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SPATIAL GENETIC STRUCTURE OF SYMPATRIC POPULATIONS OF *EREMANTHUS* SPECIES IN BRAZIL: IMPLICATIONS FOR MANAGEMENT

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There is a consensus that a complex network of factors shapes the genetic diversity in natural populations. Several processes of environmental change influence the allelic dynamics of the species. Therefore, genetic variation evaluation is essential for the conservation, management and understanding of the phylogenetic relationships among species. This study aimed to estimate the diversity and intrapopulation spatial genetic structure of three sympatric tree species of the genus *Eremanthus* in Brazil accessed by ISSR markers. The study found that *Eremanthus glomerulatus* presented the values of genetic diversity superior to the *Eremanthus erythropappus* and *Eremanthus incanus*. The genetic diversity of Nei (*h*) ranged from 0.36 to 0.40 and the Shannon genetic diversity index (*I*) from 0.50 to 0.57. Coancestry was observed in these three species with implications for seed collection. The data obtained in this study can guide conservation projects for the genus *Eremanthus*, contributing to the sustainable management of populations that coexist in the same habitat.

Keywords: Candeia, Cerrado, molecular markers, restoration of degraded areas, genetic diversity, forest management

INTRODUCTION

The genetic knowledge of natural populations is essential to understand the structure and dynamics of alleles of species and consequently for the design of conservation strategies, improvement and sustainable management (Vieira et al. 2012a, Duarte et al. 2015). These studies are essential for the definition of priority areas for conservation (Vieira et al. 2015), proper management of species (Arruda et al. 2015), strategies for seed collection and restoration of degraded areas with native species (Thomas et al. 2014). The conservation of protected areas and plant species requires knowledge of the genetic structure, explaining inter and intraspecific diversity in an ecological context (Fajardo et al. 2014, Pádua et al. 2021).

Molecular markers based in DNA genomic were used to assess the genetic diversity in several organisms. The inter-simple sequence repeat (ISSR) is a molecular marker useful in studies of intra and interspecific genetic diversity (Fajardo et al. 2018, Fajardo et al. 2014, Pádua et al. 2021) with the advantage of not requiring prior information of the DNA sequence, having greater repeatability when compared to other dominant markers (Yang et al. 1996) and applicable for various plant species (Nybom 2004).

Eremanthus erythropappus, *E. incanus* and *E. glomerulatus* from the Asteraceae family occurs as population clusters popularly known as "candeias". They are found in open fields and pastures in the Central Brazilian Savanna and Atlantic Rainforest (Loeuille 2015) in regions with seasonal semideciduous forest. These species are sympatric with extremely ecology and economic values for wood and essential oil. *E. erythropappus* is only exploited and produced in Brazil (Donadelli 2012). The wood is about USD330.00 to USD370.00 per cubic meter and the essential oil has a price ranging from USD50.00 to USD55.00 per kg of oil (Araújo et al. 2018). In the past years, *E. erythropappus* were

mainly and intensively exploited without any proper management plan (Araújo et al. 2018), affecting the ecology and genetics of their natural populations (Pádua et al. 2021). *E. incanus* is used more for the production of fence post, since it has small quantity and low quality essential oil such as alfabisabolol. *E. glomerulatus* does not have much commercial value but as it occurs in common areas with the other two species, its conservation is endangered by the exploitation of *E. incanus* and *E. erythropappus*.

According to the Botanic Gardens Conservation International (BGCI) and International Union for Conservation of Nature (IUCN) *E. glomerulatus* and *E. incanus* are on the Red List of Threatened species in the category of the threat of Least Concern (LC) (Global Tree Specialist Group 2019a & 2019b). These three species have been also listed as presumed threatened species of the flora of Minas Gerais state, Brazil and even although there is not sufficient information to justify classifying them as threatened, there are nevertheless some indications that might eventually endorse this condition (Mendonça & Lins 2000). In addition to the economic importance, the ecological relevance of the natural candeias populations is significant. The flowers are small and pink, hermaphrodites and organised into the dense capitulum. They have high percentage of pollen viability, but limited availability of nectar for floral visitors, usually *Apis mellifera* and *Trigona* sp. bees (Vieira et al. 2012b). The seeds are anemochoric (Vieira et al. 2012b). Currently, there is a demand for planted forests for the purpose of commercial exploitation of the species, especially *E. erythropappus* (Araújo et al. 2018).

