequency of the presence or absence of a given band

Genetic Similarity

Estimation of genetic similarity (sg_{ij}) between each pair of genotypes was made by Jaccard coefficient, using the following expression:

$$sg_{ij} = \frac{a}{a + b + c}$$

Where:

a: presence of allele in genotypes i and j
b: presence of allele only in genotype i
c: presence of allele only in genotype j

Subsequently, the similarity matrix between individuals was used to group the genotypes by generating a dendrogram by the unweighted pair group method (UPGMA). Genetic similarity analysis were performed using the NTSYS-PC 2.0 software (Rohlf 1992).

Results and Discussion

The DNA extraction method was effective for *M. urundeuva* as it resulted in pure and intact DNA for almost all samples, providing sufficient concentrations for molecular analysis (data not shown).

Seven ISSR primers were selected for the characterization of *M. urundeuva* genetic diversity. They are: JHON, MANNY, OMAR, UBC807, UBC835, UBC841 (Figure 2), UBC848 (Table 1). Together these primers generated a total of 116 loci. The primer that presented the largest number of bands was UBC848, while UBC835 presented the smallest number of amplified fragments. During ISSR reactions, recurrent amplification failures were observed for samples 8 and 13, which is possibly associated with DNA degradation during storage. Thus, these samples were disregarded.

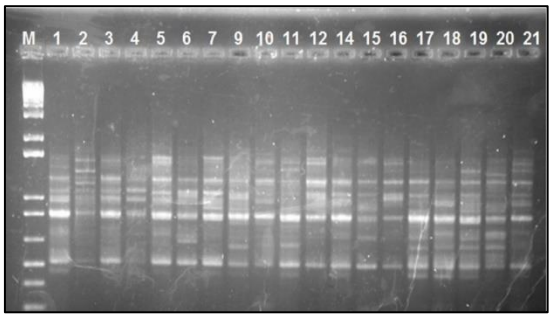

Figure 2. Pattern of ISSR fragments resulting from primer amplification UBC 841 from nineteen trees *of Myracrodruon* urundeuva Allemão. M = Ladder; 1-21 identifications of the trees.

When observing other studies that used ISSR markers to characterize the genetic diversity of other species, it was noticed that the number of primers used is quite variable. In a study with *Mauritia flexuosa* L. f. (Buriti), nine primers were used, which amplified a total of 97 bands with 78.3% polymorphism (Rossi et al. 2014). For *Mimosa caesalpiniaefolia* Benth. (Sabiá mimosa), seven ISSR primers were tested and generated a total of 78 loci with 52.7% polymorphism (Araújo et al, 2016). Another research with *Hymenaea courbaril* L. used 14 primers generating 87 loci with 65% of polymorphism (Rocha et al, 2017). Thus, it can be stated that the ISSR markers were satisfactory to characterize the genetic diversity of *M. urundeuva*, as they detected polymorphism and generated a number of fragments compatible with other studies. Moreover, they were reproducible, constituting an efficient method for detecting genetic variability among their genotypes.

Nei's genetic diversity and Shannon's diversity index express the genetic diversity of a given study population. These indices can range from 0 to 1, and the closer to zero the smaller the genetic diversity. In the present study, Nei's genetic diversity (*He*) was 0.27 and Shannon's diversity index (*I*) was 0.42 (Table 2).

For *M. urundeuva* there are no genetic diversity studies using ISSR markers that estimate the same indexes analyzed in this study. However, it can be considered that the genetic diversity of *M. urundeuva* has a considerable magnitude when compared to results obtained from researches with the same markers for other species. In a studie with *Hancornia speciosa* Gomes (Mangaba) using ISSR markers, the values of *He* and *I* were 0.18 and 0.26, respectively, which were considered low within population (Costa et al. 2015).

A genetic diversity study using ISSR markers in three commercial plantings of *Theobroma grandiflorum* (Willd. ex Spreng.) K.Schum (Cupuaçu) found *He* values of 0.114; 0.108 and 0.104, respectively, and *I* values were 0.117; 0.162 and 0.156 (da Silva et al. 2016). In a study with *Copernicia prunifera* (Mill.) H. E. Moore (Carnauba), *He* was 0.327 and *I* was 0.470 (Farjado et al. 2018).

Table 1. ISSR primers used to characterize the genetic diversity of nineteen trees of *Myracrodruon urundeuva* Allemão and their respective sequences and number of generated fragments.

