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GENETIC DIVERSITY AND POPULATION STRUCTURE OF JATOBÁ: A SPECIES WITH ECONOMIC POTENTIAL FOR THE AMAZON REGION

DIVERSIDADE GENÉTICA E ESTRUTURA POPULACIONAL DE JATOBÁ: UMA ESPÉCIE COM POTENCIAL ECONÔMICO PARA A AMAZÔNIA

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ABSTRACT

Jatobá (*Hymenaea courbaril* L.) is a species that shows ecological importance and occurs in different geographic regions in Brazil. Knowing and evaluating the existence of places with higher genetic variability can help in conservation programs and actions. In this context, the present study aimed to evaluate the genetic diversity and structure in natural populations of Jatobá with occurrence in Mato Grosso Amazon through ISSR markers. Fifty-four individuals were sampled in three populations, being one in the municipality of Marcelândia (MA=24) and two in the municipality of Alta Floresta (Comunidade Central AF=17 and Pista do Cabeça PC=13 individuals). Total genomic DNA was extracted from the leaf tissue by the CTAB method. The 54 individuals were genotyped with 10 ISSR *primers*. A total of 110 fragments were amplified, being 78.2% polymorphic. The greatest diversity indices were found in the population from MA (H=0.25; I=0.37 and %P=65.45). The genetic distance was higher among the populations of the two municipalities (0.17 between MA and AF; 0.16 between MA and PC). The analysis of molecular variance (AMOVA) indicated that 31.79 % of the total variance is among populations and 68.21 % within populations. There is genetic diversity in native populations of Jatobá in Mato Grosso Amazon. We recommend that individuals from both populations be preserved, in order to ensure the maintenance of genetic variability and the effective conservation of species in Mato Grosso Amazon.
Keywords: *Hymenaea courbaril*; genetic variability; ISSR; conservation.

RESUMO

O jatobá (*Hymenaea courbaril* L.) é uma espécie que apresenta importância ecológica e ocorre em diferentes regiões geográficas do Brasil. Conhecer e avaliar a existência de locais com maior variabilidade genética pode auxiliar em programas e ações de conservação. Neste contexto, o presente estudo objetivou avaliar a diversidade genética e a estrutura em populações naturais de jatobá com ocorrência na Amazônia mato-

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grossense por meio de marcadores ISSR. Foram amostrados 54 indivíduos em 3 populações, sendo uma no município de Marcelândia (MA=24) e duas no município de Alta Floresta (Comunidade Central AF=17 e Pista do Cabeça PC=13 indivíduos). DNA genômico total foi extraído de tecido foliar pelo método CTAB. Os 54 indivíduos foram genotipados com 10 *primers ISSR*. Foram amplificados um total de 110 fragmentos, sendo 78,2% polimórficos. Os maiores índices de diversidade foram encontrados na população de MA (H=0.25; I=0.37 e %P=65.45). A distância genética foi maior entre as populações dos dois municípios (0.17 entre MA e AF; 0.16 entre MA e PC). A análise de variância molecular (AMOVA) indicou que 31.79% da variância total está entre populações e 68.21% dentro de populações. Há diversidade genética nas populações nativas de jatobá na Amazônia mato-grossense. Recomenda-se que sejam preservados indivíduos de ambas as populações, a fim de garantir a manutenção da variabilidade genética e a conservação efetiva da espécie na Amazônia mato-grossense.
Palavras-chave: *Hymenaea courbaril*; variabilidade genética; ISSR; conservação.

INTRODUCTION

Jatobá (*Hymenaea courbaril* L.) is an arboreal species belonging to the family Fabaceae that is distributed throughout Latin America. Due to the presence a floured pulp that involves its embryo (BUSATTO et al., 2013), the species is an important food resource for fauna, that disperse its seeds. Such fact reinforces the importance of preserving and performing plantations of the species in degraded areas intended for the recomposition of the arboreal vegetation (LIMA; ZANELLA; CASTRO, 2010).