Based on the ecological and economic importance of these species, safeguarding adequate genetic conservation is essential. Among the strategies of genetic conservation is the possible *in-situ* conservation for species to maintain their evolutionary process and ensure the conservation of other species of living organisms in the environment (White et al. 2007). However, these conservation areas were often surrounded by agricultural areas and composed of forest fragments whose genetic diversity may be reduced or the present of intra-populational spatial genetic structure.

The study aimed to assess the genetic diversity through ISSR markers of three sympatric species of *Eremanthus* in the south of Minas Gerais, to analyze the spatial genetic structure of plants within populations and between species and to generate information useful for the *in-situ* genetic conservation of these species. The study hypothesised that the natural species populations in the fragmented landscape have an aggregated spatial genetic structure among nearby trees with implications for seed collection and management.

MATERIALS AND METHODS

Study and sampling location

Foliar material was collected in the Quedas do Rio Bonito Ecological Park, located approximately 13 km from the municipality of Lavras, MG (21°19'S, 44°59'W) at an altitude ranging between 950 and 1200 m. The Park is located in the Serra do Carrapato region, which is part of the Serra da Bocaina complex. The local climate according to Köppen classification is a transition between Cwb and Cwa climate seasoned with dry winters, with an average annual precipitation of 1529.7 mm and an average annual temperature of 19.4 °C.

The Quedas do Rio Bonito Ecological Park is a valuable sample location for the primitive vegetation of the Alto Rio Grande region because of its five main physiognomic types such as forest, cerrado, rupestrian field, altitude field and candeal which are well represented and reasonably preserved (Oliveira-Filho & Fluminham-Filho 1999). The main rocks are quartzites and mica schists in the highest parts and leucocratic granitic gneisses and quartzites in the lowest parts (Curi et al. 1990).

In order to quantify the genetic diversity of *E. erythropappus*, *E. glomerulatus* and *E. incanus* sixty adults trees of each species (n = 180 trees) were sampled. These samples were georeferenced in a plot of 600 m^2 (Figure 1). Leaves from each individual were collected, identified, placed in boxes containing silica gel and taken to laboratory for DNA extraction.

Genomic DNA extraction and ISSR amplification

The DNA extraction protocol from Moog and Bond (2003) with modifications was used. A total of 20 primers from the UBC set #9 (Vancouver, Canada) (Table 1) were tested and samples

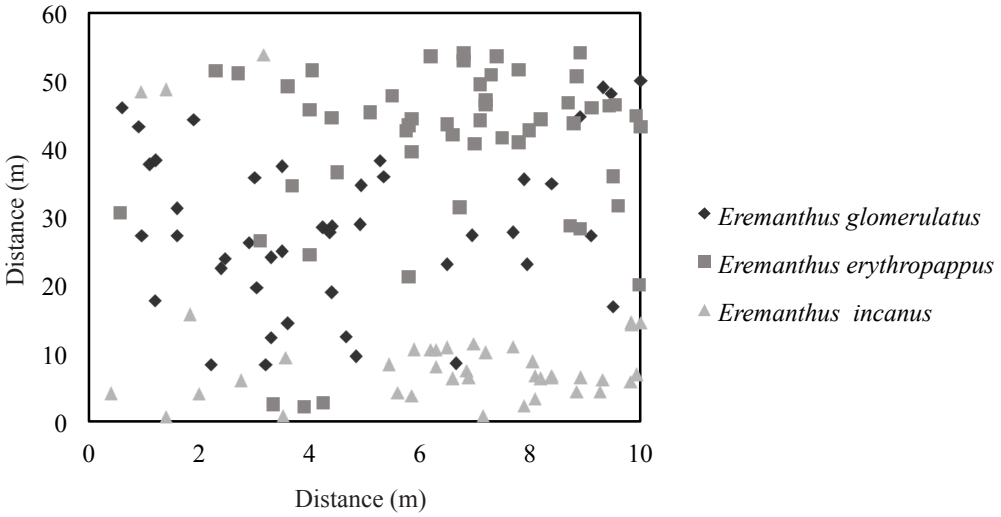


Figure 1 Distribution of individuals in the plot

Table 1 ISSR primers used to characterise the genetic diversity and spatial distribution of *Eremanthus erythropappus* (EE), *Eremanthus glomerulatus* (EG) and *Eremanthus incanus* (EI) and their respective sequences and number of locus

