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Genetic diversity of *Plathymenia reticulata* Benth. in fragments of Atlantic Forest in southeastern Brazil

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ABSTRACT. Studies of genetic diversity in natural populations are important for the definition of conservation strategies, especially in populations reduced by processes of fragmentation and continuous forest extraction. Molecular markers stand out as interesting tools for these studies. The objective of this research was to characterize the diversity and genetic structure of *Plathymenia reticulata* (Fabaceae), occurring in two fragments of the Montana Semideciduous Forest in the southern of Espírito Santo State, Brazil, using inter-simple sequence

repeat (ISSR) molecular markers. DNA samples from 149 individuals were analyzed using 10 ISSR primers, generating 156 fragments, of which 101 were polymorphic (64.74%). The individuals sampled were classified into three units: adult trees (A), a mixture of progenies (B), and young regenerating individuals (C). The number of loci used (N = 101) was greater than that established as optimal number (N = 88), indicating precision in analyses. The genetic diversity index of Nei (H' = 0.407) and the Shannon index (I = 0.594) were found to have high genetic diversity. Besides, through the diversity parameters evaluated, it was possible to confirm that in the areas of natural regeneration and progeny mix there is genetic diversity equivalent to that found in adults. The analysis of molecular variance indicated that most of the genetic variation is found within the groups (96.53%). Genetic differentiation among adult trees was low (Φ_{ST} = 0.03) indicating that high gene flow rates (N_m = 12.70) are counteracting the effects of genetic drift. The data obtained allowed to evaluate the potential of adult trees as matrices for seed collection and to obtain seedlings with confirmed genetic variability.

Key words: Matrix trees; Tropical forest; Molecular markers; Genetic variability; "Vinhático"

INTRODUCTION

The biological mechanisms of the species, as the system of reproduction and dispersion, influence the genetic structure of the populations (Loveless and Hamrick, 1984). However, fragmentation of habitats and illegal selective cuts can also alter the composition and genetic structure, leading to modifications within and between natural populations (Debout et al., 2011). Such anthropic activities may lead to the reduction and isolation of populations and individuals, leading to an increase in self-fertilization, correlated crosses, and changes in the dynamics of processes such as gene flow and genetic drift (Dal Bem et al., 2015).

The growing timber demand and population expansion in Brazil have threatened the conservation of ecosystems, resulting in changes in tropical forests, particularly in the Atlantic Forest domain and its associated ecosystems (Peres et al., 2010). In this way, studies in reduced populations have been developed in large scale in search of the conservation of vegetal species and expansion of the forest cover. In this scenario, studies of diversity and genetic structure of fragmented populations, identification of individuals as seed matrices for recovery and restoration of degraded areas, quantification of levels of genetic variation within and between populations, and genetic comparison among adult and regenerating individuals, evidencing the important relationship between the proximity of the forest matrix and areas undergoing regeneration have been published (Higa and Silva, 2006; Rodrigues et al., 2009; Martins et al., 2014; Dal Bem et al., 2015; Pádua et al., 2016).

The tree species *Plathymenia reticulata* Benth., popularly known as "vinhático", belongs to the family Fabaceae (Lacerda et al., 2002). The individuals are diploid (2n = 26), hermaphrodite, with cross-pollination mediated mainly by bees and wasps, and seeds are dispersed by the wind (Warwick and Lewis, 2003). The species presents the potential for economic exploitation, due to its wood of greater quality, and is also used in mixed

plantations in the restoration and recovery of degraded areas (Lacerda et al., 2002). It has a wide distribution in the Brazilian states, being widely distributed in the Atlantic Forest and the Cerrado (Morim, 2015). In the State of Espírito Santo located in Southeastern Brazil, among the natural occurrences of the species the southern region of the State stands out, which in recent decades has undergone intense exploitation and population reduction due to the selective cutting of forest woods and replacement of forests by pasture and cropping. The wood extraction has caused reductions in the populations of *P. reticulata*, one of the reasons for the current classification of the species as vulnerable to extinction (IUCN, 2016).

