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Molecular and quantitative genetic analysis of the neotropical tree *Jacaranda micrantha* Cham.

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SILVICULTURE

ABSTRACT

Backgrounds: Urban and peri-urban fragments are vital for biodiversity conservation, requiring genetic assessment of tree species in fragmented forests. The aim of this study was to analyze the genetic variability and diversity for adult individuals of *J. micrantha* along an urban-rural gradient in the Araucaria Forest. Fifteen individuals were sampled, with five from each remaining forest type. Initially, 10 ISRR primers were tested. Five mother trees were chosen from each site (urban, peri-urban, and rural) with a minimum distance of 100 m. The experimental design was a RCBD with 15 progenies, three provenances, three blocks, and 20 plants per plot, totaling 900 seedlings.

Results: The average percentage of polymorphic loci was 93.33%. The urban population showed a greater loss of genetic diversity (H=0.1806). 79% of the genetic diversity was found within populations. The observed gene flow value (Nm) was 1.8790, indicating that there were no random losses of alleles within populations. The fragments did not exhibit significant differences, but there were significant differences among the progenies. The stem diameter (SD) and the height-diameter relationship (H/SD) emerged as the key traits for selecting new individuals due to their higher heritability (< 0.50), accuracy (< 0.70), and relative coefficient of variation (< 7%).

Conclusion: The urban fragment is the most affected, but gene flow between fragments prevents the random loss of alleles. The analysis suggests that these fragments form a unique population, despite geographic barriers. Thus, the three fragments can be considered when choosing superior individuals for future progeny tests in genetic improvement programs for the species.

Keywords: Caroba; Progeny test; Genetic parameters; Genetic conservation.

HIGHLIGHTS

There is genetic variability among *J. micrantha* trees for the growth variables evaluated.
Environmental changes caused by urbanization did not have a significant effect on the genetic variability of *J. micrantha* populations.
Genetic diversity was lower in the population developed in urbanized areas.
The species of *J. micrantha* studied did not show reproductive isolation, showing that the fragments form a single population.

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INTRODUCTION

Intense human activity and the advancement of the urbanization process are the main factors responsible for the fragmentation of natural forests and the isolation of remnants. Fragmentation affects the structure and function of remaining forest fragments and generally has a negative impact on the genetic variability of populations and ecological and evolutionary processes (González et al., 2020 and Rezende et al., 2018). Studies have shown that fragmentation generally leads to losses in genetic diversity and increases in inbreeding in forest species due to restricted gene flow between populations (Browne and Karubian, 2018; Butcher et al., 2005; Lowe et al., 2005, 2015; Ofori et al., 2022; Schlaepfer et al.; 2018; Toczydlowski and Waller, 2019; Wang et al., 2011; White et al., 1999 and Young et al., 1993, 1996). These impacts are even more pronounced in species that have limited mobility dispersers and pollinators, which hinders the dispersal of pollen and seeds over long distances (Lander et al.; 2010; Lowe et al.; 2015 and Sujii et al., 2021). Furthermore, the genetic structure of populations is altered, affecting growth, reproductive traits, seed production, as well as the viability and vigor of plant offspring (Aguilar et al., 2019; Lowe et al., 2015 and Rey et al., 2017).

The presence of natural fragments within urban or peri-urban areas is crucial for biodiversity conservation and the provision of essential ecosystem services to humanity (Ribeiro et al., 2022 and Zhang et al., 2019). Small remnants, such as riparian corridors in urban environments, have the potential to maintain natural habitats and support the existence of diverse species (Barbosa et al., 2017 and Valente et al., 2017). The conservation of tree species in fragmented forest areas requires investigation not only into the quantity but also the genetic quality and adaptability of offspring. This is essential for assessing species viability, as fragmentation can impact the genetic structure of plants (Aguilar et al., 2019; Lowe et al., 2015 and Rey et al., 2017).

In this context, estimating genetic parameters in forest fragments is crucial for supporting, including the selection of seed trees for both conservation projects and breeding programs of native species (Costa et al., 2012; Homczinski et al., 2022; Kampa et al., 2020 and Mendes et al., 2020). The magnitude of genetic variability within a species can be assessed through the estimation of quantitative genetics parameters such as heritability, genetic and phenotypic correlations, and selection gain (Vencovsky and Barriga, 1992). On the other hand, levels of genetic diversity at the DNA level, both between and within populations and individuals, can be accessed through the analysis of molecular markers. One widely used marker type is Inter Simple Sequence Repeat (ISSR), a dominant inheritance marker, which remains popular due to its high polymorphism level, requiring small amounts of DNA per reaction, high reproducibility, low cost, and not requiring prior knowledge of DNA sequences (Chen et al., 2017; Samarina et al., 2021 and Sheikh et al., 2021).

The native species *Jacaranda micrantha* Cham., commonly known as "caroba" and belonging to the family Bignoniaceae, is an important representative of the Araucaria Forest ecosystem. It is a large deciduous tree, ranging in height from 10 to 35 m, with a trunk diameter of 40 to 60 cm. Its crown tends to be sparse and globular (Lorenzi, 2009 and Saueressig, 2014). The species occurs naturally in northeastern Argentina, eastern Paraguay, and the states of Goiás, Minas Gerais, Rio de Janeiro, São Paulo, and the southern region of Brazil (Lorenzi, 2009). Additionally, it has great landscape potential due to its beautiful flowering, making it suitable for afforestation in extensive areas and for planting aimed at the restoration of degraded areas, in addition to its ecological importance (Lorenzi, 2009 and Saueressig, 2014).

