Research Article
doi: 10.1590/01047760202228013012
vol(28), 2022

CERNE

Does the faxinal system help to maintain the genetic diversity of *Curitiba prismatica* (D.Legrand) Salywon & Landrum?

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SILVICULTURE

ABSTRACT

Background: *Curitiba prismatica* (D.Legrand) Salywon & Landrum belongs to the Myrtaceae family and it is popularly known as murta. Endemic to the Mixed Ombrophilous Forest of south Brazil, is predominant in Faxinal System, which is a traditional agricultural system in the State of Paraná. This species also has several uses and potentials, such as wood for fence posts and essential oils with pharmacological properties. For this reason, this study aimed to select molecular markers and assess whether the Faxinal System contributes to the maintenance of the genetic diversity of the species, helping in its management and conservation. As such, 120 adult reproductive individuals were sampled, 60 in two areas managed in the Faxinal System, and 60 in two conservation areas.

Results: Initially, 30 ISSR markers were selected, but only eight showed considerable variability, resulting in 68 polymorphic loci. The results show that the average diversity within populations is 80.54%. The Shannon (*I*) and Nei's (*He*) diversity indices were 0.53 and 0.36, respectively. According to Nei's genetic identity, the populations form two groups. With the analysis of the genetic structure of the populations, which indicated the existence of two distinct genetic groups ($K = 2$).

Conclusion: Thus, the populations in the Faxinal System had higher rates of genetic diversity, despite constant human activity within the system. Therefore, the Faxinal System contributes to the conservation of *C. prismatica* genetic diversity; however, considering the economic potential of the species and to minimize impacts on the existing fragments, there is a need to work with the local population to ensure sustainable forest management of the species.

Keywords: Araucaria forest; Conservation; Forest management; ISSR

HIGHLIGHTS

The average diversity within populations is 80.54%.
The populations form two groups, Faxinal and the other populations.
The Faxinal System had higher rates of genetic diversity, despite the human activity.
Faxinal System contributes to the conservation of *C. prismatica* genetic diversity.

SILVA, R. A. R.; VIEIRA, F. A.; TAMBARUSSI, E. A.; CHAGAS, K. P. T.; WATZLAWICK, L. F.; KOEHLER, H. S. Does the faxinal system help to maintain the genetic diversity of *Curitiba prismatica* (D.Legrand) Salywon & Landrum? CERNE, v. 28, e-103012, doi: 10.1590/01047760202228013012

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Open Access
CERNE
ISSN 0104-7760
UFLA UNIVERSIDADE FEDERAL DE LAVRAS

INTRODUCTION

The information generated by dominant and codominant molecular markers can be used to study genetic diversity, providing relevant information for the management of forest species (Li et al., 2018). However, this information is often ignored in the preparation of management plans, even though it may contribute to their sustainability (Ottewell et al., 2016). For the management of forest species, it is important to understand the genetic diversity involved in their populations (Ramos et al. 2016). Individuals with high levels of genetic diversity should be prioritized in sustainable management (Ratnam, 2014); this could have a direct impact on long-term survival (Brzosko et al., 2011).

For example, selective logging regimes applied in populations of trees could cause three important effects: an increase in the spatial distance between remnants after harvesting; a reduction in the total number of individuals; and spatial isolation of remaining populations (Dal Bem et al., 2015). When information on the genetic diversity of species is not considered, one of the main problems resulting from selective logging is the loss of alleles (Dal Bem et al., 2015; Vinson et al., 2015; Soliani et al., 2016). Thus, these processes can result in reductions in effective population size, heterozygosity, gene flow, and reproductive isolation. Besides, can lead to an increase in inbreeding and coancestry (Sebbenn et al., 2008).

Among the current management systems used in Southern Brazil, the Faxinal System (or Faxinais) is unique a traditional production system used for more than a century. Faxinal System are based on a consortium of extensive animal production, subsistence agriculture, and low impact forest extraction. Over the last few decades, the communities that use have experienced high rates of rural exodus and significant changes in production. Farmers began to fence their properties and switch from the traditional system to the cultivation of monocultures, which has consequently increased forest fragmentation.

One of the species common in the Faxinal System is *Curitiba prismatica*, popularly known as murta (Watzlawick et al., 2011; Albuquerque, 2015). The species is a member of the Myrtaceae family, endemic to the Mixed Ombrophilous Forest, and its distribution is restricted to the States of Paraná and Santa Catarina, Brazil (Lima, 2020). The species can reach up to eight meters in height, the flowers are hermaphrodit and the berry-like fruits are dark purple and elongated, produced annually and dispersed by birds (Salywon and Landrum, 2007; Backes and Irgang, 2009; Lorenzi, 2014; Lima, 2020). The species has high economic potential due to the production of an essential oil from leaves that can be used in the pharmaceutical industry (Gardin, 2017).