In this perspective, several molecular tools can be used to analyze the diversity and genetic structure of plant species. The ISSR (inter-simple sequence repeat) molecular markers stand out being widely used because they are universal, of a dominant nature, with low cost, and a good reproducibility (Ng and Tan, 2015).

For the present study, adult individuals of the species distributed along two forest remnants in the south of Espírito Santo, a mixture of progenies obtained from seeds collected in the interior of the remnants and regenerating individuals around the area under natural regeneration, were sampled to evaluate the importance of the proximity of a forest matrix with areas in the process of natural regeneration. Thus, the objective of this study was to characterize the genetic diversity and structure of *P. reticulata* in the southern region of Espírito Santo and to investigate through genetic data, obtained by ISSR markers, the following questions: i) did the exploitation of *P. reticulata*, as a result of selective cutting, influence the genetic diversity indexes of the population? ii) did the regenerants of the species that are developing near a forest matrix and the seedlings originated from seeds of the interior of the forest fragment have a genetic variability similar to the adult trees of the population?

MATERIAL AND METHODS

Study area

The study was carried out in a region originally contemplated by the phytophysiognomy of Montana Semideciduous Forest in the south of the State of Espírito Santo, Brazil (coordinates 20°53' South latitude and 41°42' West longitude) (Martins and Cavararo, 2012).

The sampling areas are composed of two forest fragments and pasture areas nearby in the process of natural regeneration. One of the fragments (approximately 93 hectares) is known as Floresta da Rosal and is located in the Permanent Preservation Area (PPA) of Usina Hidrelétrica Rosal (UHE Rosal), belonging to CEMIG (Companhia Energética de Minas Gerais) (Rezende et al., 2009). The second forest fragment is privately owned and has approximately 110 hectares. The separation between these forest areas is approximately 100 m, and they are interconnected by small forest remnants and pasture areas (Figure 1). In the last two decades, the entire area has undergone a process of selective exploitation of timber resources. During this period, among the tree species existing in the area, the species *P. reticulata* had its wood extensively explored.

Sampling

To characterize the genetic diversity, 149 individuals of *P. reticulata* were identified. Sampling involved individuals in three developmental stages, separated into three sample

units: 67 healthy adult trees and in reproductive age (A), 42 seedlings from seeds collected in the interior of the fragments - mixture of progenies (B), and 40 regenerating individuals in areas of natural regeneration around the fragments (C).

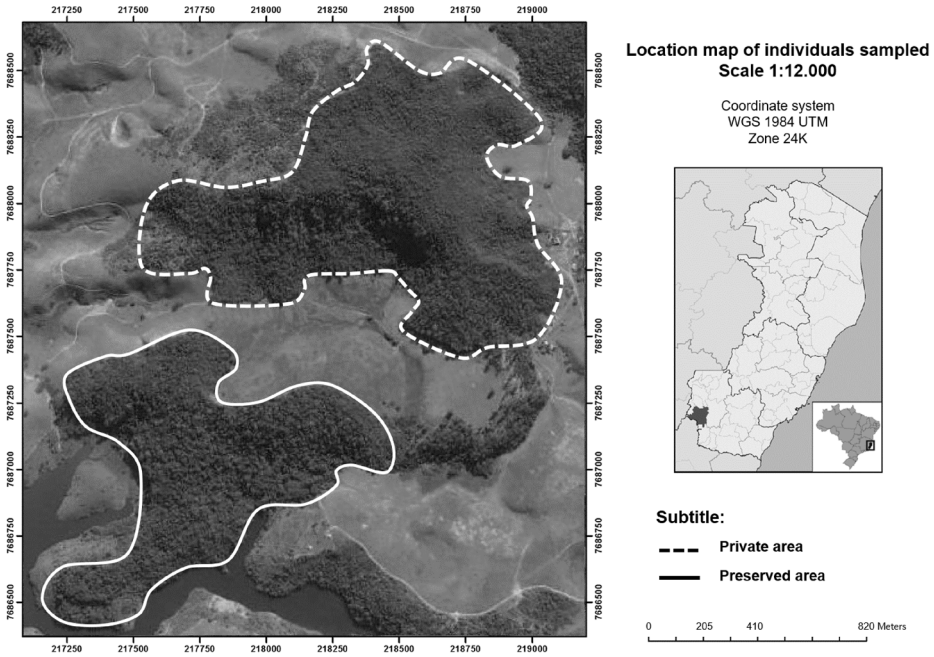

Figure 1. Map of location of the study area evidencing the two forest fragments studied, Espírito Santo, Brazil.