However, no genetic studies have been conducted on this species so far, especially regarding the effects of fragmentation on the genetic structure of natural populations of *J. micrantha*. Given this knowledge gap, the present study aims to evaluate the evolutionary potential of plant populations of *J. micrantha* that remain in urban, periurban and rural remnants, their adaptability to constantly changing environments, and consequently their ability to ensure species conservation. Therefore, the aim of this study was to analyze the genetic variability of juvenile traits and the genetic diversity of adult individuals of *J. micrantha* along an urban-rural gradient in the Araucaria Forest.

MATERIAL AND METHODS

Description of the Study Area

The collection of *J. micrantha* seeds was carried out in different fragments of the Araucaria Forest (AF): urban, peri-urban and rural. The urban and peri-urban areas are located in Irati county, Parana state, under the geographic coordinates 25º 28' 02" S and 50º 39' 04" W, and in the rural area of Palmeira County, Parana state, under the geographic coordinates 25º 25' 46" S and 50º 00' 23" W (Figure 1). The urban fragment was characterized as a forest remnant in the urban perimeter of the county, while the periurban fragment is a forest remnant located at a minimum distance of 500 meters from the urban perimeter of the county. The rural fragment corresponds to a non-urbanized forest remnant, destined to agricultural and livestock activities, extractivism, rural tourism, forestry and environmental conservation. These areas have similar altitudes, ranging from 812 m in Irati to 867 m in Palmeira. The relief of these places is formed by hills and mountains, the climate of the region is characterized as Cfb, subtropical, humid, mesothermal, with cool summers, with the occurrence of severe and frequent frosts and without a dry season (ICMBIO, 2013).

ISSR (*Inter Simple Sequence Repeat*) genetic diversity

Fifteen individuals were sampled, with five from each remaining forest type (urban, peri-urban, and rural). Leaf samples were collected and stored in 2 mL plastic tubes containing 2% CTAB (cationic hexadecyl trimethylammonium bromide) and kept in a freezer at -20 ºC until DNA extraction. The modified protocol of Doyle and Doyle (1987) was used for DNA extraction. Initially, 10 primers obtained from the protocols of Wolfe (2005) were tested (Table 1).

For the optimization of PCR reactions, a cocktail was used containing 10% (v/v) buffer (Invitrogen™) consisting of 20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% (v/v) glycerol; 2.0 and 2.33 mM $MgCl_2$ (Invitrogen™); 0.2 mM dNTPs (Ludwig Biotec); 1 – 1.5 µl (at 10 pmol) of each primer; 1 unit of Taq DNA polymerase (Invitrogen™), and 1 µl of DNA. The reactions were performed in a Multigene Thermal Cycler (Labnet International, Inc.), from the following amplification conditions: 94 °C for 90 sec, followed by 35 amplification cycles, with each cycle subjecting the samples to 94 °C for 40 sec, annealing at 46 to 52 °C for 45 sec, and extension at 72 °C for 90 sec; 94 °C for 45 sec; 44 °C for 45 sec, with a final extension at 72 °C for 5 min.

The resulting amplification products were subjected to agarose gel electrophoresis with a concentration of 2% in 1X TBE buffer, for 135 minutes at 90 V. To assist in band analysis, 3 µg of labeled 100 bp DNA ladder (Invitrogen™) were used. Additionally, previously successfully amplified samples were used as controls to obtain a robust and unambiguous band pattern.

