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EDITED BY  
Alessandro Alves-Pereira,  
State University of Campinas, Brazil

REVIEWED BY  
Mara Fernandes Moura,  
Instituto Agronômico de Campinas  
(IAC), Brazil  
Igor Hamoy,  
Federal Rural University of the  
Amazon, Brazil

\*CORRESPONDENCE  
Marcia Flores da Silva Ferreira  
marcia.ferreira@ufes.br

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# Genetic diversity of juçara palm: An alternative for selection and conservation in cash crop for fruit production

Liana Hilda Golin Mengarda, Guilherme Bravim Canal,  
Marina Santos Carvalho, Adésio Ferreira and  
Marcia Flores da Silva Ferreira\*

Laboratório de Genética e Melhoramento Vegetal, Departamento de Agronomia, Centro  
de Ciências Agrárias e Engenharias, Universidade Federal do Espírito Santo, Alegre, ES, Brazil

The diversity of juçara palm (*Euterpe edulis*) must be investigated at the molecular level to enable the adoption of appropriate management strategies that may associate breeding studies with conservation. With the aim of describing the genetic diversity and structure of a population of *E. edulis* in a commercial plantation area, the genotyping of 199 individuals was performed using nine nuclear microsatellite markers. We detected inbreeding in the population and the diversity within the plots was high showing diversity among the individuals. The individuals from matrices germplasm of the locality Santa Marta, were divergent. The diversity indices observed in the studied population were close to those reported in the literature for natural populations; thus, the *ex situ* conservation of the population in this managed is feasible, as is their potential use in breeding studies.

## KEYWORDS

diversity, plant breeding, SSR, *Euterpe edulis* Mart., threatened species

## Introduction

The juçara palm (*Euterpe edulis* Mart., Arecaceae) is a species from the Brazilian Atlantic Forest that plays an important ecological role, but this palm is threatened with extinction. Biome fragmentation, with a consequent reduction of gene flow, and the illegal cutting of the species to obtain heart of palm have been reducing the genetic variability of this species in the forest remnants (Seoane et al., 2005; Vieira et al., 2010; Santos et al., 2015). The fragmentation and the exploitation can significantly impact the loss of diversity, in the other hand the maintenance to the gene flow and allelic exchanges are crucial to define conservation efforts of the *E. edulis* (Montagna et al., 2018).

The use of Juçara palm for the production of fruit pulp represents a sustainable alternative to the illegal extraction of heart of palm (Silva et al., 2013), providing continuous and permanent income generation. The incentive provided by fruit pulp production increases interest in the commercial planting of the species, the planting of agro-ecological crops (Danelli et al., 2016), and reforestation. When evaluating the population biology of *E. edulis* in managed landscape, De Souza et al. (2021) checked it was found the sites assessed outside protected areas also contribute to the conservation of this palm. Such actions, if strategically performed, can increase the genetic variability of the species, aiding conservation. In addition, the establishment of sustainable commercial crops can advance the genetic improvement of the species by allowing the selection of divergent and productive matrices.

The fruit of the juçara palm have a high nutraceutical value and great potential as a functional food. In addition, the pulp, has nutritional characteristics similar to or better than those of açai pulp (*E. oleracea*; Borges et al., 2011; Silva et al., 2013; Schulz et al., 2016). The market for juçai pulp is expanding. Thus, the economic advantage of preserving the species *via* sustainable management, including planting, is apparent.

In the south of Espírito Santo State, Brazil, a heterogeneous area of *E. edulis* cultivation was established over approximately 30 years through natural regeneration and the planting of seedlings originating from seeds. Currently, this area consists of approximately 30,000 plants, which are managed with the objective of fruit pulp production. This managed area may represent a conservation site and a potential source of genetic variability available for breeding studies. However, the genetic diversity of this managed area requires characterization to evaluate this potential.