The trunk of *Hymenaea courbaril* is used as wood in civil construction and in the furniture industry (MELO; MENDES, 2005). Parts of the plant are used as therapeutic medicine for several diseases (CARVALHO, 1994). In spite of the potential in the national and international market, the natural populations of the species have been reduced in the Brazilian Amazon due to the fragmentation caused by the advance of the agricultural frontier.

The responses of plant species to the fragmentation process are highly variable, depending their characteristics and environmental changes (LEWIS et al., 2005). Therefore, knowing the population genetic structure and detecting areas where there is a larger genetic variability of the species are fundamental to establish collection and conservation strategies of plant genetic resources of the species.

Cataudella et al. (2010) affirms that the choice of natural populations for a program of management and conservation has as first and most important step the characterization of these populations. Thus, the genetic criteria based on the use of molecular markers are among the main methods of characterization. According to the same author, the most important genetic parameters that provide precious information for conservation projects of threatened species are: the gene flow, the measures of genetic diversity and the degree of spatial genetic structure of the populations.

Molecular biology has provided valuable tools for genetic diversity analyzes and population structure in plant species, being the molecular markers one of these tools that detect differences in the DNA level among two or more individuals and help in the molecular characterization of the analyzed material (GIUSTINA et al., 2014; ROSSI et al., 2014).

Among the methods that use molecular markers, the ISSR (*Inter Simple Sequence Repeat*) has been widely used because it demonstrates a fast and efficient technique with high reproducibility and high indices of polymorphism, providing a large number of low cost data for the researcher and the ISSR markers may be transferred to any kind of plant (RIVAS et al., 2013; GIUSTINA et al., 2014; ROSSI et al., 2014).

In this context, the present study aimed to evaluate the genetic diversity and structure in natural populations of Jatoba, with occurrence in Mato Grosso Amazon through ISSR markers.

MATERIAL AND METHODS

The study area and the plant material sampling

The study areas are located in the north region of Mato Grosso state, comprising the localities: Marcelândia (MA) (11º02'48"S e 54º29'57" O) in the municipality of Marcelândia- MT state; Comunidade

Central (AF) (9º 53' 43" S e 55º 54' 30" O) and Pista do Cabeça (PC) (10º 21' 24" S e 56º 26' 01" O) located in the municipality of Alta Floresta – MT state (Figure 1). The climate of the region is of Equatorial type, hot and humid, characterized by average annual temperature above 24ºC and rainfall above 2,500 mm. The average altitude ranges from 200 to 290 m. The majority soils belong to the class of the oxisols with plain, soft undulated relief. The activities with more importance in the local economies are agriculture and logging. Basically, the vegetation is characterized by savanna, ombrophilous forest and the seasonal forest (MATO GROSSO, 2006).

The collection sites were selected where the species has natural occurrence. Each site is formed by a population of study, totaling three populations. Fifty-four individuals were sampled, being distributed in MA - 24 individuals, AF - 17 individuals and PC - 13 individuals. All collection areas showed plants distributed along right and left margins of highways or roads, in an extension of 200 meters on each margin we sampled all individual adults in reproductive age.

Of each selected individual was collected leaf material with help of a slingshot or trimmer. Preferably young leaves were collected, without mechanical damage or signs of disease. All the material was identified in field and stored in silica gel. The material was transported to the Laboratory of Plant Genetics and Molecular Biology of the University Campus of Alta Floresta - UNEMAT, and stored in a freezer (-20 °C) until the extraction of DNA.