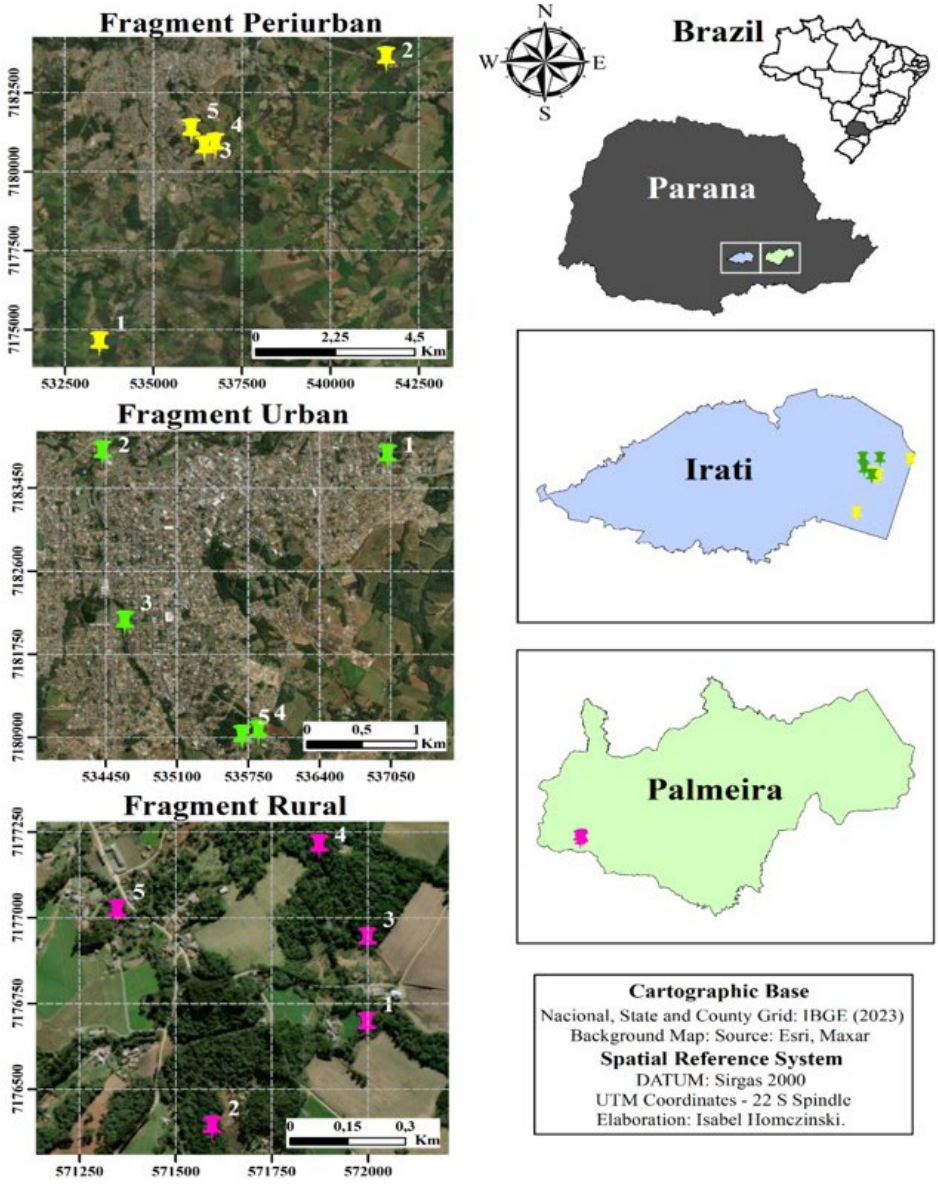

Figure 1: Geographic location of *Jacaranda micrantha* seed collection in the county of Irati and Palmeira - Pr.

Table 1: ISSR primers used in DNA amplification, primer sequene and size of the amplified fragments from three populations and 15 genotypes of *Jacaranda micrantha* collected in the county of Irati and Palmeira – state of Parana, Brazil.

Primer type	Primer sequence (5' – 3')	Size range bp
P-807	$(AG)_8T$	600-900
P-808	$(AG)_8C$	600-1000
P-827	$(AC)_8G$	400-900
P-834	$(AG)_8YT$	300-1000
P-835	$(AG)_8YC$	300-900
P-848	$(CA)_8RG$	600-700
P-855	$(AC)_8YT$	400-1000
P-857	$(AC)_8YG$	600-1000
P-866	$(CTC)_6GT$	500-900
P-873	$(GACA)_4$	300-900

* Y = (C, T); R = (A, G); H = (A, C, T); B = (C, G, T); V = (A, C, G); D = (A, G, T).

The gel was subsequently photographed under ultraviolet light using the Syngene photo documentation system (Synoptics Ltda). For statistical analysis of the data, only robust and unambiguous bands were considered, while bands with low intensity or overlapping with other bands were discarded. The analysis of the agarose gel photographs resulted in binary matrices indicating the presence (1) or absence (0) of dominance. From the gel genotyping, a binary matrix was generated indicating the presence (1) or absence (0) of band fragments, which was used to calculate estimates of genetic diversity. Descriptive analyses of the data, including the total number of bands, number of polymorphic bands, number of monomorphic bands, and percentage of polymorphic bands, were also performed.

The analysis of genetic diversity was conducted using the software POPGENE (Population Genetic Analysis), version 1.3 Yeh et al. (1997). The parameters used for analysis included the number of observed alleles (*na*), effective number of alleles (*ne*), Nei's genetic diversity (*H*) (Nei, 1978), and Shannon's index (*I*). Additionally, total genetic diversity (*Ht*), genetic diversity within populations (H_s), genetic differentiation coefficient (G_{st}), and the effective gene flow between populations (N_m) were also calculated. Analysis of molecular variance (AMOVA) was performed using the GenAlEx V6.5 software (Peakall and Smouse, 2006).

The genetic dissimilarity matrix among the evaluated individuals was calculated using the Russel e Rao coefficient based on the binary matrix. The genetic dissimilarities were used for cluster analysis using the UPGMA (Unweighted Pair-Group Method Average) method, implemented in the R software (R Core Team, 2023) through the *"MultivariateAnalysis"* package (Azevedo, 2021). The coefficient of cophenetic correlation (CCC) was calculated between the genetic dissimilarity matrix and the cophenetic values matrix to assess the consistency of the clustering. CCC values above 0.8 indicate good representativeness between the distances (Bussab et al., 2015).

Genetic variability

For seed collection, five seed trees were selected in each of the collection sites (forest remnant: urban, peri-urban and rural), with a minimum distance between the matrices of 100 m, to avoid related individuals (Sebbenn, 2003). For the selection of seed trees, a phenotypic evaluation was carried out considering the height and diameter of the trunk, phytosanitary aspects and vigor, with the aim of selecting healthy individuals with great seed production capacity.

The fruits were collected in February, as soon as they started to discolor and open spontaneously. They were then sun-dried to ensure complete seed release, and the seeds were identified and separated at the matrix level for later sowing (Saueressig, 2014). The sowing was performed in 120 cm^3 plastic tubes containing Mecplant® substrate and 4 g Kg^{-1} of Osmocote® 14-14-14 Classic slow-release fertilizer (NPK - nitrogen, phosphorus, and potassium), with one seed per tube. The tubes were placed in a greenhouse for 30 days at 25 ± 4 ºC, relative humidity ≥ 80%, and intermittent mist irrigation system. After this period, they were transferred to a shade house where they remained until evaluation at 150 days.