Primer	*Sequence (5'–3')	Locus		
		EE	EG	EI
BECKY	CACACACACACACAYC	-	8	-
JHON	AGAGAGAGAGAGAGY C	-	11	-
MANNY	CACCACCACCACRC	7	9	8
OMAR	GAGGAGGAGGAGRC	-	14	7
UBC807	AGAGAGAGAGAGAGAGT	10	-	12
UBC808	AGAGAGAGAGAGAGAGC	8	-	12
UBC809	AGAGAGAGAGAGAGAGG	8	-	15
UBC810	GAGAGAGAGAGAGAGAT	-	10	-
UBC827	ACACACACACACACACG	10	8	8
UBC834	AGAGAGAGAGAGAGAGYT	14	11	8
UBC835	AGAGAGAGAGAGAGAGYC	13	12	11
UBC841	GAGAGAGAGAGAGAGAYC	11	7	-
UBC842	GAGAGAGAGAGAGAGAYG	-	11	5
UBC855	ACACACACACACACACYT	-	8	9
UBC857	ACACACACACACACACYG	8	-	10
UBC860	TGTGTGTGTGTGTGTGRA	-	-	8
UBC864	ATGATGATGATGATGATG	10	-	-
UBC889	DBDACACACACACACAC	10	-	-
UBC898	CACACACACACACARY	-	8	-
UBC901	GTGTGTGTGTGTYR	14	5	6
	Total	123	122	119

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

with good quality of amplification and a greater number of fragments were selected. PCR was performed in a solution containing 20 ng of template DNA, 10 × PCR buffer (500 mM Tris-HCl pH 8.0, 200 mM KCl, 0.25 mM BSA, 200 mM Tartrazine and 1% Ficoll), 2.6 mM $MgCl_2$, 0.25 mM of each dNTP, 0.125 U *Taq* DNA polymerase, 0.4 µM of ISSR primer and ultrapure water to bring the reaction volume to 12 µL. Amplification was performed in a GeneAmp PCR System 9700 thermal cycler with an initial denaturation at 94 °C for 3 min, followed by 37 cycles of denaturation at 94 °C for 1 min, annealing at 47 °C for 2 min and extension at 72 °C for 2 min and a final extension at 72 °C for 7 min. The amplified products were separated using a horizontal electrophoresis in 1.5% agarose gel (w/v) at 120 V for 150 min in 1X TAE buffer (Tris-Borato EDTA). After the gel staining process in ethidium bromide, visualisation of the amplification products was performed under ultraviolet and a binary data matrix was constructed with the presence (denoted by 1) or absence (denoted by 0) of the fragments.

The optimal number of markers and genetic diversity

The number of ISSR markers sufficient to obtain accurate estimates of genetic diversity were estimated using bootstrap in GENES program (Cruz 2001). In the analysis, the correlation estimates of similarity matrix values with other matrices generated with different numbers of markers and the stress value (E) which indicated the fit between the original matrix and the simulated matrix were obtained. In the condition where the E estimate was less than 0.05 the number of markers for the analyses were considered sufficient (Kruskal 1964, Dias & Kageyama 1998).

The loci were considered polymorphic (% *P*) when the frequency of the most common allele did not exceed 0.95 (Nei 1978). The characterisation of intrapopulation genetic diversity was estimated by the genetic diversity, Shannon index (*I*), Nei's genetic diversity (*h*), number of observed alleles (*Na*) and effective number of alleles (*Ne*) in PopGene program (Yeh et al.1997). The genetic similarity between each pair of genotypes was analyzed using the Jaccard coefficient, followed by the simplified representation of the distances using a dendrogram obtained by the agglomerative hierarchical method of the unweighted pair-group method using arithmetic averages (UPGMA) in NTSYS-PC 2.0 program (Rohlf 1992).