Adult individuals (sample unit A) were georeferenced and assessed densitometrically [height estimated by visual assessment and diameter at breast height (DBH), measured at 1.30 m from the soil with diametric tape]. The adults were sampled in two forest fragments, 19 remaining trees (7 to 35 m high and 15 to 119 cm of DBH, numbered from 1 to 19 for the analyses) located within the Rosal fragment, in the PPA (A1 subunit) and 48 individuals (10 to 40 m high and 15 to 65 cm DBH, numbered 20 to 67 for the analyses) in the particular fragment (A2 subunit). This particular area, of greater extension, has a significant number of adult individuals of *P. reticulata*, besides accessibility for seed collections.

The progeny mixture (sample unit B) was obtained by randomly collecting 360 seeds of 20 adult individuals (of reproductive age) in the two fragments. The seeds were collected from mature fruits after the natural fall in the soil. An approximately similar number of fruits were collected in each tree, the number of seeds being variable for each fruit.

The regenerating individuals (sample unit C) were sampled in three areas of natural regeneration around the fragments, each one being called a subunit of regenerants (C1, C2, and C3, distant each other for approximately 550 m), totaling 40 individuals.

DNA extraction

Leaf samples of the individuals from each sample unit were collected and lyophilized

for the extraction of genomic DNA by the CTAB (cetyltrimethylammonium bromide) method originally proposed by Doyle and Doyle (1990). The concentration and purity of total DNA were estimated by spectrophotometry in the NanoDrop apparatus (Thermo Scientific 2000C).

PCR amplification

Ten ISSR primers developed by the University of British Columbia, Vancouver, Canada, were used for the genetic characterization of *P. reticulata* individuals.

Polymerase chain reactions (PCR) were performed at the Laboratory of Biochemistry and Molecular Biology, Universidade Federal do Espírito Santo, UFES (Brazil). The final volume of the reactions was 20 µL, containing a 1X buffer (10 mM Tris-HCl, pH 8.5, and 50 mM KCl), 2.5 mM $MgCl_2$, 1 mM dNTP, 0.2 mM of each primer, 1 U Taq DNA polymerase, and 50 ng genomic DNA. The amplifications were performed in an Applied Biosystem thermocycler, Veriti model, programmed for an initial stage of denaturation at 94°C for 5 min followed by 35 cycles consisting of 3 steps: 45 s at 94°C, 45 s at 52°C and 90 s at 72°C, and a final extension at 72°C for 7 min.

Amplification products were separated by electrophoresis on 2% agarose gels in 1X TBE (0.089 M Tris, 0.089 M boric acid and 0.002 M EDTA), to 100 V for 5 h. The gels were stained by immersion in ethidium bromide solution (0.50 µg/mL) and subsequently photographed under UV light (ChemiDoc MP Imaging System - Bio Rad). The molecular size of the amplified fragments was estimated with a 100-bp molecular weight marker (Ladder).

Statistical analyses

The electrophoretic profiles visualized on the gels were transformed into binary matrices, assigning values equal to 1 for the presence of band and 0 for the absence. A descriptive analysis of the data was performed, including a total number of bands (TNB), a number of polymorphic bands (NPB), the percentage of polymorphic bands (PPB) per primer, and size variation of generated fragments in base pairs (TBP). The optimum number of polymorphic fragments indicated for studies of genetic diversity with the species *P. reticulata* was estimated using the bootstrap analysis, obtaining the correlation (r) estimates and the stress value (E) as described by Kruskal (1964).

Genetic dissimilarity indexes based on the complementarity of the Jaccard coefficient were used to estimate the genetic comparison between pairs of adult individuals at the two sampling locations. The number of genetic groupings was determined by hierarchical method UPGMA (unweighted pair group method with arithmetic mean). The cut-off point (Pc) for determining the number of groups formed was established by the statistical criterion proposed by Mojema (1977), by the formula Pc = m + KSD, where m = the mean distance values of the melting levels corresponding to the stages; K = 1.25; SD = standard deviation. The fit between the distance matrix and the dendrogram was verified by the cophenetic correlation coefficient (r). All analyses described above were performed with the aid of the Genes software (Cruz, 2013).