The experimental design used was a randomized complete block design (RCBD) with 15 treatments (progeny) and three provenances, with five progenies from each provenance (forest remnants: urban, peri-urban, and rural), three blocks, and 20 plants per plot, totaling 900 seedlings. The evaluated variables were: stem diameter (SD, mm), shoot height (H, cm), total height (TH, cm), and the H/SD ratio. The relationships between variables (H/SD) were determined by simple division.

The genetic parameters were analyzed using the statistical package lme4 (Bates et al., 2015) in the R statistical environment (R Core Team, 2023). The analysis of quantitative traits considered the block effects as fixed and progeny and provenance effects as random, using two mixed statistical models. The first model assessed at the provenance level (1), and the second at the progeny level (2). Where: *b*= fixed effect associated with the block; *t* = random effect of the progeny; *bt*= interaction between block and progeny; *p*= random effect associated with the provenance; *e* = residual error.

$$y = Xb + Zt + Wbt + Sp + e, \quad (1)$$

$$y = Xb + Zt + Wbt + e, \quad (2)$$

The significance of the model effects was verified using the Likelihood Ratio Test (LRT) with a chi-square (χ^2) test at a 5% level of significance in the R statistical

environment (R Core Team, 2023) using the "lmerTest" package (Kuznetsova et al., 2017). The estimates of the genetic parameters were obtained at the progeny level, meaning that the analysis considered the combined data from the evaluated provenances (urban, peri-urban, and rural) since there was no significant difference at the provenance level.

For the estimation of variance components, the Restricted Maximum Likelihood (REML) method was used. The estimated variance components were: $\hat{\sigma}_a^2$: additive variance; $\hat{\sigma}_g^2$: genetic variance; $\hat{\sigma}_e^2$: environmental variance; $\hat{\sigma}_d^2$: within-progeny error variance. The estimated genetic parameters were the heritability at the individual plant level $\left(\hat{h}_a^2\right)$ (3), average heritability within progenies $\left(\hat{h}_d^2\right)$ (4), average heritability among progenies $\left(\hat{h}_m^2\right)$ (5), individual additive genetic coefficient of variation $\left(C\hat{V}_{gi}\right)$ (6), coefficient of genetic variation among progenies $\left(C\hat{V}_{gp}\right)$ (7), environmental coefficient of variation $\left(C\hat{V}_e\right)$ (8) and the relative coefficient of variation $\left(\hat{b}\right)$ (9). Additionally, to support the selection of the best progenies, the accuracy in progeny selection $\left(\hat{r}_{aa}\right)$ (10), was estimated following Vencovsky and Barriga (1992). Where: $\hat{\sigma}_g^2$= is the genetic variance; $\hat{\sigma}_e^2$= is the environmental variance; $\hat{\sigma}_d^2$= is the variance within progenies; b = is the number of repetitions/block; n: is the number of individuals/plot; $\overline{x}$ = is the average of progenies.

$$\hat{h}_a^2 = \frac{4\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2 + \hat{\sigma}_d^2} \quad (3)$$

$$\hat{h}_d^2 = \frac{3\hat{\sigma}_g^2}{\hat{\sigma}_d^2} \quad (4)$$

$$\hat{h}_m^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2 / b + \hat{\sigma}_d^2 / nb'} \quad (5)$$

$$C\hat{V}_{gi}(\%) = \frac{\sqrt{4\hat{\sigma}_g^2}}{\overline{x}}.100 \quad (6)$$

$$C\hat{V}_{gp}(\%) = \frac{\sqrt{\hat{\sigma}_g^2}}{\overline{x}}.100 \quad (7)$$

$$C\hat{V}_e(\%) = \frac{\sqrt{\hat{\sigma}_d^2}}{\overline{x}}.100 \quad (8)$$

$$\hat{b} = \frac{C\hat{V}_{gp}}{C\hat{V}_e} \quad (9)$$

$$\hat{r}_{aa} = \sqrt{\hat{h}_a^2} \quad (10)$$

RESULTS

Genetic diversity

The 10 primers produced a total amplification of 45 bands, ranging from 300-1000 bp. The average number of bands per primer ranged from 02 (P-848) to 06 (P-835), with an average of 4.5 per primer. Out of the 45 amplified bands, 42 were polymorphic, and only 03 bands were of monomorphic nature, of which only primers P-807, P-827, and P-866 exhibited monomorphic bands. Thus, the percentage of polymorphic loci ranged from 75% to 100% with an average value of 93.33% (Table 2).

Table 2: Descriptive analysis of the 10 ISSR primer amplification pattern in genomic DNA samples from three populations and 15 genotypes of *Jacaranda micrantha* collected in the county of Irati and Palmeira – state of Parana, Brazil.