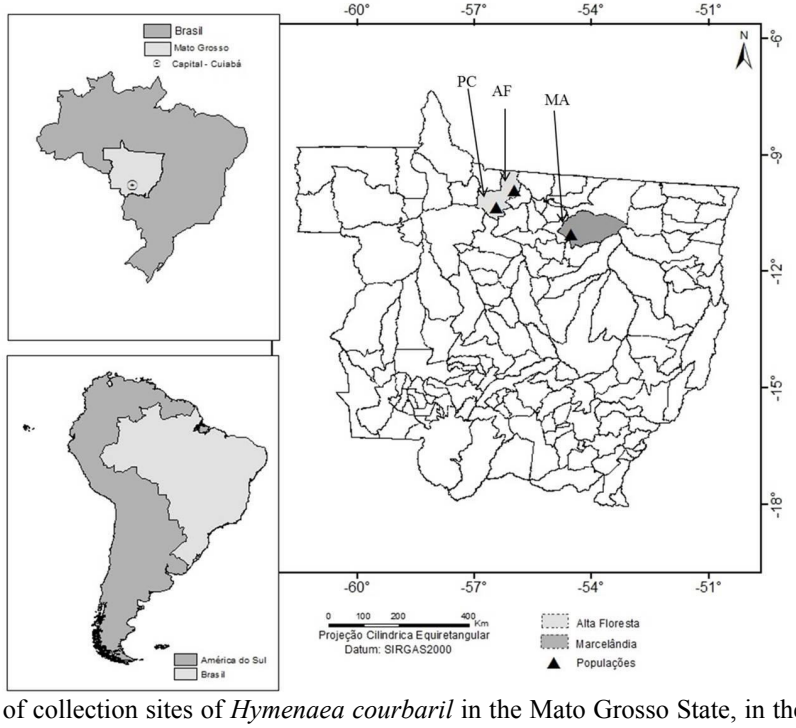

FIGURE 1: Location of collection sites of *Hymenaea courbaril* in the Mato Grosso State, in the municipalities of Alta Floresta and Marcelândia. AF=Comunidade Central, PC=Pista do Cabeça and MA=Marcelândia.

FIGURA 1: Localização dos pontos de coleta de *Hymenaea courbaril* no Estado de Mato Grosso, nos municípios de Alta Floreta e Marcelândia. AF=Comunidade Central, PC=Pista do Cabeça; MA=Marcelândia.

Extraction and quantification of DNA

The total genomic DNA was extracted from approximately 100 mg of folioles by following the method Cetyl trimethylammonium bromide (CTAB) described by Doyle and Doyle (1987) with modifications: increased concentration of polyvinylpyrrolidone (PVP) from 1% to 2%, CTAB from 2% to 5% and β-mercaptoethanol from 0.2% to 2%, furthermore the time of incubation at 65°C was reduced from 60 min to 30 min. The quality of the extracted DNA was evaluated through the technique for electrophoresis in 1% agarose gel stained with ethidium bromide (0.2 mg/mL). For quantification the bands was compared with standard DNA (lambda phage). The DNA samples were diluted to 10 ng/μL.

Amplification and genotyping of loci ISSR

All individuals were genotyped with 10 ISSRs markers. The amplification reactions (PCR) were performed in the thermocycler Biocycler with a final volume of 20 µL, being 1 µL DNA (± 10 ng), 2 µL buffer (10x 1M KCl; 1M Tris pH 8.3; 1M $MgCl_2$; 10% Tween 20), 2 µL $MgCl_2$ (25 mM), 3 µL primer (2 mM), 4 µL dNTP (1 mM), 1 µL DMSO and 0.2 µL Taq polymerase (5 U/µl).

The amplification program was consisted of 4 minutes at 94°C (initial denaturation); 35 cycles of 1 minute at 94°C (denaturation); 1 minute at 49-58.8°C (depending on the primer), 3 min 72°C (extension) and 7 minutes at 72°C (final extension).

Amplification products were separated by electrophoresis in agarose gel 1.5% in running with buffer TBE 1x at 90V constant voltage for approximately four hours. The gels were stained with ethidium bromide (0.6 ng/mL). For comparison of the sizes of the amplified fragments, the DNA ladder of 100 pb (Invitrogen™) was used. Then the gel was photo documented under ultraviolet light using the transilluminator.

Data analysis

For the construction of a binary matrix, the amplified fragments were evaluated and coded as binary characters: presence (1) and (0) absence of bands. Based on the binary matrix were calculated the percentage of polymorphism obtained with each primer and the genetic diversity of the locus or PIC (polymorphic information content) that is an estimate used for evaluation of the discriminatory power of one locus.