Molecular genetic diversity was estimated using the Popgene 1.32 software (Yeh and Boyle, 1997), assuming for calculations that the loci were in Hardy-Weinberg equilibrium. The following diversity parameters were estimated: percentage of polymorphic loci (PPL), genetic Nei's index of diversity (H') (Nei, 1973), and Shannon index (*I*) (Shannon and Weaver, 1949).

Analysis of molecular variance (AMOVA) was calculated according to Excoffier et al. (1992), to reveal the genetic diversity distribution within and between sample units, with the aid of the Arlequin software, version 3.11. The historical gene flow among the adult trees was estimated indirectly from the estimates of genetic divergence between groups (G_{ST}), as a function of N_m, by the formula $N_m = 0.5 (1 - G_{ST}) / G_{ST}$ (McDermott and McDonald, 1993) using the Popgene 1.32 program (Yeh and Boyle, 1997).

Bayesian analysis with the STRUCTURE 2.3 software (Pritchard et al., 2000) was used to infer the number of genetic groups (K) in which the genotypes are structured. To verify the most probable number of groups, K values ranging from K = 1 to K = 9 were tested. Twenty races were performed for each K value, with 1,000,000 Monte Carlo interactions via Markov chains (MCMC), with an initial burn-in of 250,000 interactions. The most probable K value was determined by the ΔK method proposed by Evanno et al. (2005), using the STRUCTURE HARVESTER software (Earl and Vonholdt, 2012). After the selection of the best K, a consensus of the 20 interactions was made by the Clumpp software (Jakobsson and Rosenberg, 2007) and then the graphic result was obtained by the Distruct software (Rosenberg, 2004).

RESULTS AND DISCUSSION

The ten ISSR primers used to genotype the 149 individuals of *P. reticulata* sampled, produced a total of 156 fragments, being 101 polymorphic (64.74% of polymorphism) (Table 1). The highest NPB was observed in the primer UBC 818 (88.23%) and the lowest in primer UBC 855 (28.57%).

Table 1. ISSR primers selected for *Plathymenia reticulata* presenting sequences (5'-3') of each primer, total number of amplified bands (TNB) per primer, ISSR (including all sample units), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), size variation of the fragments generated in base pairs (TBP) established based on a marker of 100 bp.

Primer	Sequences (5'-3')*	TNB	NPB	PPB (%)	TBP (max-min)
UBC 807	AGA GAG AGA GAG AGA GT	21	16	76.19	2080-250
UBC 810	GAG AGA GAG AGA GAG AT	9	6	66.66	1000-400
UBC 812	GAG AGA GAG AGA GAG AA	22	13	59.09	2000-300
UBC 818	CAC ACA CAC ACA CAC AG	17	15	88.23	1000-400
UBC 827	ACA CAC ACA CAC ACA CG	19	9	47.36	1900-450
UBC 834	AGA GAG AGA GAG AGA GYT	17	14	82.35	1400-250
UBC 840	GAG AGA GAG AGA GAG AYT	14	11	78.57	1000-250
UBC 842	GAG AGA GAG AGA GAG AYG	13	9	69.23	900-350
UBC 855	ACA CAC ACA CAC ACA CYT	14	4	28.57	1500-500
UBC 868	GAA GAA GAA GAA GAA GAA	10	4	40.00	1000-500
Total		156	101	64.74	-

*A = Adenine; T = Thymine; C = Cytosine; Guanine; Y = (C or T). UBC: ISSR primers, developed by the University of British Columbia, Vancouver, Canada.

The molecular markers ISSR used in this study showed efficiency in detecting polymorphism in *P. reticulata*, revealing a greater genetic variability identified by the high PPB. The results are similar to those found by other authors in genetic analyses using dominant markers in tree species. Analyzing the genetic diversity of *Bertholletia excelsa*, Ramalho et al. (2016) used eight ISSR primers, obtaining 52 fragments being 46 polymorphic. In *Theobroma grandiflorum*, 15 ISSR primers were selected generating 102 amplified fragments, of which 53 were polymorphic (52% polymorphism) (Silva et al., 2016).