Primers	Sequence *(5'- 3')**	Number of loci
JOHN (AG)7-YC	AGAGAGAGAGAGAGYC	16
MANNY (CAC)4-RC	CACCACCACCACRC	16
OMAR (GAG)4RC	GAGGAGGAGGAGRC	17
UBC807 (AG)8-T	AGAGAGAGAGAGAGAGT	18
UBC835 (AG)8YC	AGAGAGAGAGAGAGAGYC	13
UBC841 (GA)8YC	GAGAGAGAGAGAGAGAYC	15
UBC848 (CA)6RG	CACACACACACACACARG	21
Average		16,57
Total		116

R- purine (A or G); Y-pyrimidina (T or C).
*Sequences from the University of British Columbia (UBC primer set # 9) and according to Chiron et al, 2009.

Table 2. Genetic diversity index estimated for nineteen trees of *Myracrodruon urundeuva* Allemão: Nei's genetic diversity (He); Shannon's diversity index (I) and percentage of polymorphic loci (P%).

Genetic diversity index	Estimated value
He	0,2706 ± (0,1599)
I	0,4226 ± (0,2000)
P	116 (99,15 %)

( ) Standard deviation

The percentage of polymorphic loci (P) indicates the amount of loci for which more than one allele has been observed, without the frequency of the most frequent allele being greater than 95%. For the studied population, the percentage of polymorphic loci was 99.15%. To characterize the genetic variability of *Bertholletia excelsa* Bonpl. (Brazil nut) with ISSR, 52 fragments were amplified with an average of P of 88.6% using eight primers (Ramalho et al. 2016). In the diversity study for *Rollinia mucosa* (Jacq.) Baill. (Biribazeiro), 118 bands were recorded and 81.3% generated polymorphism (Lorenzoni 2014). ISSR primers generated 102 polymorphic fragments (52%) for genetic analysis of three commercial cultivars of *Theobroma grandiflorum* (da Silva et al. 2016). For a *Copernicia prunifera* population, ISSR primers were also used which generated 79 loci with 72% polymorphism (Farjado et al. 2018).

In a genetic diversity analysis using AFLP markers (amplified fragment length polymorphism) in three progenies tests of *M. urundeuva* , 34, 50 and 53 polymorphic loci (137 total loci) were obtained (Freitas et al. 2005). Using izoenzyme markers to evaluate *M. urundeuva* genetic diversity in two anthropogenic populations in the semiarid region of northeastern Brazil, the average of polymorphic loci were 12.5% and 25.0% (Lacerda et al. 1999). Comparing these values with the values obtained in the present study, it can be stated that the individuals of *M. urundeuva* selected for this study have a considerable rate of polymorphism, which also indicates their genetic diversity.

Genetic similarity values ranged from 0.67 to 0.15 (Table 3). The lowest similarity was observed between accessions 1 and 15 (0.15), and the highest similarity between accessions 6 and 7 (0.67) (Figure 3). Possibly, the greatest similarity between individuals 6 and 7 is related to their physical proximity in the study area (Figure 1).

The dendrogram show the constitution of two large groups among the 19 genotypes. Group I is composed only of individuals 1 and 2, and group. II already has the majority of individuals, being formed by the remaining 17 individuals (Figure 1). In group II, there is still a subdivision into two smaller groups (IIa and IIb) formed by the individuals: 3, 5, 6, 7, 9, 19, 21, 12, 20, 15, 17, 16, 15 and 14; and 10, 11 and 4, respectively. Therefore, if there is interest in to prioritize a greater number of mother trees, the collected could be performed using trees from these two subgroups, in addition to group 1.

Through these results, it is possible to plan that seed collection be performed in the most genetically distant mother trees, resulting in seeds with greater genetic variability, since the more divergent the parents, the greater the diversity of their progenies (Manfio et al. 2012). In addition to this information, it is also necessary to distinguish individuals by sex for seed collection, taking into account the species *M. urundeuva* is predominantly dioecious (Gaino et al. 2011).