Genetic diversity studies allow the quantification of genetic variability among and within populations and of genetic divergence among individuals within a population. This knowledge makes it possible to define actions for the characterization and improvement of native species, facilitating the selection of breeding matrices with a high probability of success in crosses as well as the selection of productive cultivars and those adapted to different environments (Barbieri and Maia, 2012). These analyses also allow us to not only respond to ecological issues that are important for the adoption of conservation measures (De Souza et al., 2021; Cerqueira et al., 2022), such as the existence of population structure and the genetic relationship between individuals (Park et al., 2009), but also select divergent individuals for breeding studies, which is important in managed areas.

Microsatellite molecular markers have been used in studies regarding the diversity of natural populations of juçara palm (Gaiotto et al., 2003; Conte et al., 2006, 2008; Novello et al., 2018; Cerqueira et al., 2022; Pereira et al., 2022). These studies have revealed that the occurrence of non-random crosses is relatively common in natural populations of this species, with the possible

occurrence of complete siblings within open-pollinated families (Gaiotto et al., 2003). Additionally, a reduction in the number of reproductive individuals increases inbreeding, favouring changes in the genetic structure of the populations (Conte et al., 2006). Still, the origin of the mother plant affects some genetic parameters. In *E. edulis*, Cerqueira et al. (2022) report that progenies from mother plants of forest fragment inserted in the more forested landscape had a greater number of private and effective alleles, which, in turn, contributes to the diversity maintenance and the conservation.

Anthropic impacts on *E. edulis*, an allogamous species, have also been reported to provide changes in its allelic frequencies, especially because of the reduction of seed dispersers (Carvalho et al., 2017). Furthermore, even though fragmentation and exploitation can significantly impact the loss of diversity, the limitations to the gene flow and maintenance of allelic exchanges are crucial to define conservation efforts of the *E. edulis* (Montagna et al., 2018).

For these reasons, a characterization of the potential for conservation and selection within the area to be evaluated by the present study is important. From the point of view of the ecological viability of the managed area, the management of fruit collection in this population allows for the action of pollinators and dispersers. In addition, the managed area was established from natural regeneration, theoretically presenting great genetic variability, which can be exploited for breeding. These facts suggest that the population of the managed area is highly diverse (Novello et al., 2018; De Souza et al., 2021).

The sustainable management for fruit production in a cultivation area with genetic variability can favor conservation actions for *E. edulis* and selection of divergent matrices for genetic improvement. Toward these ends, the present study used microsatellite markers to characterize the genetic diversity and structure of the *E. edulis* population in the managed area, with the aim of contributing to the sustainable management, improvement, and maintenance of this species diversity.

## Materials and methods

### Study area and sampling

The study was conducted in an area of approximately 20 hectares with predominance of *Euterpe edulis*, totaling around 20,000 plants (1,500 plants ha<sup>-1</sup>). This area belong to two companies that process juçaizeiro fruit pulp, Açai Juçara® and Bonalotti®) and located in the municipality of Rio Novo do Sul, Espírito Santo State, Brazil (at longitude 20°48'29.7", latitude 40°56'3.5", and a mean elevation of 467 m). The climate is classified according Köppen and Geiger (1928) as tipe "Aw."

1 <https://power.larc.nasa.gov/data-access-viewer/>

The municipality has an average annual temperature of 24.08°C and an average temperature of the coldest month above 20°C, the daily averages can reach values below 18°C. The plantation is conducted for the purpose of producing fruit and processing juçara pulp (juçai), without a pre-defined spacing, no productive management treatment and no controls of diseases and pests, only mowing at harvest time. In the plantation there are a mixture of individuals from of spontaneous development and enrichment by the owners by the sowing of seeds in the area.

The study area was sampled by twenty plots distributed over the entire area. Only ten plants were collected per plot (as a subsample), totaling 200 plants (Figure 1). Only adult plants with bunches were sampled in each plot. The plots definition followed the history of regeneration in the planted area. Fragments of stipe collected from each plant were lyophilized and stored in a freezer at -30°C for the further extraction of DNA, according Carvalho et al. (2019).