Forest management strategies can be facilitated by data from dominant markers as Inter Simple Sequence Repeat (ISSR) (Chen et al., 2017). They can provide information on genetic diversity indices, allele and genotypic frequencies, gene flow, effective population size, fixation index, genetic distance, and genetic bottlenecks (Brandão et al., 2015; Costa et al., 2015). ISSRs cannot differentiate heterozygous from homozygous individuals, they have been used in studies of intra-and interpopulation genetic diversity, as well as in analyses of genetic structure (Silva et al., 2017; Fajardo et al., 2018; Laakili et al., 2018; Bocanegra-González et al., 2019; Freire et al., 2019).

The objective of this study was to select ISSR markers appropriate to detect genetic polymorphism in *C. prismatica*, as well as analyze through molecular markers the influence of the Faxinal System on the genetic conservation of the species, considering that are constantly subjected to plant extraction alongside alternative land uses. Thus, the following hypothesis was tested: Faxinais have contributed to the conservation of *C. prismatica* genetic diversity because of the use of low impact forest extraction.

MATERIAL AND METHODS

Plant sampling

Leaf samples were collected from adults of *C. prismatica* in four natural populations in the States of Paraná and Santa Catarina, two within the Faxinal System, and two in conserved populations (Fig. 1). Individuals throughout the study areas were sampled, maintaining an average distance of 20 meters between them. They were, labelled, and placed in plastic containers containing silica gel. The samples were then sent to the Laboratory of Genetics and Forest Improvement at the Federal University of Rio Grande do Norte, in the municipality of Macaíba, Rio Grande do Norte State, Brazil.

According to the Köppen classification, the climate of the study region is Subtropical Humid Mesothermal (Cfb), with cool summers, no dry season, and frosts (Alvares et al., 2013). The region experiences rainfall distributed evenly throughout the year, severe frosts, and average annual precipitation between 1,500 and 1,600 mm. The average annual temperature is approximately 18 °C, with a minimum of -2 °C and a maximum of 32 °C.

The studied populations that are managed using the Faxinal System are located in the municipality of Rebouças on the second plateau of Paraná. The relief varies from slightly undulated to undulated (Embrapa, 2013), with the predominance of the Mixed Ombrophila Forest. The soils in the Faxinais are mainly classified as Litolic, Neosols, Cambisol, and Argisol (IBGE, 2012).

The Faxinal Marmeleiro de Baixo (MAR) covers an area of 2,274.9 ha, of which 556.6 ha is forested. The forest fragment includes 36 tree species, with the predominant occurrence of six that are among the ten most common species in regional floristic studies: *Ilex paraguariensis* A.St.-Hil., *Ocotea porosa* (Nees & Mart.) Barroso, *Ocotea puberula* (Rich.) Nees, *Cedrela fissilis* Vell., *Campomanesia xanthocarpa* (Mart.) O.Berg, *Casearia decandra* Jacq., and *C. prismatica* (Albuquerque et al., 2011). The Faxinal Barro Branco (BAR) has an area of 1,452 ha. In the forest fragment, the following species were predominant: *Casearia obliqua* Spreng., *I. paraguariensis*, *Ocotea odorifera* (Vell.) Rohwer, *Cinnamodendron dinisii* Schwacke, *C. prismatica*, and *C. decandra* (Andrade, 2014).

The Floresta Nacional de Irati (Flona) (IRA) is a Conservation Area administered by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), located on