The optimal number of loci for the reliable characterization of genetic diversity in the present study was from 88 polymorphic fragments when correlation (r) reached 0.98 and stress (E) assumed a value of 0.04 (Figure 2). Values of E < 0.5 and correlation closer to 1 are pointed out by Kruskal (1964) as indicative of precision of the estimates. In this study, 101 polymorphic loci were found for *P. reticulata*, a number above that established as optimal (N = 88), revealing that the number of primers used and the number of fragments obtained were sufficient and accurate for genetic diversity analysis of the species.

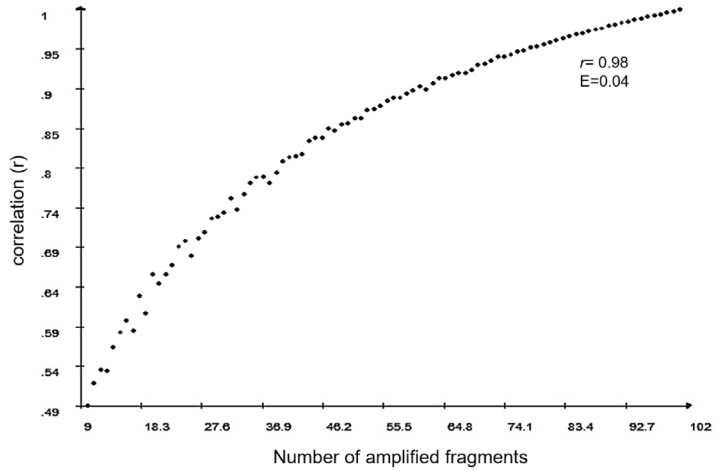

Figure 2. Correlation coefficient (r) obtained by the bootstrap analysis and the number of amplified fragments for the 149 individuals of *Plathymenia reticulata* using 10 ISSR primers.

The genetic dissimilarity matrix obtained by the complementarity of the Jaccard coefficient, revealed variability among adult individuals (unit A) of *P. reticulata*, with the formation of nine groups and a cut-off point of 85.23% (Figure 3).

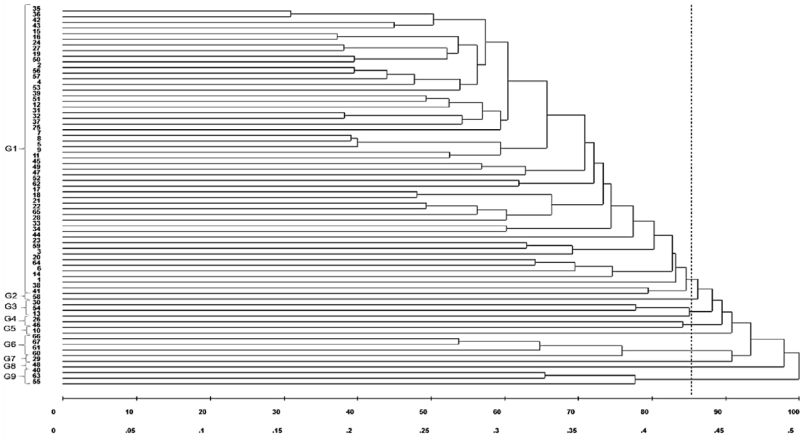

Figure 3. Dendrogram obtained by the UPGMA method representing genetic dissimilarity among the 67 adult *Plathymenia reticulata* individuals, forming nine groups. Numbers from 1 to 19 correspond to the individuals of the A1 subunit and from 20 to 67, of the A2 subunit. Cutting point (Pc): 85.23%.

Dissimilarity values ranged from 0.15 to 0.64, with a mean of 0.40, with the smallest genetic distances being found between pairs of subjects 35 x 36 and the largest distances between 25 x 55. The value of the cophenetic correlation coefficient (r) was 0.808, which revealed a fit between the graphical representations and the original genetic distance matrix that is, the dendrogram showed a true representation of the original data. The heterogeneity of the samples, evidenced by the distribution of the genotypes in different groups, can be explored in future forest restoration and recovery programs that wish to obtain seed lots with maximum genetic variability. Dissimilarity indices can be used to select the most diverse individuals for this purpose.