Some older plants – identified as 182, 183, 184, 185, and 186 – were also collected, regardless of the presence of bunches. Plots 1–19 consisted of plants from mostly natural regeneration, with the plants from plots 1, 2, 3, 7, and 19 being the oldest (over more than 30 years), according to the landowner. Plot 20 represented the most recent area (12–15 years), established with the planting of seedlings propagated from seeds collected from plants in the district of Santa Marta, in the municipality

of Vargem Alta, Espírito Santo State, Brazil (about 50 Km far from sampling area).

## Molecular analyses

The genomic DNA of the 200 individuals was extracted according to the CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle, 1990), with modifications. After extraction, the DNA quality and concentration were estimated using a spectrophotometer (NanoDrop 2000/2000c, Thermo Fisher Scientific, Waltham Massachusetts, EUA), and the DNA integrity was verified by 0.8% agarose gel electrophoresis.

The samples were genotyped with nine microsatellite loci (Gaiotto et al., 2001; Table 1 and Supplementary Table 1). Polymerase chain reactions (PCRs) were performed in a volume of 15 µL containing 30 ng of genomic DNA, 1x Buffer IO (Synapse), 0.15 µM of each primer, 1.5 mM MgCl<sub>2</sub>, 0.25 mM dNTPs, and 1/2 U of Taq DNA polymerase. The following thermocycling programme was used: 94°C for four min, 30 cycles composed of 94°C for one and a half minutes, the annealing temperature of the primer (according Table 1) for 1 min, and 72°C for 1 min, and a final extension of 72°C for 7 min.