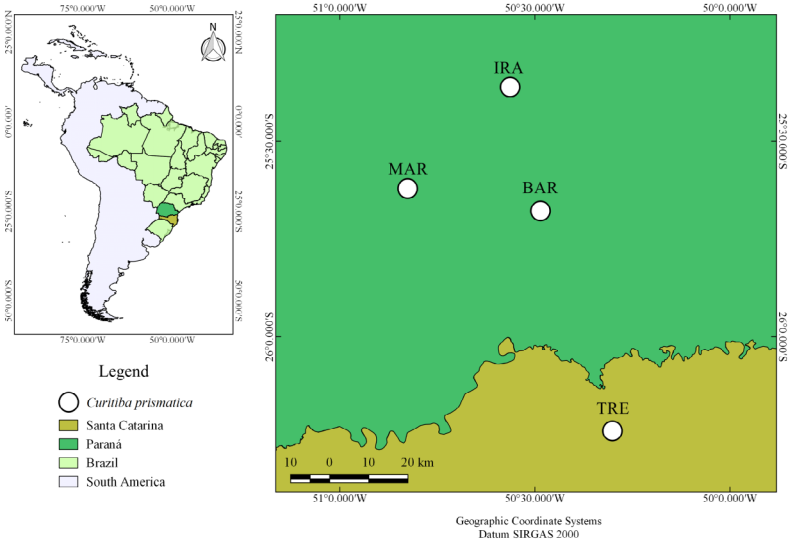

Fig. 1 Geographic location of the sampled populations of *Curitiba prismatica* in Paraná and Santa Catarina States, Brazil. Flona de Irati (IRA), Faxinal Marmeleiro de Baixo (MAR), Faxinal Barro Branco (BAR), and Flona de Três Barras (TRE).

the second plateau of Paraná, between the municipalities of Fernandes Pinheiro and Teixeira Soares, in the Colonial Microregion of Irati, Paraná State. It has a total area of 3,495 ha, 57.60% of which is covered by native forest, and 37.50% by forest plantations. The native forest is well conserved and includes 113 tree species, with the most common being *Araucaria angustifolia* (Bertol.) Kuntze, *I. paraguariensis*, *O. odorifera*, *Nectandra grandiflora* Nees & Mart, *O. porosa*, and *C. decandra* (Rode et al., 2010).

The Floresta Nacional de Três Barras (Flona) (TRE) is considered the highest national forest in terms of elevation in Southern Brazil, with an area of 4,458 ha, 31.46% of which is native forest. The predominant soil in the region is an association between Latosol and Cambisol. The tree species native to the area were extensively exploited by the Southern Brazil Lumber & Colonization (Lumber) Company between 1910 and 1940, with a particular focus on the harvesting of *A. angustifolia*. After the creation of the Flona in 1944, there is no record of further logging in the forest.

DNA extraction, amplification, and electrophoresis

The protocol described by Doyle and Doyle (1987) was used with modifications to obtain the genomic DNA. The result material was quantified in a microplate spectrophotometer (Epoch™). DNA amplification reactions were carried out with a Veriti Thermocycler, with a final volume of 12 µL, containing: 2 µL of diluted genomic DNA (1:50); 1.2 of 10X Buffer; 1.0 mg.mL^{-1} of BSA; 2.5 mM dNTP; 50 mM $MgCl_2$; 5 U.µL^{-1} of Taq DNA polymerase and ultrapure water. The PCR protocol included an initial denaturation for 2 min at 94 °C, followed by 37 amplification cycles for 15 s at 94 °C, 30 seconds at 47 °C, 1 min at 72 °C, with a final extension for 7 min at 72 °C, and cooling to 4 °C.

After performing the PCR, the amplification products were stained with bromophenol blue together with GelRed™ and separated on agarose gel using 1.5% horizontal electrophoresis. The gel was immersed in a TAE (Tris-Acetate-EDTA) 1X buffer solution at a voltage of 100 V for a period of 2.5 h. Subsequently, the gels were photographed under ultraviolet light using the E-Box VX2 photo-documenter.

Selection of ISSR markers

Thirty ISSR markers were tested in bulk using genomic DNA with three individuals randomly selected from the sampled populations. The markers were selected based on the best amplification results and the total number of amplified loci.

Analysis of genetic diversity

The number of observed alleles (*Na*), number of effective alleles (*Ne*), percentage of polymorphic loci, Nei's genetic diversity (*He*), and Shannon index (*I*) were estimated assuming Hardy-Weinberg equilibrium using the POPGENE software version 1.3 (Yeh et al., 1997). The genetic distance was classified into categories, according to Nei (1978): low, when the distance is less than 0.05; average, between 0.05 and 0.15; and high when greater than 0.15. A comparison of the Nei and Shannon diversity index values between populations was conducted through analysis of variance in the Assistat 7.7 software (Silva and Azevedo, 2016), considering Tukey's test at 5% probability.

The genetic similarities estimated between populations were observed using the software POPGENE version 1.3 (Yeh et al., 1997). From this, a Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) genetic identity dendrogram was constructed using the Sequential Agglomerative, Hierarchical and Nested Clustering (SAHN) routine. The dendrogram was developed with the NTSYS program, version 2.11 (Rohlf, 2000).