Primer type	Total bands	Polymorphic bands	Monomorphic bands	% of polymorphic loci
P-807	4	3	1	75.00
P-808	5	5	0	100.00
P-827	6	5	1	83.33
P-834	5	5	0	100.00
P-835	6	6	0	100.00
P-848	2	2	0	100.00
P-855	5	5	0	100.00
P-857	4	4	0	100.00
P-866	4	3	1	75.00
P-873	4	4	0	100.00
TOTAL	45	42	3	93.33

The number of observed alleles (*na*) ranged from 1.5111 to 1.7333, while the number of effective alleles (*ne*) ranged from 1.2995 to 1.4943 among populations. Genetic diversity loss was observed in the evaluated populations, with the urban population showing the lowest values for Nei's index (*H*) (0.1806) and Shannon's index (*I*) (0.2722) (Table 3). There was variation in the percentage of polymorphic loci (PPB) and the number of polymorphic loci (NPL) among the evaluated populations, with the urban population exhibiting the lowest polymorphism (51.11%) and the lowest number of polymorphic loci (23), while the rural population had the highest percentage of polymorphism (73.33%) and number of polymorphic loci (33) (Table 3).

The average total diversity (H_t = 0.3092) was higher than the average diversity within populations (H_e = 0.2442). The genetic divergence (G_{st}) was 0.21, indicating that 21% of the genetic diversity was found among populations and 79% within populations (Table 4). The observed gene flow value (*Nm*) was 1.8790, indicating that there were no random losses of alleles within populations.

Table 3: Summary of genetic variations revealed through ISSR primers in three populations of *Jacaranda micrantha* collected in Irati and Palmeira – state of Parana, Brazil.

Genetic Variations	P1 – Periurban	P2 – Rural	P3 – Urban	All populations
na (mean ± SD)	1.6889 ± 0.4682	1.7333 ± 0.4472	1.5111 ± 0.5055	1.9333 ± 0.2523
ne (mean ± SD)	1.4943 ± 0.4169	1.4917 ± 0.3962	1.2995 ± 0.3468	1.9333 ± 0.2523
H (mean ± SD)	0.2736 ± 0.2119	0.2785 ± 0.1998	0.1806 ± 0.1936	0.3092 ± 0.1587
I (mean ± SD)	0.3988 ± 0.2950	0.4106 ± 0.2779	0.2722 ± 0.2826	0.4677 ± 0.2070
NPL	31	33	23	42
PPB	68.89%	73.33%	51.11%	93.33

Na: observed nº of alleles; *Ne*: effective nº of alleles; H: Nei's genetic diversity; *I*: Shannon's information index; NPL: nº of polymorphic loci; PPB: Percentage of polymorphic loci.

Table 4: Coefficient of genetic differentiation for three populations of *Jacaranda micrantha* collected in Irati and Palmeira – state of Parana, Brazil.

Primer type	Ht	Hs	Gst	Nm
P-807	0.3447	0.2520	0.2662	5.99
P-808	0.2281	0.2180	0.0590	5.03
P-827	0.3232	0.2867	0.1320	9.15
P-834	0.2925	0.2094	0.2927	1.42
P-835	0.3238	0.2932	0.0866	11.04
P-848	0.2537	0.1648	0.3504	0.93
P-855	0.3904	0.2918	0.2233	5.75
P-857	0.3872	0.2218	0.3693	1.45
P-866	0.2101	0.1538	0.2396	3.44
P-873	0.3007	0.2690	0.1058	11.25
Mean ± SD	0.3092 ± 0.0252	0.2442 ± 0.0191	0.21	1.8790

Ht: Total gene diversity; *Hs*: gene diversity within population; *Gst*: coefficient of gene differentiation; *Nm*: estimate of gene flow from *Gst* or *Gcs*. E.g., *Nm* = 0.5(1 - *Gst*)/*Gst*.

The analysis of molecular variance (AMOVA) of the three populations also showed that the majority of the genetic variation was within populations (89%), with 11% of the variation attributed to differences between populations (Table 5).

Table 5: Analysis of molecular variance (AMOVA) in three populations of *Jacaranda micrantha* collected in Irati and Palmeira – state of Parana, Brazil.

Source	df	SS	Variance component	Total variation (%)	*P*-value
Among population	2	23.467	11.733	11%	0.011
Within population	12	86.800	7.233	89%	0.011
Total	14	110.267		100%	

From the UPGMA cluster figure, constructed based on the genetic distance matrix, it was observed that the three populations became genetically closer at each step. The first cluster consisted of the peri-urban and urban populations, and subsequently, the rural population joined the other populations (Figure 2).

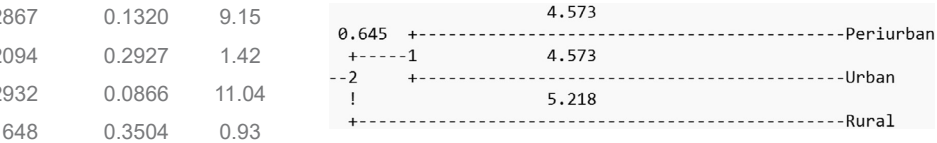

Figure 2: UPGMA cluster figure of three populations of *Jacaranda micrantha* collected in Irati and Palmeira – state of Parana, Brazil.

According to the Russell and Rao dissimilarity, individuals periurban-2 and rural-2 were the closest individuals with a distance of 0.49. On the other hand, individuals periurban-5 and urban-1 were the most divergent individuals, with a distance of 0.80. The grouping of individuals using the Mojena method formed three groups, with two groups consisting of only one individual (individual 5 from the peri-urban population and individual 1 from the urban population), and the third group was formed by the remaining individuals (Figure 3). The cophenetic correlation was 0.86, with a p-value of 0.001. This result reinforces the clustering of populations (Figure 2), which shows a genetic proximity among the evaluated individuals.