The genetic diversity parameters calculated for *P. reticulata* reveal high diversity in the species (Table 2).

Table 2. Genetic diversity of *Plathymenia reticulata*.

Sample units	N	PPL (%)	H'	*I*
Unit A	67	64.74	0.419 ± 0.096	0.605 ± 0.108
Subunit A1	19	58.97	0.380 ± 0.152	0.548 ± 0.206
Subunit A2	48	63.46	0.416 ± 0.107	0.600 ± 0.133
Unit B	42	60.26	0.348 ± 0.146	0.515 ± 0.192
Unit C	40	64.74	0.383 ± 0.120	0.563 ± 0.142
Subunit C1	23	60.26	0.379 ± 0.144	0.550 ± 0.192
Subunit C2	9	50.64	0.310 ± 0.195	0.452 ± 0.269
Subunit C3	8	48.72	0.287 ± 0.199	0.421 ± 0.276
Species	149	64.74	0.407 ± 0.089	0.594 ± 0.099

Sample size (N), percentage of polymorphic loci (PPL), Nei index (H'), Shannon index (*I*). Data are reported as means ± standard deviation.

The C2 and C3 subunits had the lowest PPL (C2 = 50.64% and C3 = 48.72%). These subunits of regenerants also presented the lowest values for the other parameters of genetic diversity. Considering that the parameters analyzed are sensitive to the sampling effect (Hmeljevski et al., 2011), the low values found may be associated with the sample size, since the C2 and C3 subunits have the smallest number of individuals sampled.

The H' of sample units varied from 0.287 to 0.419 and *I* from 0.421 to 0.605. For the species, 0.407 was obtained for the H' and 0.594 for *I*, values considered satisfactory for *P. reticulata*. According to the literature, the Shannon index can vary between 0 and 1, with 1 being the maximum genetic diversity of a population (Lewontin, 1972). The value found (0.594) suggests that *P. reticulata* presents high levels of genetic variation. These results may be related to the ecological aspects of the species such as anemochorous dispersion and cross-fertilization (Lacerda et al., 2002), which according to Loveless and Hamrick (1984) are factors that increase intrapopulation genetic diversity.

The genetic diversity found in the individuals present in the protected area was H' = 0.380 and *I* = 0.548, and in those sampled in the particular area was H' = 0.416 and *I* = 0.600. The data emphasize the importance of conservation and sustainable use of the species so that such diversity is not lost through possible anthropogenic actions. Besides, the results indicate that even with recent wood extraction in the study area, genetic diversity in the *P. reticulata* population remains high. The selective logging occurred in the area did not cause significant genetic erosion, because the seed bank present in the soil is possibly allowing the regeneration of the individuals, ensuring the maintenance of the genetic variability of the species.

For the mixture of progenies (unit B) and natural regenerants (unit C) significant values

of genetic diversity were also identified. The analyses carried out on the mixture of progenies indicate the genetic variation that can be found in future batches of seedlings produced from the seeds collected in the trees present in the studied fragments. Considering that there is an important relationship between the proximity of the forest matrix and areas in the process of regeneration (Martins et al., 2014), the results indicate that the geographic proximity to the forest fragment is important for the maintenance of three regeneration areas (average of 200 m in distance), collaborating in this way, so that the regenerating individuals maintain a genetic diversity equivalent to the one found between the adults. Furthermore, the species has its seeds dispersed by the wind, which increases the chance of the propagules being disseminated around the whole fragment, explaining the result obtained. Therefore, the data indicate that the regenerants, as well as the mixture of progenies of *P. reticulata*, have genetic variability similar to the adult trees sampled. The data also reveal the potential of adult individuals to be used as matrices for seed collection.

The partition of genetic variation revealed greater variation within the groups than among them (Table 3).

Table 3. Analysis of molecular variance of the general population (species) and among sample units of *Plathymenia reticulata*.