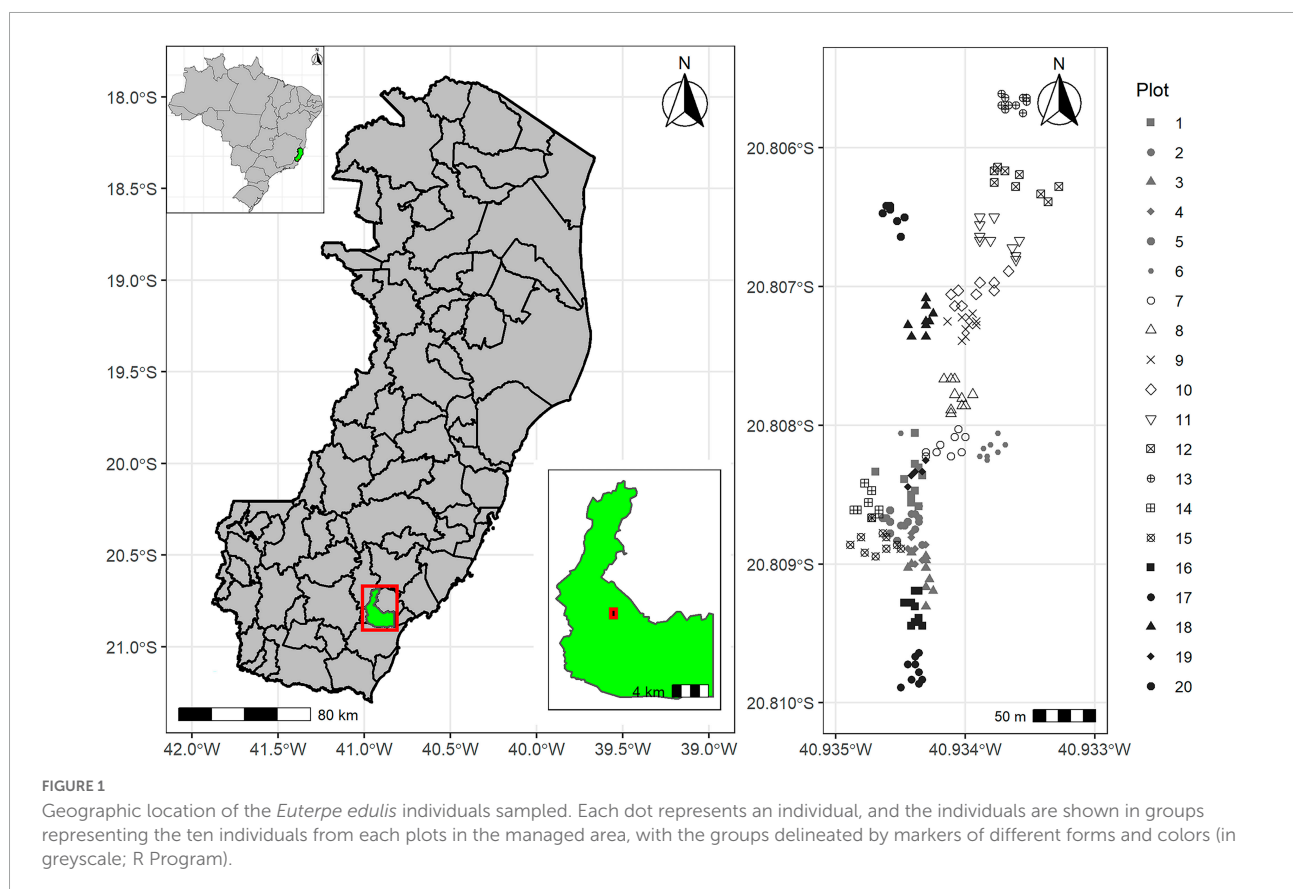


TABLE 1 Genetic diversity indices for *Euterpe edulis* microsatellite loci in 199 plants.

SSR	Motif	Ta	AS	A	H <sub>o</sub>	H <sub>e</sub>	f	PIC
EE5	(AG) 24	56	90–144	9	0.64	0.74	0.13	0.71
EE8	(AG) 20	62	92–108	2	0.53	0.45	−0.17	0.35
EE9	(AG) 19	62	77–103	4	0.50	0.53	0.07	0.43
EE23	(A) 14 (AG) 23	60	90–160	6	0.40	0.66	0.38	0.60
EE41	(AG) 21 (TG) 13	62	100–147	7	0.46	0.74	0.38	0.71
EE45	(AG) 28	62	89–119	4	0.52	0.57	0.09	0.53
EE47	(AG) 20	60	235–275	6	0.58	0.76	0.23	0.72
EE54	(AG) 25	58	108–156	4	0.29	0.65	0.55	0.58
EE63	(AG) 18	62	100–155	6	0.78	0.68	−0.14	0.64
Mean				5.33	0.52	0.64	0.17	0.58

SSR, simple sequence repeat; Motif, simple sequence repeat motif; Ta, annealing temperature (°C); AS, allele size (base pairs); A, number of alleles obtained by direct counts of alleles along the 199 samples in each of the loci; H<sub>e</sub>, expected heterozygosity; H<sub>o</sub>, observed heterozygosity; f, fixation index; PIC, polymorphic information content.

For the genotyping of individuals, the amplification products were subjected to capillary electrophoresis with the 35–500 bp dsDNA kit (Fragment Analyzer<sup>TM</sup>) and analysed by PROSize 2.0 software (Advanced Analytical Technologies, Inc.).

## Diversity and genetic structure

Descriptive analyses of the nine microsatellite loci were performed for the 20 plots. For each locus, the boundary disequilibrium and the Hardy-Weinberg equilibrium, assessed by allelic permutations within the plots, were estimated by Fisher's exact test (Weir, 1996), using FSTAT v. 2.9.3.2 (Goudet, 2002). The following parameters were estimated: number of alleles (A) per locus (mean), expected heterozygosity (H<sub>e</sub>), observed heterozygosity (H<sub>o</sub>), fixation index (f) and number alleles in polymorphic locus in the plot (PA) obtained by the GDA software (Lewis and Zaykin, 2002). Also were estimated the allele number of the plot/total number of alleles (A/ToA), and the polymorphic information content (PIC; Botstein et al., 1980).

The estimates of genetic differentiation among and within the plots were obtained by analysis of molecular variance (AMOVA) by the Arlequin 3.5.2.2 program with two hierarchical levels, using the mean for the nine loci and the 199 individuals in the 20 plots (Excoffier et al., 2005).