Genetic variability

Based on the obtained results, there was no statistically significant difference among the populations (urban, peri-urban, and rural). In other words, the distinct environmental conditions existing in the three remaining forest remnants where parent trees were selected did not exhibit a statistically significant difference (Table 6).

Thus, the model employed to generate the variances solely considered the progenies, treating them as a single seed collection zone utilizing all sampled parent trees. Consequently, it is evident that there existed genetic

variability among the parent trees for all assessed traits (Table 6). These outcomes imply that these traits in the juvenile phase (nursery phase) prove effective in discerning the genetic variability among the progenies, thereby serving as evaluative traits for the selection of superior *J. micrantha* progenies.

Regarding to the estimation of genetic parameters, the average heritability was considered high ($\hat{h}^2_m$ > 0.50) for the traits SD, TH, and H/SD, whereas the trait H exhibited a moderate heritability (0.15 < $\hat{h}^2_m$ < 0.50). These results were also observed for individual heritability ($\hat{h}^2_a$) and within-progeny heritability ($\hat{h}^2_d$) (Table 7).

The individual genetic coefficient of variation $\left(C\hat{V}_{gi}\%\right)$ was considered high (>7%) for DC, HT, and H/DC, while for the progeny coefficient $\left(C\hat{V}_{gp}\%\right)$, only the traits SD and H/SD had values greater than 7%. The experimental precision for all traits $\left(C\hat{V}_{e}\%\right)$ was considered high (>10%) for all evaluated traits, resulting in a relative coefficient of variation $\left(\hat{b}\right)$ ranging from low to moderate (<0.50). The accuracy was considered of moderate to high magnitude, with values ranging from 0.42 for H/SD to 0.74 for SD, indicating that at 150 days of age, genetic factors have a moderate to high influence on trait expression.

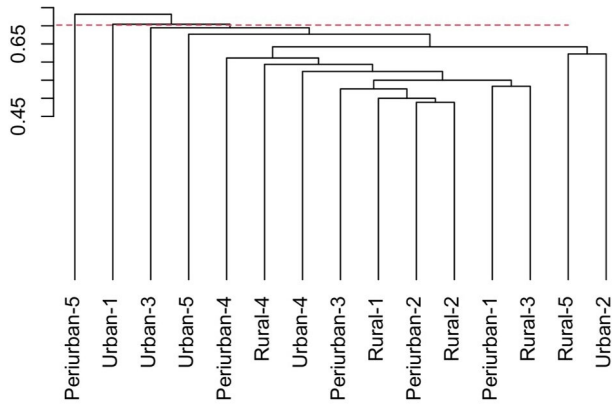

Figure 3: Representative dendrogram of phenotypic dissimilarity between 15 *Jacaranda micrantha* matrices in three remnants of Araucaria Forest, in Irati and Palmeira, state of Paraná, obtained by the UPGMA method, for estimating the reference cut value in the dendrogram using the Mojena Method, with K = 0.702, from ISSR molecular markers. The red line represents the "cutoff point".

Table 6: Likelihood ratio test (LRT) of the model considering the populations and the model only the progenies for the variables stem diameter (SD), shoot height (H), total height (TH) and the relationship between shoot height and stem diameter (H/DC) of *Jacaranda micrantha*, evaluated at 150 days.

Model considering the populations						
Effect	**LRT**	**gl**	**Pr**	**LRT**	**gl**	**Pr**
	SD (mm)			**TH (cm)**		
Progenies	5.134	1	0.02346	3.503	1	0.06126
Population	0.05298	1	0.818	0.1339	1	0.7144
Plot	82.13	1	1.276e-19	36.05	1	1.927e-09
Block	F=23.21	2	1.138e-06	F=1.747	2	0.1928
	H (cm)			**H/SD**		
Progenies	4.819	1	0.02814	5.593	1	0.01803
Population	0.0007	1	0.9778	-2.022e-09	1	1
Plot	15.16	1	9.89e-05	70.95	1	3.659e-17
Block	F=1.761	2	0.1904	F=19.58	2	4.799e-06
Model considering only the progenies						
Effect	**LRT**	**gl**	**Pr**	**LRT**	**gl**	**Pr**
	SD (mm)			**TH (cm)**		
Progenies	5.977	1	0.014449	4.396	1	0.03602
Plot	82.13	1	1.276e-19	36.05	1	1.927e-09
Block	F=23.21	2	1.138e-06	F=1.747	2	0.1928
	H (cm)			**H/SD**		
Progenies	5.267	1	0.02173	5.897	1	0.01517
Plot	15.16	1	9.89e-05	70.95	1	3.659e-17
Block	F=1.761	2	0.1904	F=19.58	2	4.799e-06

LRT= the likelihood ratio test statistic; gl= degrees of freedom for the likelihood ratio test; Pr= the p-value.

Table 7: Estimation of genetic parameters for the variables stem diameter (SD), shoot height (H), total height (TH) and the relationship between shoot height and stem diameter (H/DC) of Jacaranda micrantha, evaluated at 150 days.