Groups	Sources of variation (%)		Statistics F
	Between groups	Within groups	Φ_{ST}
Adults (A1 x A2)	3.4661	96.5338	0.0346
Regenerants (C1 x C2 x C3)	9.9698	90.0301	0.0997
Adults x Regenerants	6.8345	93.1654	0.0683
Adults x Mixture of progenies	7.6907	92.3092	0.0769
Regenerants x Mixture of progenies	9.5317	90.4682	0.0953
Species	8.0361	91.9638	0.0803

In the total population (species), including all sample units, 91.96% of the genetic variation was identified within the groups, and only 8.03% of the variation was observed between them. The regenerants and the mixture of progenies are derived, for the most part, from the cross between the sampled adults, which may be contributing to the greater variation observed within the groups. Moreover, the data reveal that the historical genetic variation existing in adults has been maintained in the generations (Martins et al., 2008).

AMOVA was also performed considering the comparison between the sample units of each category. According to Wright (1978), the data reveal low differentiation between adults (A1 x A2, $\Phi_{ST} = 0.034$) and moderate differentiation for the other comparisons: regenerating (C1 x C2 x C3, $\Phi_{ST} = 0.099$), adults x regenerants ($\Phi_{ST} = 0.068$), adults x mixture of progenies ($\Phi_{ST} = 0.076$), and regenerants x mixture of progenies ($\Phi_{ST} = 0.095$).

The low genetic differentiation identified among the groups of adult trees with the highest proportion of variation observed within the groups (96.53%) may be mainly due to the allogamous character of the species. According to Hamrick and Godt (1996), tree species, preferably allogamous, such as *P. reticulata*, have high diversity within populations and less differentiation between them. Besides, the low genetic differentiation between the A1 x A2 groups indicates that there is practically no reproductive subdivision between the sampled locations; the whole area represents a single deme that constitutes a single group of individuals, near enough to reproduce among themselves (Templeton, 2011). Thus, the low value of Φ_{ST} shows that the trees are not genetically isolated due to the occurrence of gene

flow. In this study, the historical gene flow calculated indirectly based on the estimator of genetic divergence between groups (G_{ST} = 0.0379), presented N_m equal to 12.70. According to Wright (1951), a value of $N_m > 1$ is sufficient for that gene flow to override the effects of genetic drift, avoiding differentiation between groups. The low value of G_{ST} and the high N_m value found are related to the low genetic divergence between the adult individuals at the two collection sites and allow to affirm that the forest extraction occurred in the study area was not effective to cause evident genetic differentiation.

Moderate genetic differentiation was found for adults x mixture of progenies, adults x regenerants, and regenerants x mixture of progenies, which may be associated with the contribution of differentiated individuals to each of the groups. The moderate differentiation observed between adults and the mixture of progenies may be related to the genetic contribution of pollen grains from trees that were not sampled, mainly adults located in the particular area where not all the trees were demarcated.

Similarly, in adult x regenerants a moderate differentiation was observed, which reinforces the idea that non-sampled individuals are contributing to the genetic patrimony of these individuals. There is also the possibility that some of the adult individuals are contributing to the genetic heritage in a differentiated way. In this perspective, even if the anemochorous dispersion of the species allows the propagules to reach the surroundings of the whole fragment, trees closer to each area may be contributing as a source of seeds, more expressively (Guariguata and Ostertag, 2001). This perception can be confirmed by the value of Φ_{ST} found for the analysis between the three regeneration areas C1 x C2 x C3 (Φ_{ST} = 0.099), the highest value found in the present study. The result suggests that few individuals, which do not represent the total genetic patrimony, are contributing in a differentiated way to the genetic constitution of each one in the regeneration areas. This result can be explained by the founder effect that consists of the contribution of few individuals in the colonization of a new area, in which such individuals have a limited sample of the genetic variation present in the original population (Mayr, 1963).

The result of the Bayesian approach indicated that the population is structured in two groups (best K = 2) (Figure 4). The colors assigned in the analysis reveal the proportion of ancestry of the genotypes and indicate that the genetic characteristics are being shared between the individuals of each sample unit (Pritchard et al., 2000). Regarding genome, the C1 area is having differentiated contribution about one of the groups when compared to the areas C2 and C3. Therefore, the data allow confirming the occurrence of the founder effect and the moderate differentiation found between the regeneration areas.