For characterization of genetic variability, the program POPGENE (Population Genetic Analysis) version 1.32 (YEH; YANG; BOYLE, 1999) was used. We estimated the Nei's genetic diversity (H) (NEI, 1978), the percentage of polymorphic loci (P%) and the Shannon index (I) for each population and for the set of populations. The analysis of genetic diversity among populations was performed by Nei's method (NEI, 1978), estimating the total heterozygosity (Ht), the average gene diversity within populations (Hs), the average genetic divergence between the populations (G_{ST}) and the gene flow (Nm).

The analysis of molecular variance (AMOVA) was used to reveal the distribution of the genetic diversity within and between populations as described by Excoffier, Smouse and Quattro (1992), with the help of the program ARLEQUIN 3.01 (EXCOFFIER; LAVAL; SCHNEIDER, 2006).

In order to form the dissimilarity matrix, the arithmetic complement of Jaccard index was used. The genetic dissimilarity matrix was used for the clustering analysis of genotypes by UPGMA method (*Unweighted Pair-Group Method Average*) that was computed with the aid of the program Genes (CRUZ, 2008).

The program "Structure" (PRITCHARD; STEPHENS; DONNELLY, 2000) based on bayesian statistic was used to infer the number of clusters (K). We performed twenty runs per value of K, 200,000 initial interactions ("burn-ins") and 500, 000 Monte Carlo simulations through Markov chain (MCMC). For the definition of the most probable K value, we used the criteria described by Pritchard and Wen (2004) and Evanno, Regnaut and Goudet (2005).

RESULTS AND DISCUSSION

The ten primers amplified a total of 110 fragments in the 54 genotypes of Jatobá, being 86 polymorphic bands (78.2%). The numbers of bands amplified per primer ranged from 7 to 13, with an average of eleven per primer (Table 1). The primer DiAC3'G showed the highest percentage of polymorphism in the three analyzed populations. Alves et al. (2007), reported that the tropical tree species showed a big number of alleles per locus. Rossi et al. (2014), evaluated genotypes of *Mauritia flexuosa* L. and found an average of 10.7 bands per primer. Santana et al. (2011), observed an average of 10 bands per primer when studied 17 accessions of umbu-cajazeiras belonging to BAG tropical fruit of Embrapa Mandioca e Fruticultura, based on ISSR markers. However, Rivas et al. (2013), studied native populations of *Theobroma subincanum* Mart. and obtained results lower than those found in the present study, with the average number of bands per primer of 6,69.

TABLE 1: ISSR primers used in natural populations of *Hymenaea courbaril* with descriptions of sequence and the amplified results as the number of total bands (TB), polymorphic bands (PB), percentage of polymorphic bands (P%) and PIC = polymorphic information content.

TABELA 1: *Primers* ISSR utilizados em populações naturais de *Hymenaea courbaril* com descrições de sequência e os resultados amplificados como o número total de bandas (TB), bandas polimórficas (PB), % de bandas polimórficas (%P) e PIC = conteúdo informativo polimórfico.

Primers	Populations											
	AF				PC				MA			
	TB	PB	%P	PIC	TB	PB	%P	PIC	TB	PB	P%	PIC
DiAC3'T	12	8	66.67	0.54	12	8	66.67	0.53	12	7	58.33	0.46
DiAC5'DBD	13	3	23.08	0.29	13	2	15.38	0.38	13	8	61.54	0.53
DiAG3'YC	8	4	50.00	0.46	8	5	62.50	0.56	8	6	75.00	0.50
DiCA5'CR	10	4	40.00	0.39	10	5	50.00	0.45	10	7	70.00	0.48
DiCA5'CY	13	9	69.23	0.37	13	7	53.85	0.39	13	9	69.23	0.13
DiCA5'BDB	13	6	46.15	0.14	13	4	30.77	0.07	13	7	53.85	0.17
DiAC3'YT	8	5	62.50	0.20	8	5	62.50	0.15	8	3	37.50	0.34
TriACA3'RC	13	9	69.23	0.23	13	8	61.54	0.30	13	11	84.62	0.23
DiAC3'G	13	11	84.62	0.36	13	10	76.92	0.29	13	12	92.31	0.31
DiAC3'C	7	3	42.86	0.49	7	4	57.14	0.43	7	7	100.00	0.42
Total	110	62	56.36	3.47	110	58	52.73	3.55	110	77	70.00	3.57
Mean	11	6.2	55.43	0.35	11	5.8	53.73	0.36	11	7.7	70.24	0.36

Where: Y = C or T; R = A or G; B = C, G or T; D = A, G or T; AF = Comunidade Central; PC = Pista do Cabeça; MA = Marcelândia.