Spatial genetic structure (SGS)

The coefficient of coancestry (kinship, F_{ij}) was estimated between pairs of individuals for each distance class with the use of the SPAGEDI program (Hardy & Vekemans 2005). In the analysis, a coefficient equal to zero indicated inbreeding. Based on the standard error of the mean of the estimates obtained by jackknife resampling, the confidence intervals were constructed at 95% probability (Hardy & Vekemans 2005). A total of 1000 permutations within each class were made to test the occurrence of spatial genetic structure (SGS). In addition, the extent of SGS using the *Sp* statistic (Vekemans & Hardy 2004) in the program SPAGeDi was also estimated.

RESULTS

The optimal number of ISSR markers

Twelve ISSR primers were selected to characterise the genetic diversity of *E. erythropappus*, 13 for *E. glomerulatus* and *E. incanus* generating a total of 123, 122 and 119 markers respectively (Table 1). Bootstrap analysis indicated that 87 markers were sufficient to calculate accurate estimates of genetic diversity parameters for *E. erythropappus* (r = 0.95, E = 0.04), as well as for *E. glomerulatus* with 85 markers (r = 0.96, E = 0.04) and *E. incanus* with 65 markers (r = 0.94, E = 0.04) (Figure 2).

Genetic diversity

E. glomerulatus presented the highest values of genetic diversity for all parameters studied, followed by *E. erythopappus* and *E incanus* (Table 2). The genetic diversity of Nei (*h*) ranged from 0.36 (*E. erythropappus*) to 0.40 (*E. glomerulatus*) and the Shannon genetic diversity index (*I*) from 0.50 (*E. incanus*) to 0.57 (*E. glomerulatus*). The percentage of polymorphic loci was above 85% for the three species.

Genetic similarity

Based on the genetic similarity generated from the samples (Figures 3, 4 and 5), the greatest

Table 2 Diversity index estimated for *Eremanthus erythropappus* (EE), *Eremanthus glomerulatus* (EG) and *Eremanthus incanus* (EI)

Diversity index	Species		
	EE	EG	EI
h	0.36 (0.13)	0.40 (0.12)	0.34 (0.17)
I	0.53 (0.17)	0.57 (0.16)	0.50 (0.23)
Na	1.94 (0.23)	1.95 (0.22)	1.86 (0.35)
Ne	1.62 (0.29)	1.71 (0.27)	1.58 (0.34)
LP	116	116	102
%P	94.31	95.08	85.71

h = Nei index, *I* = Shannon index, *Na* = number of observed alleles, *Ne* = effective number of alleles, LP = polymorphic loci, *%P* = percentage of polymorphic loci, ( ) = standard deviation

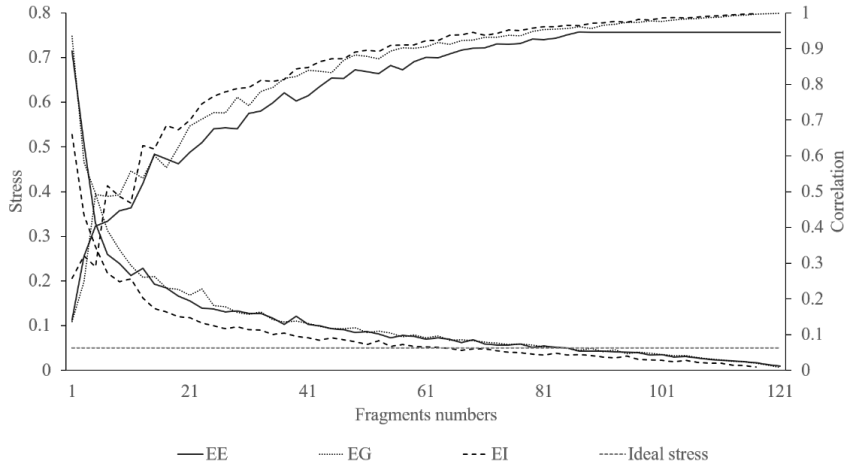


Figure 2 Analysis of optimal number of fragments for *Eremanthus erythropappus* (EE), *Eremanthus glomerulatus* (EG) and *Eremanthus incanus* (EI)