Table 3. Jaccard's genetic similarity matrix with the 19 studied individuals of *Myracrodruon urundeuva* Allemão based on seven ISSR markers.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**9**	**10**	**11**	**12**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
1	1																		
2	0.3636	1																	
3	0.3750	0.3673	1																
4	0.1746	0.2800	0.2933	1															
5	0.3455	0.3261	0.6167	0.2208	1														
6	0.2745	0.3684	0.5556	0.3667	0.5283	1													
7	0.3167	0.3333	0.6452	0.3026	0.6500	0.6667	1												
9	0.2444	0.3636	0.4423	0.3036	0.4400	0.5435	0.5200	1											
10	0.1930	0.3261	0.4677	0.3538	0.3134	0.4151	0.4531	0.2941	1										
11	0.2037	0.3721	0.4426	0.3492	0.3710	0.4510	0.5000	0.3830	0.5490	1									
12	0.3214	0.2708	0.4058	0.2533	0.4688	0.3607	0.4559	0.4800	0.2222	0.2879	1								
14	0.1639	0.3061	0.3429	0.4000	0.3188	0.4717	0.4118	0.2545	0.4167	0.4643	0.2297	1							
15	0.1563	0.2692	0.3857	0.2877	0.3429	0.3729	0.3562	0.3585	0.3538	0.3492	0.3429	0.3582	1						
16	0.2131	0.2553	0.3857	0.3429	0.3623	0.4138	0.4348	0.4038	0.2941	0.2687	0.4242	0.3188	0.3239	1					
17	0.2708	0.3158	0.3548	0.3279	0.2656	0.3529	0.3871	0.3043	0.3393	0.3091	0.3065	0.3000	0.3065	0.3065	1				
18	0.2857	0.3111	0.4844	0.3143	0.4603	0.4630	0.4265	0.4286	0.3438	0.3607	0.3731	0.3692	0.3529	0.3939	0.4906	1			
19	0.2553	0.3235	0.4423	0.3704	0.3889	0.5385	0.4717	0.4857	0.3061	0.3478	0.3750	0.3913	0.3333	0.3774	0.4318	0.4894	1		
20	0.3077	0.2963	0.4000	0.2885	0.3830	0.5000	0.4255	0.4286	0.1957	0.3000	0.4600	0.2667	0.3261	0.3654	0.2667	0.4318	0.4222	1	
21	0.2439	0.2353	0.5000	0.2963	0.3830	0.4211	0.4400	0.4118	0.3696	0.3333	0.3462	0.3600	0.4118	0.4151	0.3261	0.4375	0.4286	0.3590	1

Collecting seeds taking into account the genetic diversity of species is essential to avoid future problems such as associated anomalies (anatomical deformities), dwarfism, physiological disorders, albinism or even mortality due to low weather resistance or pest attacks and other pathological agents, besides ensuring the preservation of the different alleles present in the species. Thus, it is recommended the selection of trees that present greater genetic dissimilarity and that belong to different groups for seed collection (Sebbenn 2003). With the results of this study, we can affirm that there is considerable genetic diversity among the studied trees. Therefore, they can be selected as seed source, provided that trees from different groupings and with low genetic similarity between them be selected to prevent inbreeding and to keep the genetic diversity between their progenies.

Conclusions

The ISSR markers detected polymorphism and genetic diversity among the genotypes of the potential mother trees of *M. urundeuva* (Allemão).

The UPGMA clustering method revealed the existence of two large groups that can be considered in order to collect seeds between divergent genotypes.