The assessment of population genetic structure, aiming at conducting an analysis of molecular variance (AMOVA) of the populations, as well as estimates of the fixation index (F_{ST}) were performed in the ALERQUIN 3.1 software (Excoffier et al., 2007). Additionally, we conducted Mantel's test and resampling using the Monte Carlo method (1,000 permutations) to assess the correlation between Nei's genetic distance (1978) and geographical distance, as well

as the principal coordinates analysis (PCoA) of individuals in the GenAlEx program (Peakall and Smouse, 2006).

To obtain the number of genetic groups (*K*), Bayesian analysis was performed using the Structure v.2.2 software (Pritchard, 2002). The number of *K* populations was defined according to the Δ*K* method (Evanno et al., 2005), implemented in the Structure Harvester software (Earl and Vonholdt, 2012).

To identify population decreases, or reductions in the effective size of the population over generations, we used the infinite allele model (IAM), according to Kimura and Crown (1964), and the stepwise mutation model (SMM), according to Kimura and Otha (1978). Furthermore, to identify recent population decreases, the signal test (α = 0.05) was applied based on the allele frequency in the program Bottleneck 1.2.02 (Cornuet and Luikart, 1996).

RESULTS

Number of loci

Among the 30 tested ISSR markers, 8 showed satisfactory PCR amplification, ranging from 5 to 12 loci, for a total of 68 polymorphic loci (Tab. 1). Of the selected markers, UBC844 amplified the most polymorphic loci (12) and UBC843 the least (05).

Tab. 1 Number of polymorphic loci, and the value of the polymorphic information content (PIC) for each ISSR markers from *Curitiba prismatica*.

ISSR primer	Sequence (5' - 3')	Loci	PIC
UBC813	CTCTCTCTCTCTCTCTT	9	0.50
UBC818	CACACACACACACACAG	7	0.42
UBC821	GTGTGTGTGTGTGTGTT	9	0.49
UBC822	TCTCTCTCTCTCTCTCA	9	0.48
UBC826	ACACACACACACACACC	9	0.40
UBC843	CTCTCTCTCTCTCTCTRA	5	0.41
UBC844	CTCTCTCTCTCTCTCTRC	12	0.43
UBC859	TGTGTGTGTGTGTGTGRC	8	0.47

R = purine (A or G) and Y = pyrimidine (C or T).

Genetic diversity

The intrapopulation genetic diversity estimated for the *C. prismatica* populations are shown in Tab. 2. The percentage of polymorphic loci (*P*) varied from 54.41% in the population sampled from the IRA to 80.88% in BAR. The number of observed alleles (*Na*) varied from 1.54 to 1.81, and the effective number of alleles (*Ne*) ranged from 1.40 to 1.54.

Considering all four populations, Nei's diversity index (*He*) was 0.36, and we found no significant differences for *He* among the four populations. For the Shannon index (*I*), only the BAR population presented a value significantly different from IRA, with a diversity of 0.32. The BAR population had the greatest diversity (0.46); however, it did not differ statistically from the MAR and TRE populations. For all four populations, the result for *I* was 0.53.

According to the AMOVA, there is greater genetic diversity within populations (80.54%) than between populations (19.46%). The fixation index was positive (F_{ST} = 0.19). According to the Mantel test, there was no significant correlation between genetic and geographic distances (r = 0.45; P = 0.058).

The distribution of the spatial structure of individuals in the evaluated populations according to Nei's genetic distance is shown in Fig. 2. Individuals from the MAR and BAR populations showed greater genetic variability than the other populations.

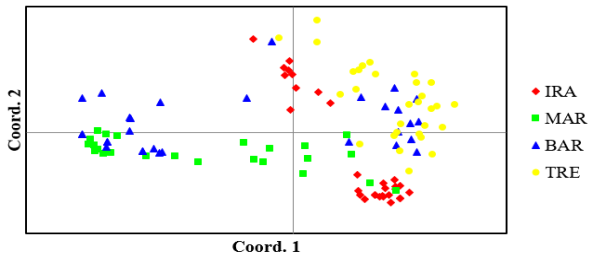

Fig. 2 Principal Coordinate Analysis (PCoA) among individuals from *Curitiba prismatica*, located in conserved populations and Faxinal System. Flona de Irati (IRA), Faxinal Marmeleiro de Baixo (MAR), Faxinal Barro Branco (BAR), and Flona de Três Barras (TRE).