The population subdivision was estimated by Wright's (1951) statistic according to the Weir and Cockerham (1984) methods, and the diversity indices were obtained among (F<sub>ST</sub> and G<sub>ST</sub>) and within (F<sub>IS</sub>) the 20 plots, as was the correlation between alleles within an individual relative to the whole population (F<sub>IT</sub>). Estimates of the genetic divergence among plots (D<sub>ST</sub>), the proportion of total diversity among plots (G<sub>ST</sub>), and the f values within plots (G<sub>IS</sub>) were also obtained by FSTAT for Windows, v. 3, 2, 2002 (Goudet, 2002).

Additionally, pairwise F<sub>ST</sub> values were obtained for plot pairs using Arlequin software 3.5.2.2. F<sub>ST</sub> values up to 0.05

are considered indicative of low differentiation, those between 0.05 and 0.15 indicate moderate differentiation, and those above 0.15 indicate high genetic differentiation (Goudet, 2002). Subsequently, Nei's (1978) distance matrix was estimated for the plots, and a grouping was performed according to the unweighted pair group method with arithmetic mean (UPGMA) clustering by and neighbor join techniques using the Genes software.

An analysis of population STRUCTURE among the 20 plots was performed using the inference of genetic groups by the Bayesian [Monte Carlo Markov chain (MCMC)] approach implemented in the software STRUCTURE v. 2.2 (Pritchard et al., 2000). The number of subpopulations was predefined as K = 20 (which would represent the maximum number of clusters, equivalent to the number of plots sampled), with the same number of runs (20) and a burn in of 500,000 replications, followed by one million MCMC steps. The models of allele Correlated allele frequencies) and Admixture were used in the analysis. The most probable K value was determined according to Evanno et al. (2005) by the application Structure Harvester (Earl and Vonholdt, 2012). The CLUMP software was used to align the 20 independent runs of STRUCTURE obtained for the best K value and to generate graphical outputs. Based on the genetic distance matrix between individuals, a principal coordinate analysis (PCoA) was performed using DARwin 6.0.14 software (Perrier and Jacquemoud-Collet, 2006). Also, the UPGMA clustering of individuals was done using Genes software.

## Results

One sample was excluded from the analysis because the sample did not amplify with any of the nine primers; therefore, the sample size was 199 individuals. In nine microsatellite loci with no linkage and with not Hardy-Weinberg equilibrium we detected 48 alleles, with two to nine alleles per locus (mean of

5.3; **Table 1**). Most of the *loci* had a high *PIC* ( $\geq 0.50$ ). The  $H_o$  ranged from 0.29 to 0.78, and the  $H_e$  from 0.45 to 0.76. The *f* (fixation index) value ranged from  $-0.17$  (EE08) to 0.55 (EE54).

The average allele number ranged from 3.0 to 4.2 per plot (**Table 2**). The value of  $H_o$  varied from 0.40 (plots 5 and 18) to 0.65 (plot 8; mean = 0.52); the value for  $H_e$  varied from 0.51 (plot 3) to 0.66 (plot 20; mean = 0.58). The  $H_e$  value exceeded the  $H_o$  value only in plots 8 and 16. Even in the plots with the most positive *f* values (plots 4, 5, 18, and 20, **Table 2**).

According to AMOVA results (**Supplementary Table 2**) most of the genetic variation between individuals was observed within the plots (91.64%), with little variation among the plots (9.36%). The estimate of  $F_{ST}$  was 0.08, significant at the 1% probability level.

According to the degree of population subdivision estimated by the *F* statistics, little differentiation among plots ( $G_{ST} = 0.08$ ) and moderate differentiation within the plots ( $F_{IS} = 0.12$ ) were observed. The value of  $F_{IT}$  was 0.19.