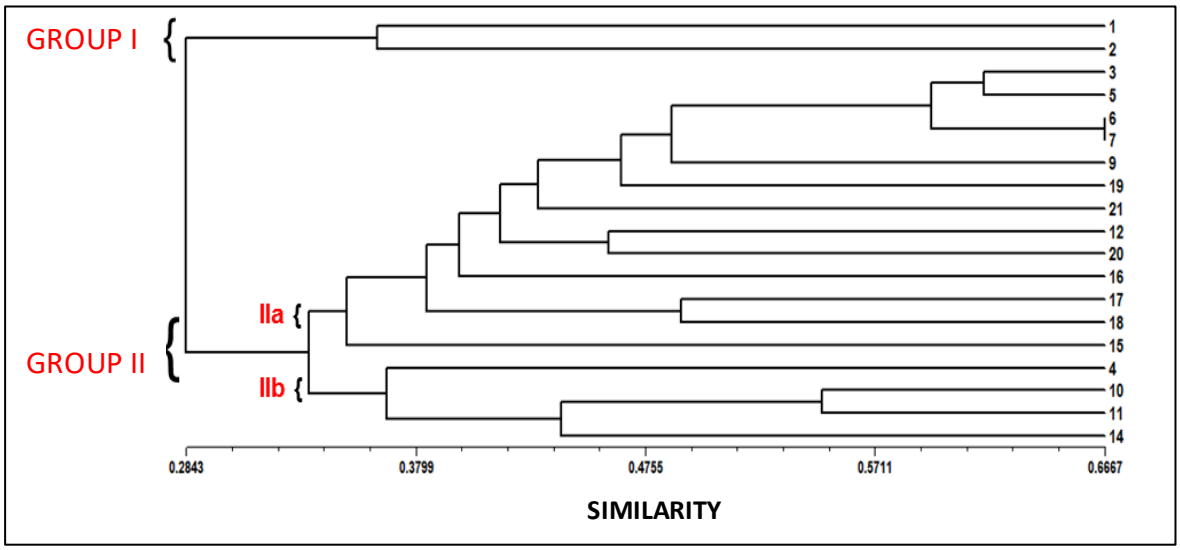

Figure 3 - UPGMA Dendrogram representing the genetic similarity between nineteen trees of *Myracrodruon urundeuva* Allemão.

Individuals 1 and 15 were the most divergent and therefore should be prioritized for seed collection for future genetic conservation programs, recovery of degraded areas, establishment of protected areas and even genetic improvement. Given the considerable genetic diversity shown in the studied population and because *M. urundeuva* is considered an endangered, its in situ conservation is highly recommended.

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References

Araújo FS, Pacheco MV, Vieira FA, Ferrari CS, Félix F C, Chagas, K P T. (2016) ISSR molecular markers for the study of the genetic diversity of *Mimosa caesalpiniaefolia* Benth. *Idesia*, 34: 47-52

BRASIL. Ministério do Meio Ambiente, Recursos Hídricos e Amazônia Legal (1997). Manejo da bacia hidrográfica do rio Coxipó-açu para a conservação

de seus recursos hídricos. Cuiabá: UFMT, ABEAS/SRH, 113p.

Caixeta, ET, Ferrão LFV, Maciel-Zambolim E (2013) Molecular Markers In: *Biotechnology applied to plant breeding*. Viçosa. P. 31-68.

Carlini EA, Duarte-Almeida J, Rodrigues E, Tabach R (2010) Antiulcer effect of the pepper trees *Schinus terebinthifolius* Raddi (aroeira-da-praia) and *Myracrodruon urundeuva* Allemão, Anacardiaceae (aroeira-do-sertão). *Brazilian Journal of*

Pharmacognosy, 20(2): 140-146. doi.org/10.1590/S0102-695X2010000200001

Carvalho, PER (2003) Brazilian tree species, Embrapa Informação Tecnológica**,** Colombo-PR. v.1, 177p.

Chiron GR, Oliveira RP, Santos TM, Bellvert F, Bertrand C, van den Berg C. (2009). Phylogeny and evolution of *Baptistoni*a (Orchidaceae, Oncidiinae) based on molecular analyses, morphology and floral oil evidences. *Plant Systematics and Evolution*, 281 (1-4): 35-49. doi.org/10.1016/j.bse.2009.12.038

Costa DF, Vieira FA, Fajardo CG, Chagas KPT (2015) Genetic diversity and selection of ISSR primers in a natural mangaba population (*Hancornia speciosa* Gomes) (Apocynaceae). *Revista Brasileira de Fruticultura*, 37: 970-976. doi.org/10.1590/0100-2945-246/14

Costa RB, Almeida, EV, De Freitas ACA, Schirmbeck AG, Rodrigues NB, Roa RAR (2011) Hybrid vigour of aroeira parent trees for the seeds germination character. *Multitemas*, 39: 155-162. doi: 10.20435/multi.v0i39.371

Da Silva BM, Rossi APB, Dardengo JFE, Araújo VAAC, Rossi FS, Oliveira LO, Clarindo WR (2016) Genetic diversity estimated using inter-simple sequence repeat markers in commercial crops of cupuassu tree. *Ciência Rural*, 46(1): 108-113. doi: 10.1590/0103-8478cr20141634

Dias CFT, Bertini CHCM., Silva APMS, Cavalcanti JJV (2015) Genetic variability in early-cycle erect cowpea analysed with RAPD and ISSR markers. In: *Revista Ciência Agronômica*, 46(3): 563-572. doi: 10.5935/1806-6690.20150039

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochem Bull*, 19:11–15.