According to the dendrogram based on Nei's genetic identity, two groups were identified with a cutoff value close to 0.86: the first group was composed of the IRA and TRE populations, and the second included the MAR and BAR populations (Fig. 3). Two distinct genetic groups (*K* = 2) were also identified according to the Δ*K* values (Fig. 4).

Therefore, the four populations evaluated were structured into two genetic groups. Additionally, the analysis

Tab. 2 Estimates of genetic diversity for the four natural populations of *Curitiba prismatica*.

Population	*L / P%*	*Na*	*Ne*	*He**	*I*
MAR	45/66.18	1.66±0.48	1.40±0.38	0.23±0.20a	0.35±0.29ab
BAR	55/80.88	1.81±0.40	1.54±0.35	0.31±0.18a	0.46±0.25a
IRA	37/54.41	1.54±0.50	1.41±0.44	0.22±0.23a	0.32±0.32b
TRE	42/61.76	1.62±0.49	1.43±0.39	0.25±0.21a	0.36±0.30ab
Average	44.8/65.8	1.66±0.47	1.45±0.39	0.25±0.21	0.37±0.29
Total	67/98.53	1.99±0.12	1.61±0.28	0.36±0.12	0.53±0.15

Polymorphic loci (L), percentage of polymorphic loci (P%), number of alleles observed (Na), number of effective alleles (Ne), Nei's genetic diversity index (He), Shannon index (I). Values represent the average ± standard deviation. *The data showed no statistically significant difference by the Tukey test at 5% probability. Flona de Irati (IRA), Faxinal Marmeleiro de Baixo (MAR), Faxinal Barro Branco (BAR), and Flona de Três Barras (TRE).

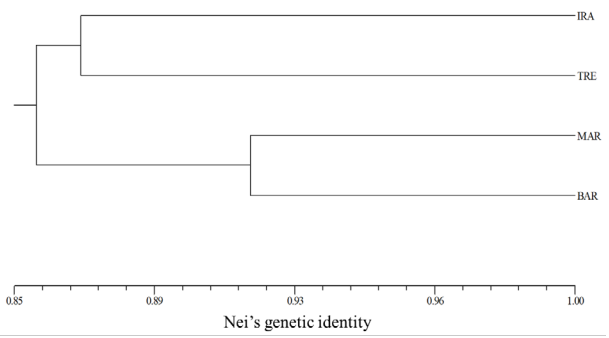

Fig. 3 Dendrogram of Nei's genetic identity, by the UPGMA method, between four *Curitiba prismatica* populations. Flona de Irati (IRA), Faxinal Marmeleiro de Baixo (MAR), Faxinal Barro Branco (BAR), and Flona de Três Barras (TRE).

of the occurrence of population decreases for *C. prismatica* demonstrated that the MAR, BAR, and TRE populations have experienced genetic bottlenecks, based on the IAM model. However, considering the SMM, a significant genetic bottleneck was only found for the BAR population.

DISCUSSION

The number of markers (n = 8) and loci (n = 68) found in the present study were similar to those used in other studies on the genetic diversity of tree species (Rossi et al., 2017; Fajardo et al., 2018; Moreira et al., 2018; Souza et al., 2018; Bocanegra-González et al., 2019). To determine the effectiveness of each molecular marker in terms of detecting polymorphism, the Polymorphic Information Content (PIC) showed that all markers are moderately informative, according to the classification proposed by (Botstein et al. 1980).

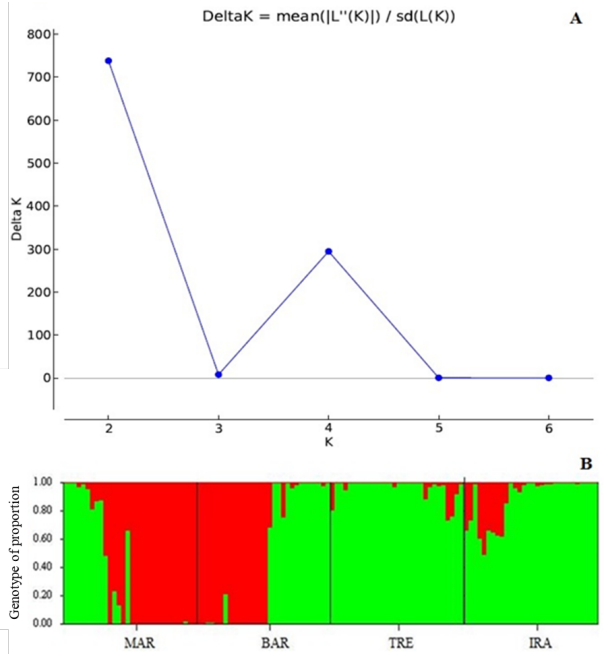

Fig. 4 Genetic structure of the Curitiba prismatica populations. A. K value obtained by the ΔK method using a Bayesian-based, according to Evanno et al. (2005), demonstrating the number of genetic groups. B. Representation of the genetic groupings of the populations, delimited by the vertical bar and the colors that represent the groups. Flona de Irati (IRA), Faxinal Marmeleiro de Baixo (MAR), Faxinal Barro Branco (BAR) and Flona de Três Barras (TRE).