The primer that showed the highest polymorphic information content (PIC) for the population AF was DiAC3'T (0.54), for the population PC was DiAG3'YC (0.56) and for MA was DiAC5'DBD (0.53). Whereas the primers that showed the lowest PIC values were DiAC3'YT (0.14) for AF, DiCA5'BDB (0.07) for PC and DiC5'CY (0.13) for MA (Table 1).

According to the classification suggested by Botstein et al. (1980), markers with PIC values above 0.50 are considered very informative, values between 0.25 and 0.50 are considered moderately informative and values lower than 0.25 are considered little informative. Of the 10 primers used, 70% had PIC above 0.25 in the populations from AF and MA, and 80 % in the population from PC (Table 1). Therefore, we recommended these primers for studies with *Hymenaea courbaril*.

The highest diversity indices estimated from Nei (H) and Shannon (I) as well as the greatest percentage of polymorphism were found in the population from Marcelândia (H=0.25; I=0.37 and P%=65.45), followed by Comunidade Central population (H=0.17; I=0.26 and P%=50) while Pista do Cabeça population showed the lowest genetic diversity and consequently the lowest polymorphism (H=0.16; I=0.23 and P%=42.73). In the level of species, the genetic diversity was H=0,27 and I=0.41 (Table 2). Similar results were found by many works that studied native species from Amazon, such as *Mauritia flexuosa* (ROSSI et al., 2014), *Theobroma subincanum* (RIVAS et al., 2013) and *Theobroma speciosum* Willd. Ex Spreng (GIUSTINA et al., 2014). Thus, revealing the importance of the Amazon Forest as a reservoir of intraspecific genetic diversity and the need for conservation of this Biome.

TABLE 2: Genetic diversity within populations of *Hymenaea courbaril*. H (Nei's genetic diversity); I (Shannon diversity index); P (percentage of polymorphism).
TABELA 2: Diversidade genética dentro das populações de *Hymenaea courbaril*. H (diversidade genética de Nei); I (índice de diversidade de Shannon); P (% polimorfismo).

Population	H	I	P
Marcelândia	0.25	0.37	65.45
Comunidade Central	0.17	0.26	50
Pista do Cabeça	0.16	0.23	42.73
Level of species	0.27	0.41	78.18

The total heterozygosity (Ht) estimated was of 0.26, revealing that the species presents reserve of genetic variability in the natural populations in Mato Grosso Amazon.

The gene flow (Nm) estimated or the number of migrants per generation in the populations of *Hymenaea courbaril* was 1.53 (Table 3), characterizing that the populations studied are not genetically isolated. According to Smouse and Sork (2004), the value of gene flow calculated based on genetic divergence reflects the gene flow that occurred during a long period. The estimate does not indicate that is having gene flow in a particular reproductive event, but it calculates the levels of gene flow that must have occurred to produce the observed patterns of genetic structure.

TABLE 3: Genetic parameters of *Hymenaea courbaril*. HT (total heterozygosity); HS (genetic diversity within populations); G_{ST} (genetic divergence between populations); Nm (gene flow).
TABELA 3: Parâmetros genéticos de *Hymenaea courbaril*. HT (heterozigosidade total); HS (diversidade genética dentro das populações); G_{ST} (divergência genética entre as populações); Nm (fluxo gênico).