Through the results obtained, it is possible to affirm that there is high genetic diversity present in the population. The analyzed adult individuals showed potential for seed collection because they retain a satisfactory genetic diversity. Besides, it was confirmed the importance of the proximity of the forest matrix in the maintenance of the regeneration areas, since between the natural regenerants and the progeny mixture there is genetic diversity equivalent to that found in adults, which indicates that genetic variability is being maintained in the species. Thus, it is possible to certify the genetic variation that can be found in future lots of seedlings obtained from the collection of seeds in trees present in the forest remnants studied. The results also indicated that the fragments are important sources of genetic variability, and considering that there is no reproductive subdivision among the adults in the two localities, it can be affirmed that the occurrence of fragmentation and extractivism did not cause isolation and differentiation of the individuals.

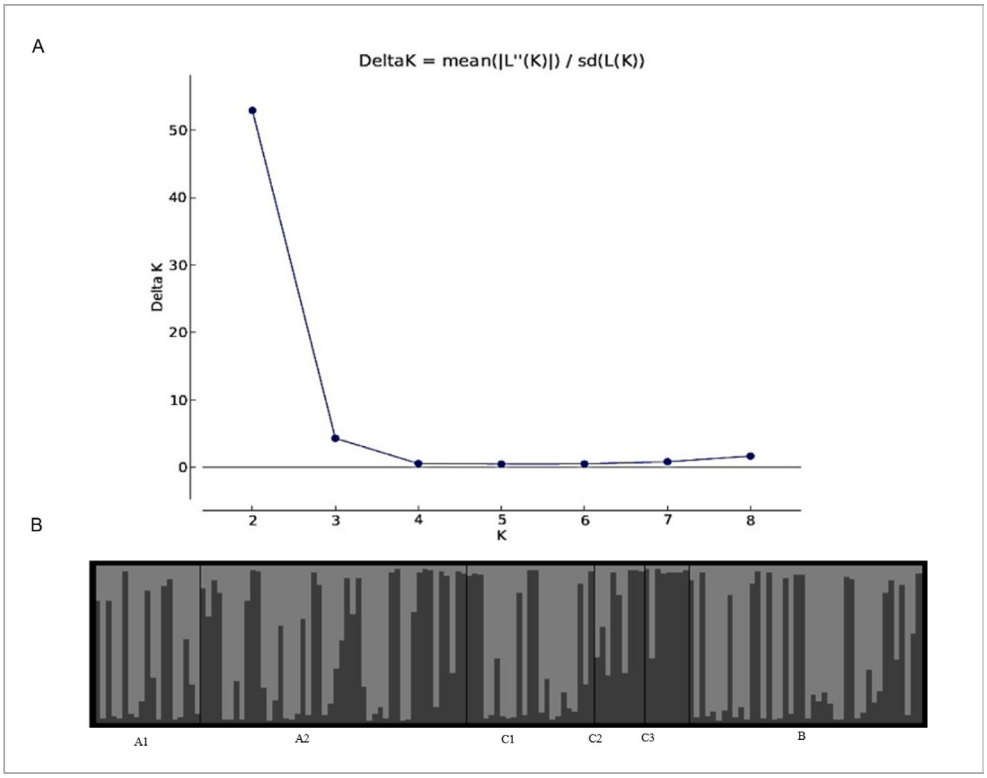

Figure 4. Bayesian approach (software STRUCTURE) of 149 individuals of *Plathymenia reticulata*. **A.** Graph of the values of ΔK for each value of K. Best K = 2. **B.** Bar chart identifying the number of clusters about sample units. The letters A1, A2, C1, C2, C3, and B refer to the sample units and subunits evaluated.

CONCLUSION

ISSR markers were efficient in detecting polymorphism and in quantifying genetic diversity in *P. reticulata*. The studied population presents high genetic diversity that remains in the species, considering adults, a mixture of progenies, and young regenerants. Thus, despite the recent extraction scenario, no genetic erosion was observed.

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