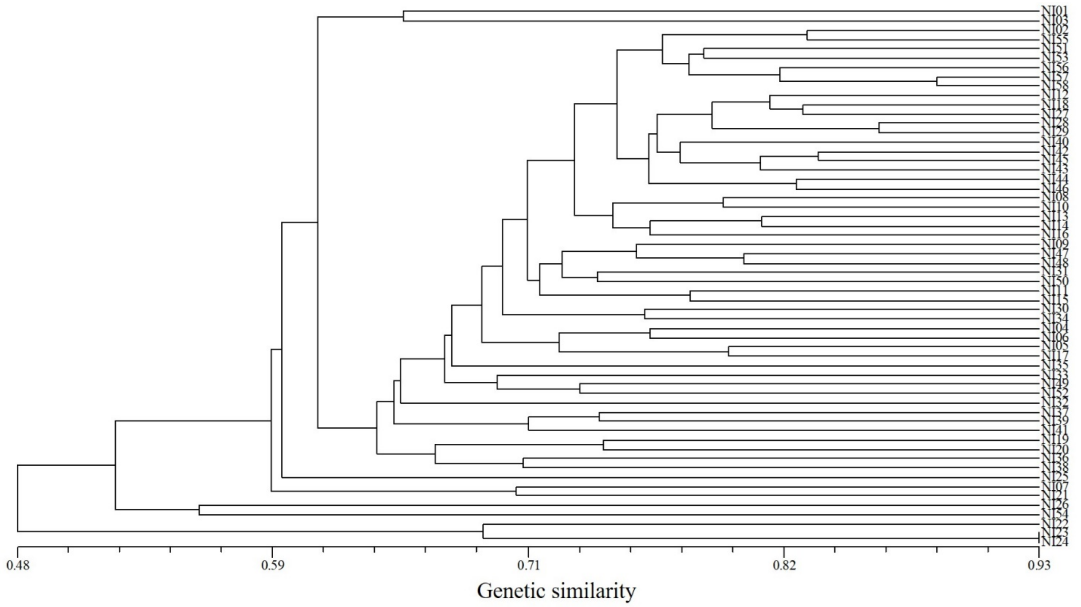


Figure 3 UPGMA dendrogram representing the genetic similarity between individuals of the species *Eremanthus erythropappus*

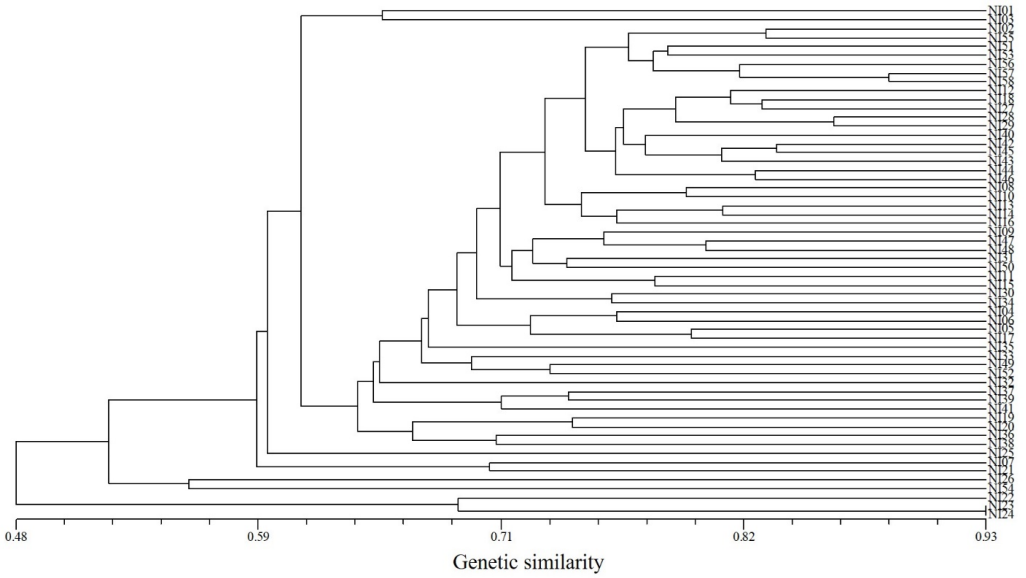


Figure 4 UPGMA dendrogram representing the genetic similarity between individuals of the species *Eremanthus glomerulatus*