	HT	HS	G_{ST}	Nm
Average	0.26	0.19	0.25	1.53
Standard deviation	0.03	0.02		

The genetic diversity among populations (G_{ST}) was 0.25, demonstrating that 24.6% of the genetic variability is between populations. This result can be justified by gene flow found in the populations. The G_{ST} values obtained are consistent with others found for tropical allogamous species. Estopa et al. (2006), reported that 21% of the genetic variability of *Eremanthus erythropappus* MacLeish are between populations

The genetic distance was higher among the populations of the two municipalities (0.17 between MA and AF; 0.16 between MA and PC) that are distant from each other more than 200 km (Table 4 and Figure 2) while the lowest was between the two populations of the municipality of Alta Floresta (0.03 between PC and AF), that are distant 67 Km between themselves. The pattern found suggests that the increasing in the geographical distance is accompanied by an increase in the genetic divergence. Rossi et al. (2014) also verified that natural populations of *Mauritia flexuosa* with the greatest genetic distances presented the largest geographical distances.

TABLE 4: Geographical distance, genetic distance and Nei's genetic identity (NEI, 1978) among native populations of *Hymenaea courbaril*. Marcelândia (MA), Comunidade Central (AF) and Pista do Cabeça (PC).
TABELA 4: Distância geográfica, distância e identidade genética de Nei (1978) entre populações naturais de *Hymenaea courbaril*. Marcelândia (MA), Comunidade Central (AF) e Pista do Cabeça (PC).

Population	Nei's identity	Genetic distance	Geographical distance
MA with AF	0.84	0.17	209 Km
AF with PC	0.97	0.03	67 Km
MA with PC	0.85	0.16	217 Km

Distance obtained from the Google Earth program.

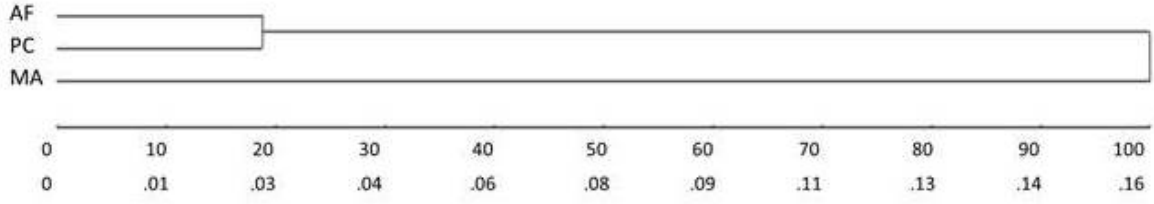

FIGURE 2: UPGMA dendrogram of three populations of *Hymenaea courbaril* based on genetic diversity matrix.
FIGURA 2: Dendrograma UPGMA de três populações de *Hymenaea courbaril* com base na matriz de diversidade genética.

The analysis of molecular variance (AMOVA) indicated that 31.79% of the total variance is between populations and 68.21% within populations, demonstrating that the higher genetic differentiation is at intrapopulation component than at interpopulation component (Table 5). These results corroborate with the several studies performed with tropical species which observed the greatest genetic diversity in intrapopulation level (ROSSI et al., 2014). Genetic variability studies in natural populations with occurrence in tropical regions have demonstrated that plants preserve great variability within populations (PAIVA, 1998).

TABLE 5: Analysis of molecular variance (AMOVA) of the tree populations of *Hymenaea courbaril* studied based 10 ISSR markers.
TABELA 5: Análise de variância molecular (AMOVA) das três populações de *Hymenaea courbaril* estudada a partir de 10 marcadores ISSR.

Source of variation	GL	SQ	CV	VT (%)	F_{ST}
Between populations	2	163.57	4.18	31.79	0.32
Within populations	51	457.19	8.96	68.21	
Total	53	620.76	13.14		

Where: Degree of freedom (GL), Sum of Squares (SQ) component of variation (CV) Total variation (VT) and P are chances of a variance component greater than the observed values by chance. The probabilities were calculated 1023 random permutations. P <0.001.

The interpopulation genetic variation of 31,79% suggests that there is still gene flow in at least some populations, such as in the PC and AF populations from the municipality of Alta Floresta that demonstrated low genetic differentiation values. A low genetic differentiation rate may be a remaining result of the period when landscape was not so fragmented, suggesting that the fragmentation of the area studied is recent because the colonization of the municipality started in 1976 (ROSA; PERIN; ROSA, 2003).