Polymorphism, which is considered an important measure of diversity, was different among populations and these results were lower than that observed for other tree species in the Atlantic Forest biome, such as *Hancornia speciosa* Gomes (Apocynaceae), with an approximate polymorphism of 81.00% based on a study of 105 individuals (Fajardo et al., 2018), and *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae), with polymorphism of 93.00% for 64 individuals (Freire et al., 2019). In the present study we found that the managed areas have high levels of genetic diversity, thus contributing to the maintenance of the species over time.

C. prismatica seeds have a high germination rate (Rego et al., 2011), as well as a relatively quick seedling growth rate (Mello and Peroni, 2015), and it is likely that the Faxinal System present more favorable environmental conditions for the occurrence of the species. However, due to the extensive fragmentation of the forest over the last century, the studied populations show significant decreases in population size. This is related to the modification of effective niche of the species due to the use of intensive agriculture, which has altered the surrounding matrix.

Regarding the genetic diversity indices, the BAR population stood out from the others, considering the average number of observed and effective alleles, as well as the Nei (0.31) and Shannon (0.46) diversity indices. Therefore, it appears that the genetic bottleneck identified in the population may be recent, since it has not yet directly affected the genetic diversity of the species. Furthermore, the BAR population has a population density of 46 individuals.ha^{-1} (Andrade, 2014), which is lower than the Faxinal Marmeleiro de Baixo, with a density of 130 individuals.ha^{-1} (Albuquerque et al., 2011).

In the present study, the genetic variation within populations was greater than among populations. The results confirm that the remaining populations of the species conserve genetic variability. Similar results were reported for populations of *Senefeldera verticillata* (Vell.) Croizat (Euphorbiaceae) (Vieira et al., 2018) and *Myrcia splendens* (Sw.) DC. (Myrtaceae) (Brandão et al., 2015). Like *C. prismatica*, these species occur naturally in the Atlantic Forest and their seeds are dispersed by zoochory. In relation to the PCoA, individuals from BAR showed greater diversity, indicating that this population should be prioritized for conservation, through the improvement of the management system, based on the information obtained in this study and other complementary approaches, for example, analysis of the spatial genetic structure.

As for the sharing of alleles, the Bayesian analysis identified two genetic groups ($K = 2$), which was corroborated by the cluster analysis based on Nei's genetic identity. The MAR and BAR populations, which occur in the Faxinal System, formed a group while the IRA and TRE populations located in conservation areas constitute a second genetic

group, despite the geographical distance of approximately 75 km between them. In the case of the IRA and TRE, it is probable that these populations were connected in the past due to extensive gene flow across the region. Thus, we can assume that despite the intense fragmentation of the Mixed Ombrophilous Forest in recent decades (Accioly, 2013), the shared alleles are still fixed in these populations. Furthermore, we found no tendency towards isolation due to the distance between populations (Fig. 2).

The present study considers the effects of the Faxinal System on the genetic conservation of *C. prismatica*. Analysis of the influence of forest management on species conservation using ISSR markers was effective, based on the percentage of polymorphism. Our results suggest that the management strategies adopted in the Faxinal System have contributed to the conservation of the species.

CONCLUSION

The population of Faxinal Barro Branco showed greater genetic diversity, despite having experienced a significant population decrease, which is likely due to the extensive fragmentation of the forest. As *C. prismatica* is an endemic species from south Brazil, it is recommended that the populations in the Flonas de Irati and Três Barras of Irati and Três Barras are maintained conserved. In the case of the Faxinal System, it is important to sensitizing the local population to ensure that sustainable forest management of the species continues as the species has economic potential that can be exploited with minimal impacts on existing forest fragments.

ACKNOWLEDGMENTS

The present work was carried out with the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Financing Code 001.

AUTHORSHIP CONTRIBUTION

Project Idea: RARS, LFW

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Review: RARS, FAV, EVT, KPTC, LFW, HSK

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