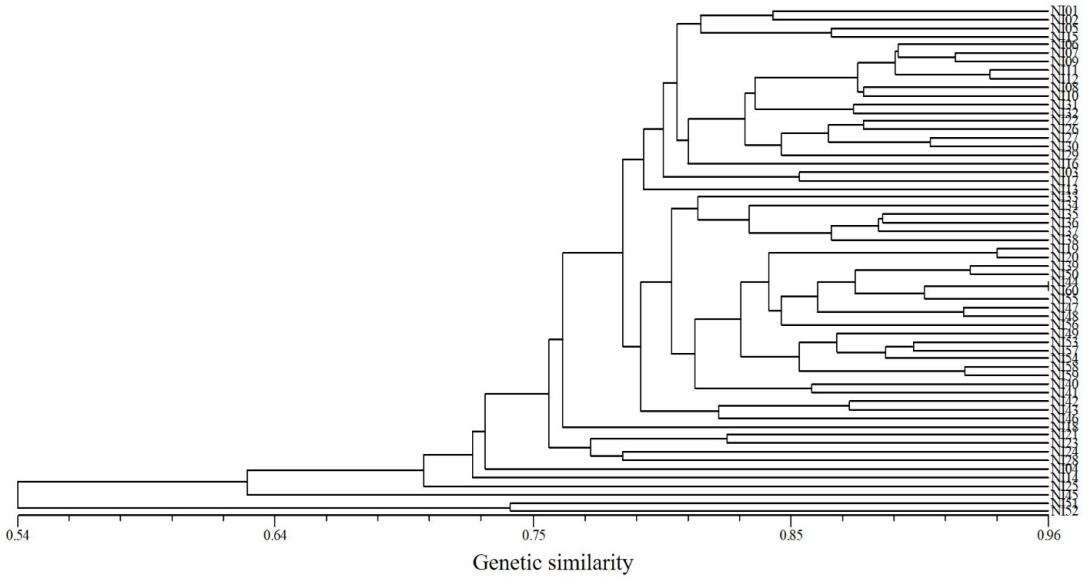
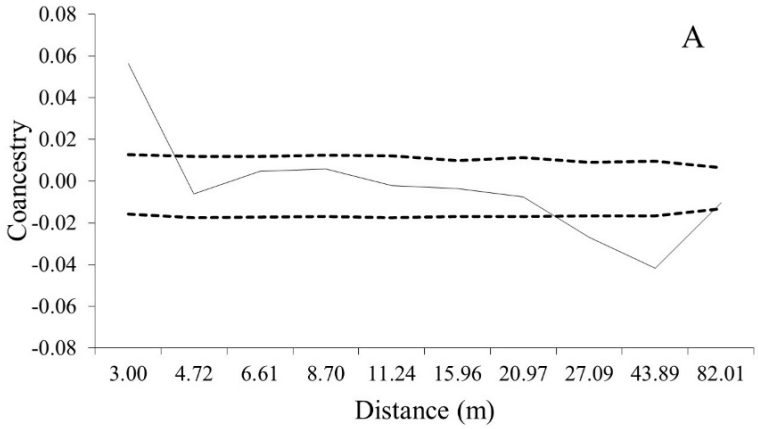


Figure 5 UPGMA dendrogram representing the genetic similarity between individuals of the species *Eremanthus incanus*

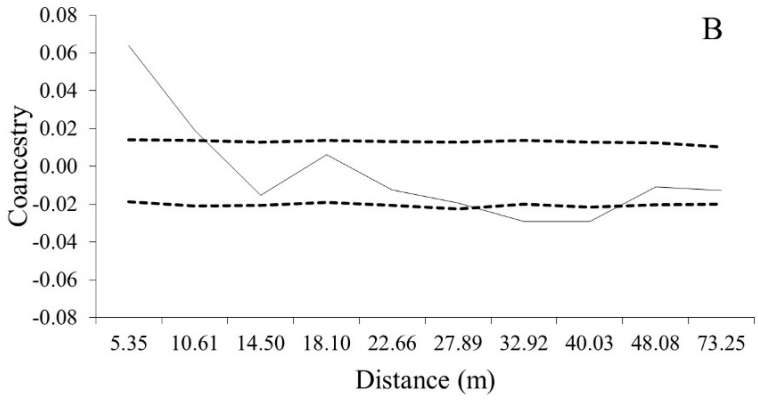
values of *E. erythropappus* individuals was 0.92 and the lowest 0.50. *E. glomerulatus* showed individuals with a similarity between 0.93 and 0.48. The genetic similarity among *E. incanus* individuals ranged from 0.54 to 0.96.