Genetic Parameters	SD	H	TH	H/DC
Média	2.045	16.6033	24.0367	9.2072
$\hat{\sigma}_a^2$	0.2230	1.2997	2.9880	6.3895
$\hat{\sigma}_g^2$	0.0557	0.3249	0.7470	1.5974
$\hat{\sigma}_d^2$	0.2768	4.3948	7.7881	8.8502
$\hat{\sigma}_e^2$	0.0709	0.3265	1.0544	2.0118
$\hat{\sigma}_f^2$	0.4034	5.0463	9.5896	12.4594
$\hat{h}_a^2$	0.5526	0.2576	0.3116	0.5128
$\hat{h}_m^2$	0.6637	0.3409	0.6082	0.6613
$\hat{h}_d^2$	0.6041	0.2218	0.2877	0.5415
$C\hat{V}_{gp}(\%)$	11.5449	3.4332	3.5957	13.7271
$C\hat{V}_{gi}(\%)$	23.0897	6.8664	7.1914	27.4541
$C\hat{V}_e(\%)$	25.7284	12.6263	11.6102	32.3110
$\hat{r}_{aa}$	0.7434	0.5075	0.5582	0.7161
$\hat{b}$	0.4487	0.2719	0.3097	0.4248

$\hat{\sigma}_g^2$: genetic variance between progenies; $\hat{\sigma}_a^2$: additive genetic variance; $\hat{\sigma}_e^2$: environmental variance; $\hat{\sigma}_d^2$: variance within progenies; $\hat{\sigma}_f^2$: phenotypic variance; $\hat{h}_a^2$: additive narrow-sense heritability; $\hat{h}_m^2$: average progeny heritability; $\hat{h}_d^2$: additive heritability within progenies; $C\hat{V}_{gi}(\%)$: individual additive genetic variation coefficient; $C\hat{V}_{gp}(\%)$: coefficient of genetic variation between progeny; $C\hat{V}_e(\%)$: coefficient of experimental variation; $\hat{r}_{aa}$: selection accuracy of progeny; $\hat{b}$: relative coefficient of variation.

DISCUSSION

Genetic diversity

The abundance of polymorphic loci is fundamental for genetic diversity studies (Cao et al., 2019). The high level of polymorphism observed in this study (93%) and the amplification of a large number of polymorphic bands demonstrate that the ISSR molecular markers and primers used in this research are suitable for studying the genetic diversity of *J. micrantha*. The percentage of polymorphic loci exceeds the average value (70%) observed in higher plants (Shan et al., 2006).

According to Oliveira et al. (2008) the Nei's (H) and Shannon's (I) diversity indices range from 0 to 1, with values closer to 0 indicating lower diversity. The results of Nei's and Shannon's genetic diversity indices (Tab. 2) indicate a low genetic diversity in the evaluated populations, with the urban fragment exhibiting the lowest values for these indices (0.1806 and 0.2722, respectively). This confirms that forest fragmentation for urban development is the primary cause of genetic diversity loss in many plant species. The diversity indices demonstrate that as human pressure decreases, there is an increase in genetic diversity, as evidenced by the less anthropized environment (rural fragment) having the highest Nei's and Shannon's indices (0.2785 and 0.4106, respectively).

Genetic diversity and the establishment of plant populations are the outcomes of factors such as historical evolution, distribution range, reproductive mode, and lifestyle, and they are closely related to adaptability and evolutionary potential (Shan et al.; 2006; Lowe et al., 2015). Theoretically, if a species harbors high genetic diversity, its ability to adapt to environmental degradation, landscape destruction, increased environmental stress, etc., enhances its genetic diversity. The loss of genetic diversity diminishes the adaptability of plants to short-term biotic, abiotic, and environmental changes, such as diseases, pests, and herbivore foraging behavior (Aguilar et al., 2019; Lowe et al., 2015 and Rey et al., 2017).

Genetic divergence (Gst) and analysis of molecular variance (AMOVA) revealed that the greatest genetic diversity was within populations (79% and 89%, respectively), while a lesser diversity was observed between populations (21% and 11%, respectively). According to Oliveira et al. (2008), in general, tree species exhibit greater genetic variation within populations than between populations. This result has also been observed by other authors for other native forest species such as *Eugenia dysenterica* DC (Aguiar et al., 2011), *Dimorphandra mollis* Benth. (Oliveira et al., 2008) and *Eremanthus erythropappus* (DC.) MacLeish (Estopa et al., 2006).

Despite the low genetic diversity observed in the evaluated fragments, they exhibited a gene flow value (Nm) greater than 1. According to Wright (1931), a gene flow value below 1 indicates genetic isolation, while a value above 1 is sufficient to prevent random loss of alleles within a population (genetic drift effect). High gene flow among populations neutralizes drift and genetic differentiation and plays an important role in maintaining random and panmictic population structures (Cao et al., 2019). Therefore, it can be concluded that there is still gene flow between the fragments, and no reproductive isolation has been observed in the studied species.

The dendrogram obtained from the UPGMA cluster analysis based on Nei's genetic similarity coefficients (Figure 2) shows that the fragments form a single population. These results are consistent with the Russel and Rao dissimilarity cluster for the selected mother trees (Figure 3), where only three distinct groups were formed, with two of them consisting of a single parent tree. This indicates that the majority of the parent trees (86%) are genetically closely related.

Genetic variability

The process of urbanization is one of the main factors contributing to the increase in forest fragmentation. However, for the species *J. micrantha*, the environmental changes caused by urbanization did not have a statistically

significant effect on the genetic variability of the evaluated populations. Therefore, the 15 parent trees were considered as a single population, and only the variability among the progenies was assessed.

These results may be attributed to the fact that the studied municipalities are located in the interior of the state and have green corridors, such as natural remnants, parks, squares, and even vacant lots, which serve as connections between remnants and provide ecosystem services within the cities. This functionality of green corridors has been observed by Zhang et al. (2019) in a study conducted in the city of Detroit, USA. The authors demonstrated that these corridors can improve both the structure and functional connectivity of remnants, ensuring biodiversity conservation and the provision of essential ecosystem services for the population.