In the pairwise analysis, the plot 20 presented the highest  $F_{ST}$  values (from 0.16 to 0.23) with the plots 1, 3, 4, 5, 6, 7, 9, and 19 and moderate genetic differentiation from the other plots ( $F_{ST}$  ranging from 0.07 to 0.14; **Table 3**). Genetic differentiation (between 0.15 and 0.21) was obtained between the plots: 4 with 13 and 17; plot 5 with 12, 13, and 17; plot 9 with 12, 13, 14, 17, and 18 plots. Among plots 1–8 and among plots 11–15,

low genetic differentiation occurred. Plot 19 showed moderate variation with plots except to 1, 2, 3, 6, 14, 16, and 18 but (**Table 3**). The greatest variation occurred between plots 20 and 3. Lesser differentiation occurred among plots 1–8 and among plots 11–15.

By the cluster analysis one group was formed by plots 1–10, and another formed by plots 11–19. Plot 20 was not grouped (**Figure 2**). In the population structure analysis,  $K = 2$  was the most probable configuration (**Figure 3**). For the plots no obvious clusters were identifiable. For  $K = 5$  among plots 1, 5, and 6, and the individuals in plot 20 were considered homogeneous. The UPGMA based on the dissimilarity among the 199 individuals showed a broad genetic variation between the individuals in the study area (**Figure 4**). The same results were shown by the PCoA analysis, in which the percent of the variation do the two PC explain in the PCoA for the axis 1 was 35.95% and for the axis 2 16.82% (**Supplementary Figure 2**).

## Discussion

The genetic diversity observed in the population of *E. edulis* had greater genetic variation within than between plots. The high content of polymorphic information and the independence of the studied microsatellites indicate a good estimate of the

TABLE 2 Indices of genetic diversity for plots in an *Euterpe edulis* managed area.

Plot	N	A	A/ToA	$H_o$	$H_e$	<i>f</i>
1	9.78	3.44	0.63	0.55	0.57	0.04
2	9.89	3.78	0.69	0.54	0.59	0.10
3	9.89	3.00	0.55	0.48	0.51	0.05
4	10.0	4.00	0.73	0.42	0.57	0.27
5	10.0	3.56	0.65	0.40	0.54	0.28
6	9.44	3.67	0.67	0.56	0.60	0.07
7	9.56	3.67	0.67	0.56	0.59	0.05
8	9.67	4.22	0.78	0.65	0.63	$-0.04$
9	9.56	3.78	0.69	0.50	0.55	0.08
10	8.56	3.89	0.71	0.60	0.61	0.02
11	9.89	3.56	0.65	0.56	0.60	0.04
12	9.67	4.00	0.73	0.54	0.63	0.14
13	9.89	3.78	0.69	0.51	0.57	0.11
14	9.22	3.33	0.61	0.51	0.59	0.14
15	9.11	3.56	0.65	0.59	0.62	0.05
16	8.78	3.22	0.59	0.54	0.53	$-0.02$
17	9.22	3.67	0.67	0.56	0.61	0.10
18	10.0	3.67	0.67	0.40	0.57	0.32
19	9.22	3.67	0.67	0.46	0.53	0.15
20	9.44	3.89	0.71	0.49	0.66	0.26
Mean	9.54	3.67	0.67	0.52	0.58	0.12

N, mean sample size; A, number of alleles (mean per plot); A/ToA, (number of alleles in a plot)/(total number of alleles);  $H_o$ , observed heterozygosity;  $H_e$ , expected heterozygosity; *f*, fixation index.