Spatial genetic structure

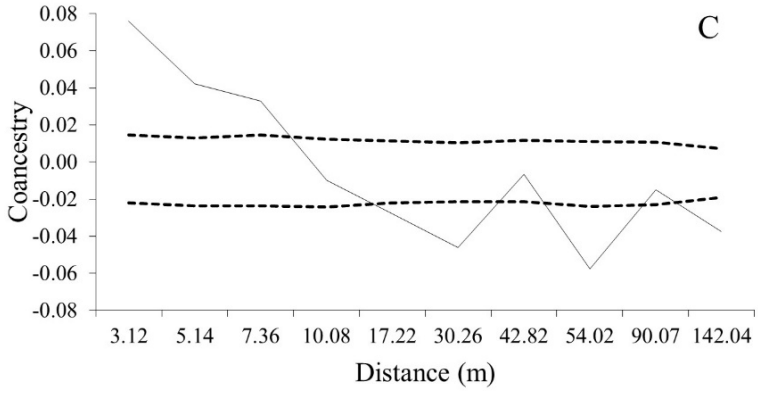
The spatial distribution of *E. erythropappus* genotypes indicated the presence of significant spatial genetic structure in the first distance class (up to 3 m), where the estimated coancestry coefficient was 0.056 (p = 0.00) (Figure 6A). *E. glomerulatus* showed spatial genetic structure of the genotypes up to class 2 (10.61 m) with coancestry values for classes 1 and 2 of 0.064 (p = 0.00) and 0.019 (p = 0.01), respectively (Figure 6B). *E. incanus* presented spatial structure up to class 3 (7.36 m) with the observed coancestry values of 0.076 (p = 0.00), 0.042 (p = 0.00) and 0.033 (p = 0.00), respectively (Figure 6C). For the three species in the other



Dotted line indicates confidence interval



Dotted line indicates confidence interval



Dotted line indicates confidence interval

Figure 6 Correlograms of the coancestry coefficient (kinship) by distance classes for individuals of three sympatric species of *Eremanthus*. *Eremanthus erythropappus* (A), *E. glomerulatus* (B) and *Eremanthus incanus* (C)

classes, the coancestry values were close to zero or negative, suggesting the random distribution of the genotypes or reduction of kinship between individuals geographically distant.

Although *E. incanus* had a kinship structure up to the third class, it was important to note that the maximum structuring distance was 7.36 m. *E. glomerulatus* exhibited a kinship structure up to the second class, where the range of this aggregation was 10.61 m. The *Sp* values revealed patterns of SGS (average $Sp = 0.027$) and the slope of the regression of F_{ij} was significant ($p < 0.01$).

DISCUSSION

The investigation involved the study of genetic diversity and spatial genetic structure of *Eremanthus* species in a natural population represented by a primitive vegetation in south of Minas Gerais State. Study results showed high values of genetic diversity for the studied species. The high genetic diversity was a characteristic of these species (Estopa et al. 2006, Freitas et al. 2008, Pádua et al. 2021). Mechanisms such as sporophytic self-incompatibility, commonly found in species of Asteraceae, might prevent inbreeding by self-pollination and cross-breeding between relatives, which led to maintaining high genetic diversity in populations of the species (Ferrer et al. 2004). Therefore, the identification of areas with natural occurrence of these species which conserved their high diversity was essential to identify future seed sources to be used in restoration processes. *Eremanthus* species occurred in early stages of succession, although they were not classified as pioneers due to their long life cycle. Some occurred in pure stands and in shallow soils that would hardly be occupied by other tree species (Scolforo et al. 2012). In addition to forest restoration issues, identifying areas with high diversity of these species was important for their breeding programmes.