The F_{ST} (interpopulation genetic divergence) was estimated at 0,32 (Table 5). According to Wright (1978), F_{ST} values from 0 to 0.05 indicate low divergence, F_{ST} values=0.05 to 0.15 indicate moderate divergence, F_{ST} values=0.15 to 0.25 indicate high divergence and F_{ST} values above 0.25 indicated very high divergence. Therefore, the data of the present study indicate that there is high interpopulation genetic divergence in the populations studied. The high divergence can be associated with genetic differentiation of the MA population in relation to the other two populations (PC and AF).

The greatest intrapopulation variation may be due to the reproductive system of *Hymenaea courbaril* presents allogamy and evidence of self-incompatibility (BAWA, 1974). The self-incompatibility registered in species is probably due to the rejection process of post-zygotic embryogenesis (GIBBS; OLIVEIRA; BIANCHI, 1999), that eliminates certain genes with high compatibility between parentals.

From the simulations carried out by the program Structure using Bayesian statistics, the most probable number of clusters (K) was defined as four, according to the methodology by Evanno, Regnaut and Goudet (2005). Thus, we observed the formation of four distinct clusters among the 54 individuals of *Hymenaea courbaril* (Figure 3).

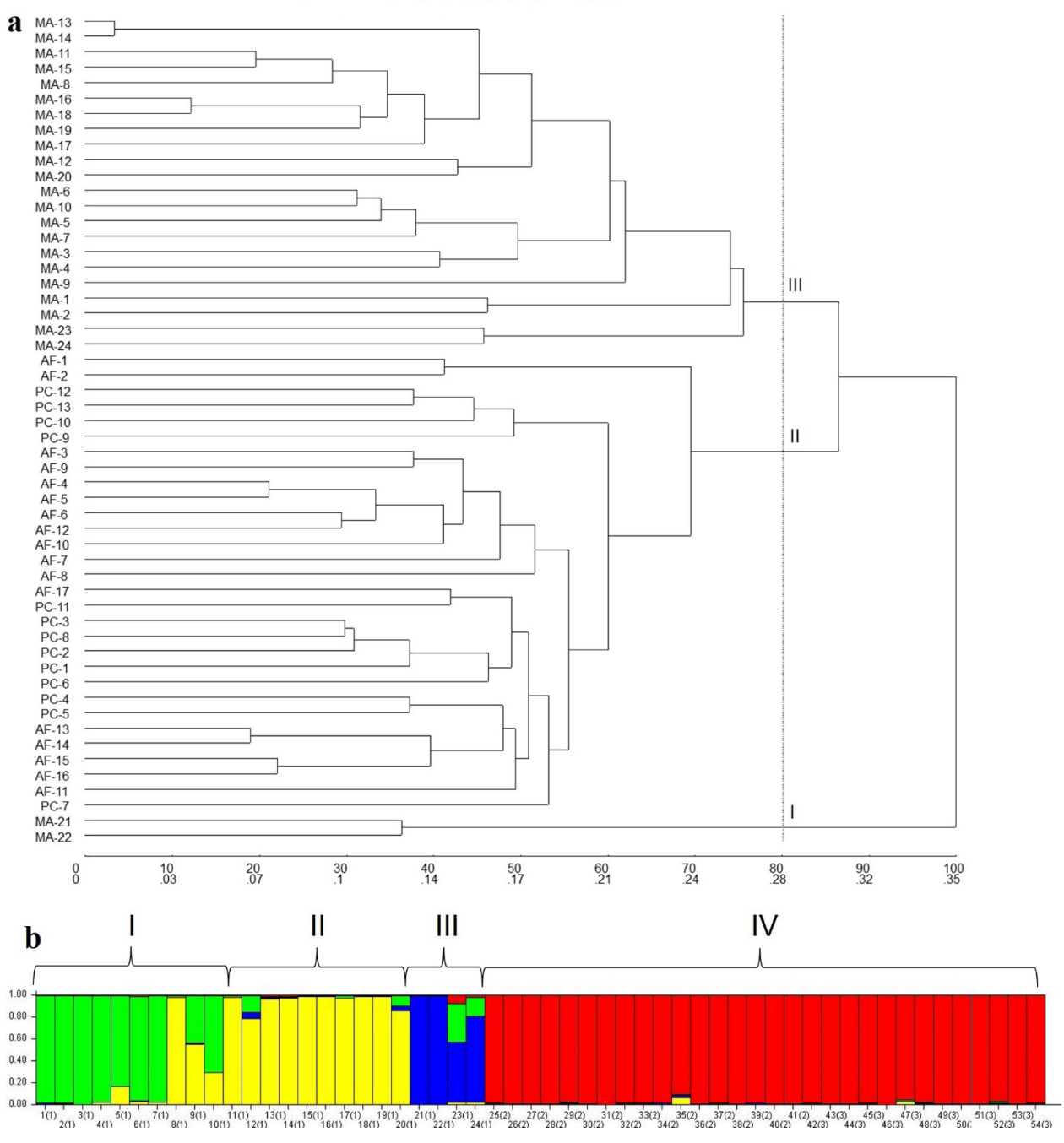

FIGURE 3: Dendrogram of genetic similarity among 54 individuals of *Hymenaea courbaril* defined by UPGMA cluster criteria, based on Jaccard coefficient. cophenetic correlation (CCC): 0.8547; Distortion (%): 1.9807; Stress (%): 14.0771(A) and genetic relationships of three populations of *Hymenaea courbaril*, estimated by the "Structure" based on 10 ISSR markers (B). The vertical lines in (B) indicate the samples and colors represent the allelic frequencies.

FIGURA 3: Dendrograma de similaridade genética entre 54 indivíduos de *Hymenaea courbaril* definidos por critérios de agrupamento UPGMA, com base no coeficiente de Jaccard, correlação cofenética (CCC): 0,8547; Distorção (%): 1,9807; Estresse (%): 14,0771 (A) e relação genética de três populações de *Hymenaea courbaril*, estimados pela estrutura baseada em 10 marcadores ISSR (B). As linhas verticais em (B) indicam as amostras e cores representam as frequ**ências alélicas.**