TABLE 3 Pairwise analysis of *FST* in 20 plots of the managed area of *Euterpe edulis*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
2	-0.003																			
3	-0.002	0.009																		
4	0.011	0.005	0.0291																	
5	0.004	0.001	0.0434	-0.018																
6	-0.008	-0.006	0.0470	-0.006	-0.002															
7	0.019	-0.002	0.0170	0.024	0.012	0.009														
8	0.022	0.027	0.0448	0.021	0.018	0.026	0.023													
9	0.060	-0.002	0.0611	0.004	0.010	0.023	0.006	0.062												
10	0.086	0.037	0.0993	0.042	0.061	0.074	0.063	-0.006	0.068											
11	0.114	0.082	0.1288	0.103	0.143	0.096	0.093	0.087	0.144	0.053										
12	0.102	0.110	0.1413	0.130	0.154	0.117	0.122	0.089	0.187	0.053	0.013									
13	0.119	0.147	0.1490	0.176	0.200	0.139	0.147	0.117	0.187	0.053	0.038	-0.015								
14	0.053	0.073	0.0900	0.098	0.110	0.160	0.083	0.087	0.187	0.053	0.036	0.010	0.010							
15	0.041	0.052	0.0954	0.041	0.062	0.031	0.071	0.089	0.093	0.053	0.048	0.050	0.082	0.025						
16	0.082	0.049	0.1245	0.057	0.010	0.027	0.075	0.117	0.062	0.053	0.055	0.078	0.082	0.080	0.039					
17	0.100	0.113	0.1556	0.159	0.185	0.010	0.132	0.087	0.209	0.053	0.055	0.004	0.006	-0.001	0.041	0.080				
18	0.073	0.070	0.1224	0.116	0.123	0.088	0.127	0.089	0.152	0.053	0.080	0.025	0.052	0.041	0.090	0.100	0.071			
19	0.012	0.048	0.0498	0.062	0.092	0.040	0.066	0.117	0.109	0.053	0.083	0.071	0.093	0.035	0.090	0.046	0.081	0.045		
20	0.189	0.135	0.2316	0.190	0.202	0.171	0.163	0.138	0.191	0.100	0.101	0.076	0.116	0.139	0.113	0.133	0.083	0.119	0.205	

In light gray statistical significance at 0.05 probability. In dark gray statistical significance at 0.01 probability.

genetic relationships in the population. The values of *f* indicated inbreeding, an outcome similar to that observed for natural populations of the species (Gaiotto et al., 2003; Conte et al., 2006).

A higher frequency of heterozygotes is a positive aspect for conservation (Conte et al., 2006). The *H<sub>e</sub>* mean value (0.58) detected in the studied population was smaller than of natural *E. edulis* populations of adult individuals. *H<sub>e</sub>* values ranging from 0.74 to 0.83 were reported for two populations of 111 and 33 individuals (Gaiotto et al., 2003); four populations of 50 individuals each (Conte et al., 2006); and for two populations of 34 and 46 individuals (Seoane et al., 2005). However, Vieira et al. (2010) reported *H<sub>e</sub>* value of 0.47 for 29 individuals in a forest fragment and recommended introduction of individuals from other populations to increase the effective size of the population and help conserve the species. Additionally, the *H<sub>o</sub>* value observed in our study was 0.52 lower than the reported in the literature reports as 0.68 and 0.63 (Seoane et al., 2005), 0.70 (Conte et al., 2006), between 0.70 and 0.66 (Gaiotto et al., 2003), and 0.59 (Vieira et al., 2010).