Our study showed that *E. glomerulatus* had the highest number of polymorphic loci, followed by the species *E. erythropappus*. Even with a smaller number of primers used (12), *E. erythropappus* presented a higher number of loci (123) in relation to *E. incanus* (119 loci) and *E. glomerulatus* (122 loci) by using 13 primers. Studies on the species of *Eremanthus* found *I* values ranging from 0.45 to 0.49 (Estopa et al. 2006), 0.44 to 0.45 (Freitas et al. 2008) and 0.39 to 0.54 (Pádua et al. 2021).

The genetic diversity in tree species occurred mainly within populations (Hamrick & Godt, 1990) if the genetic divergence was low in natural areas, due to the intense gene flow. In disturbed area through loss or fragmentation events in the present environment, the knowledge of genetic diversity of populations is crucial in genetic conservation, management and forest improvement programmes. Identifying contrasting genotypes was a strategy to delineate the sampling of plants and seeds to *ex-situ* collections (Hoban & Strand 2015) to improve the variability diversity. Reproduction mechanisms could be considered as the main responsible for genetic dissimilarity. For the studied species, anemochoric dispersion (Vieira et al. 2012b) and incompatibility for self-fertilisation (Ferrer et al. 2004) were characteristics that benefited genetic dissimilarity among individuals.

The spatial genetic structure occurred in response to the restricted dispersion of seeds and pollen of species, distribution habitat and micro environmental selection (Loiselle et al. 1995). Knowledge of population spatial genetic structure provideed important information for defining the purposes of conservation or genetic improvement. Other studies with *E. erythropappus* (Pádua et al. 2016) and tropical tree species also found a spatial aggregation of genotypes (Vieira et al. 2010, Vieira et al. 2012a, Pinheiro et al. 2017). The *Sp* statistic was useful to compare among species and the spatial genetic structure in *Eremanthus* sp. (average $Sp = 0.0270$) was similar to that observed for small trees ($Sp = 0.0259$) and gravity-dispersed species ($Sp = 0.0281$) (Vekemans & Hardy 2004). However, it was important that the analysis for each species to be conducted stringently since the aggregation pattern could vary according to the environmental characteristics of each population. Factors such as abundance of seed dispersers, density and others processes that could occur at the landscape level which affected the spatial and temporal genetic structure. A past study on *E. erythopappus* to assess spatial genetic structure in overexploited natural populations. The spatial genetic structure was observed in five of the nine studied populations (Pádua et al. 2016), showing a pattern that was not homogeneous across populations of the same species, especially if there were different degrees of conservation and exploitation.

Based on the results of spatial genetic structure, it was possible to establish conservation and management strategies in this studied area. Seed collection at the studied site aiming at *ex-situ* conservation could be performed from distances greater than 3.0 m for *E. erythropappus*, 10.0 m between *E. glomerulatus* trees and 7.0 m among *E. incanus* trees. Seeds collected in shorter distances than those detected by the analysis of spatial distribution showed some degree of kinship. A sampling of these individuals could generate inbred individuals in the following generations, compromising the fitness of the species over time.

The *Eremanthus* sp. in the studied area needed the attention from forest managers because it is a priority area for the conservation of the species in order to maintain or to increase their genetic variability. The study of Rocha et al. (2021) in forest management under a silvicultural system of seed-trees on the genetic diversity and spatial genetic structure of *Eremanthus erythropappus* showed that the mean levels of genetic diversity were quite high but not significantly different. However, their study found a positive relationship among spatial genetic structure and forest management for juvenile cohorts. The findings indicated an effect of forest management on spatial genetic structure and a tendency for increasing allelic richness in both life stages in the managed stand. The high patterns of genetic diversity might be an effect of increasing pollen/seed flow among different populations. The present study found high genetic diversity nonetheless the genotypes were spatially structured. Such area could be a source for seedlings production to the local farmers interested in restoration or commercially-planted forest, therefore it was appropriate to abide by the distance among each trees to maximise the capture of genetic diversity for the new seedlings. Species such as *Eremanthus* have long been used for commercial plantations. In this current decade, plant restoration is one of the priorities of the United Nations and the information obtained from the study will be useful for conservation practices.

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