Nonetheless, this fact can be explained by the presence and persistence of pollinators in urban areas. According to Wenzel et al. (2020), pollination services are maintained by resident pollinators in urban areas, and cities in general can host more pollinators than densely utilized agricultural areas, leading to positive population responses in urbanized environments. In summary, urbanization itself does not seem to promote the emergence of pollinators, but the presence of green fragments in urban areas provides abundant nesting sites and food sources for pollinators, thereby promoting their diversity and survival (Hülsmann et al., 2015).

The SD (stem diameter) and H/SD (height-to-diameter relationship) traits are highly recommended for progeny selection due to their high values of individual heritability ($\hat{h}_a^2$), within-progeny heritability ($\hat{h}_d^2$) and averge heritability ($\hat{h}_m^2$). According to Camara et al. (2020) The H/SD ratio, also known as the robustness index, reflects the balance between height and stem diameter growth. This index can predict the potential survival of seedlings in the field. A lower value of the index indicates that the seedlings have more lignified stems, which enhances their viability and survival in field conditions. It serves as an important criterion for assessing the adaptability and resilience of seedlings to environmental stresses and can be used as a valuable tool in selecting individuals with better field performance in breeding and reforestation programs.

High heritability coefficients indicate a high probability of genetic gain through the selection of phenotypically superior progenies (Henriques et al., 2017). Therefore, for progeny of *J. micrantha*, a mass selection strategy can be applied, which is appropriate when heritability is around 0.50.

High heritabilities for quantitative traits are characteristic of native species that have not undergone a genetic improvement process. Similar results have also been observed in other native species such as: *Mimosa scabrella* Benth (Menegatti et al., 2016), *Handroanthus avellanedae* Mattos (Sousa Santos et al., 2014) and *Dipteryx alata* Vog. Canuto et al. (2015). These results indicate that within the evaluated population, there is genetic variability within the progenies, ensuring the production of seedlings of the species with high genetic diversity.

The traits SD and the H/SD ratio exhibited individual genetic coefficient of variation ($C\hat{V}_{gi}$%) and progeny coefficient of variation ($C\hat{V}_{gp}$%) greater than 7%. According to Ferreira et al. (2016) a higher value of the coefficient of variation indicates greater genetic variability among the evaluated progenies. Therefore, these results suggest that it is possible to obtain superior individuals that can lead to greater gains in selection (Resende and Duarte, 2007).

The experimental precision for all traits, as indicated by the experimental coefficient of variation ($C\hat{V}_e$%) was high (>10%), suggesting that other factors, which were not evaluated, influenced the experiment. However, according to Moraes et al. (2015) and Paludeto et al. (2020), it is common for forest experiments conducted at the nursery or field level to exhibit $C\hat{V}_e$% values above 30%. This classification does not take into account all genetic and environmental factors.

Despite being a juvenile test (150 days), the effectiveness of phenotypic selection of *J. micrantha* progenies and the genetic gain to be obtained from selection for the evaluated traits are supported by the high accuracy values. For the SD and H/SD traits, the accuracy values were above 0.70, indicating a high level of reliability in predicting the performance of these traits (Resende and Duarte, 2007). Considering that accuracy represents the relationship between the true genetic value and the estimated value, these traits demonstrate good precision in accessing the true genetic variation based on the observed phenotypic variation. Therefore, these traits are suitable for selecting superior materials in breeding programs.

Thus, the morphological traits SD (stem diameter) and H/SD (stem height-to-diameter ratio) of *J. micranta* can be used to assess the genetic variability of the species due to their higher values of heritability, accuracy, and coefficients of variation. Furthermore, this genetic variability demonstrates the species' potential for adaptation to fragmented environments and/or environments undergoing constant change.

The results of the present research demonstrate that habitat fragmentation did not have a negative impact on the genetic variability of *J. micranta*. The evaluated progenies exhibited high genetic variability, indicating that the selected mother trees can be used for seed collection and seedling production.

CONCLUSION

The ISSR markers tested exhibit high polymorphism and can be employed for assessing the genetic diversity of the *J. micrantha* populations. Habitat fragmentation has caused a loss of genetic diversity in the *J. micrantha* population, with the urban fragment being the most affected. The analysis of Nei's and Shannon's diversity indices, as well as the molecular variance analysis (AMOVA), confirms that there is higher genetic diversity within the fragments than between them. Despite the observed loss of genetic diversity in

population fragments, there is evidence of ongoing gene flow among them, and no random loss of alleles has occurred. The cluster analysis of the evaluated fragments, including urban, periurban, and rural, suggests that they form a single population. The geographic barrier has not caused differentiation between the populations, despite being located in different municipalities. The dissimilarity analysis of the mother trees revealed the formation of three distinct groups, with one group comprising thirteen mother trees and the other two groups consisting of one mother tree each. The fragments of the urban, peri-urban, and rural populations did not exhibit significant differences, but there were significant differences among the progenies. The stem diameter (SD) and the height- diameter relationship (H/SD), also known as the robustness index, emerged as the key traits for selecting new individuals due to their higher heritability, accuracy, and relative coefficient of variation. These traits can be used as selection criteria for choosing superior individuals for future progeny tests in breeding programs.

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Review: EVT, RB, FSBP.

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