Farjado CG, Silva RAR, Chagas KTP, Vieira FA (2018) Genetic and phenotypic association of the carnauba palm tree evaluated by inter-simple sequence repeat and biometric traits. *Genethics and Molecular Research*, 17(3). doi: 10.4238/gmr18018

Freitas MLM, Aukar APA, Sebben AM, Moraes MLT, Lemos EGM (2005) Genetic variability in intra *Myracrodruon urundeuva* Fr. All. by AFLP marker. *Scientia forestalis*, 68: 21-28.

Gaino APSC, Moraes, MLT D, Moreira JP, Cardin LT, Moraes MA, Silva AMD, Freitas MLM, Sebbenn AM (2011) Mating system in *Myracrodruon urundeuva* (Anarcadiaceae): implications for conservation genetics. *Brazilian Journal of Botany*, 34(4): 545-551. doi.org/10.1590/S0100-84042011000400008

Kageyama PY, Gandara FB (1993) Population dynamics of tree species: implications for management and conservation. In: *Simpósio de Ecossistemas da costa Brasileira, Anais*, São Paulo.

Lacerda CMB, Kageyama PY, Ferraz EM (1999) Isoenzymatic diversity in *Myracrodruon urundeuva* in two anthropic situations in the semi-arid. *Scientia forestalis***,** 55: 89-95.

Lorenzi H (2008) *Brazilians trees***:** Manual for identification and cultivation of native tree plants in Brazil. 5th Edition. São Paulo: Plantarum Institute of Flora Studies Ltda. 384p.

Lorenzoni RM, Soares TCB, Santiago VF, Silva JÁ, Coelho RI (2014) Utilização de marcadores ISSR na avaliação da divergência genética entre acessos de biribazeiro. *Revista Brasileira de Fruticultura*, 36: 251-257. doi.org/10.1590/S0100-29452014000500029

Manfio CE, Motoike SY, Rezende MDV, Santos CEM, Sato AY (2012) Evaluation of macaúba progenies in the juvenile phase and estimation of genetic parameters and genetic diversity. *Pesquisa Florestal Brasileira*, 32(69): 63-68. doi 104336/2012.pfb.32.69.63

Nei M (1973) Analysis of gene diversity in sunbdivided populatios. Proceedings of the National Academy of Sciences of the United States of America. Washignton, p. 3321-3323.

Nogueira JCB (2010) *Mixed reforestation with native species: Ciliary mat*. 1st Edition. São Paulo: Forest Institute. 148p.

Paes JB, Morais VM, Lima CR (2004) Natural resistance of nine Brazilian semiarid woods to fungi under laboratory conditions. *Revista Árvore*, 28(2):275-282. doi: 10.1590/S0100-67622004000200014

Pires IE, Resende MDV, Silva RL, Resende JRMFR (2011) Genética Florestal. Edition 1th, Viçosa, MG: UFV, 318p.

Ramalho AB, Rossi AAB, Dardengo JFE, Zortea KEM, Thiago AV, Martins KC (2016) Genetic diversity among *Bertholletia excelsa* genotypes using molecular markers. *Floresta*, Curitiba, v. 46, n. 2, p. 207-214. doi: 10.5380/rf.v46i2.41970

Rocha VD, Tiago PV, Tiago AV, Pedri E C M, Cardoso, E S, & Rossi, A A B (2017) Genetic diversity among *Hymenaea courbaril* L. genotypes naturally occurring in the north of Mato Grosso State, Brazil. *Genetics and Molecular Research*, *16*(3): 1-9. doi: 10.4238/gmr16039706

Rossi FS, Rossi AA, Dardengo JFE, Brauwers LR, Silva ML, Sebben AM (2014) Genetic diversity in natural populations of *Mauritia flexuosa* L. f. (Arecaceae) with the use of ISSR markers. *Scientia Forestalis*, Piracicaba, 42(104): 631-639.

Rohlf FJ (1992). Numerical taxonomy and multivariate analysis system (version 1.70). New York: Exeter Publis Hers, 470p.

Sebbenn AM (2003) Sample size for ex situ conservation of tree species with mixed breeding system. *Revista do Instituto Florestal*, 15(2):147-162.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE, *The User-friendly Shareware for Population Genetic Analysis***.** Molecular Biology and Biotechnology Centre. University of Alberta, Edmonton, Alberta, CANADA.