The Bayesian analysis determines the clusters of individuals based on genetic distinctions, without the need for a pre-identification of the populations. Thus, the clusters of individuals may not correspond to groups of the sample survey, such as occurred in this study. The clusters 1, 2 and 3 of the "Structure"

corresponded to natural population from Marcelândia, while cluster 4 was formed by the populations from Comunidade Central and Pista do Cabeça. By the analysis of the dendogram UPGMA (Figure 3), it is possible to understand the clustering between the populations from AF and PC in the figure of the structure, because the dendogram infers that the genetic structure of these two populations presents greater genetic similarity compared to the population from Marcelândia.

According to the analysis of the three populations by the UPGMA method with a cutoff of 80%, it was observed the formation of three clusters. The cluster I was constituted by two genotypes (MA-21 and MA-22), being that these two genotypes were also isolated in the analysis of the Structure. The Cluster II was formed by genotypes from Comunidade Central and Pista do Cabeça (AF and PC), being this cluster in accordance with the results of the Structure (Figure 3). This fact reinforces that the two populations (PC and AF) have greater genetic similarity. The cluster III was constituted by others 22 genotypes from Marcelândia (MA), while in the Structure, these 22 genotypes were divided in two clusters (Figure 3). Such subdivision of the population from Marcelândia in three clusters by Structure reinforces the fact that this population presents greater genetic diversity.

CONCLUSIONS

There is genetic diversity in the three populations of *Hymenaea courbaril*, being that the population from Marcelândia shows the highest diversity indices. The molecular characterization revealed that the genetic diversity is greater in intrapopulation level than in interpopulation level, but we observed that a genetic isolation is already occurring among the populations more geographically distant. We recommended that individuals be preserved from both populations, in order to ensure the maintenance of genetic variability and the effective conservation of the species in Mato Grosso Amazon.

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