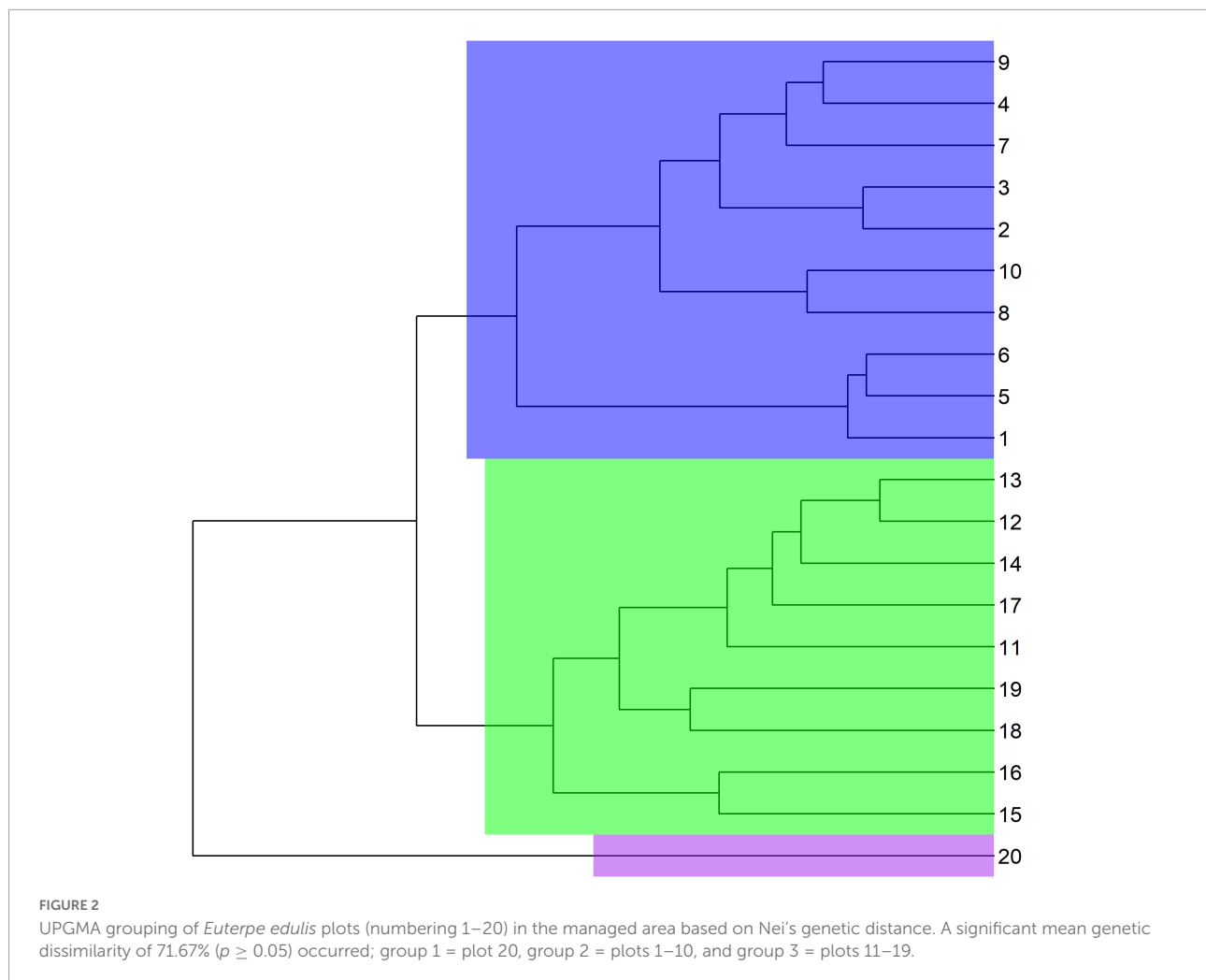
An explanation for these results is the even though the microsatellites markers in this study are the same as Gaiotto et al. (2001, 2003), the number of microsatellites markers used was half. And Gaiotto et al. had two population separated by a distance of 27 km as opposed to current study which was conducted along one population, studied by subsampling plots.

The variation in *f* values among the plots of the area ranged from -0.04 to 0.32. Positive values of fixation index indicate

inbreeding among individuals, a condition observed especially in plots 4, 5, 18, and 20 (*f* ≥ 0.26). However, the mean *f* value of the population (0.12) was the same as or close to that reported for natural populations of *E. edulis* (Gaiotto et al., 2003; Conte et al., 2006).

In a study on the effects of heart of palm exploitation on the genetic structure of natural populations of *E. edulis* that compared undisturbed with exploited populations, Conte et al. (2006) reported that the exploitation process did not alter the diversity levels or the genetic structure of the exploited populations. However, higher inbreeding levels were observed in the young plants of these populations, and this inbreeding could affect later generations. Seoane et al. (2005) observed increased inbreeding in seedlings and genetic differences in allelic frequencies, probably due to genetic drift. Other studies indicate that in natural populations of *E. edulis*, although the diversity among the individuals of the effective population (at the reproductive stage) is high, genetic drift in the progeny may exist due to anthropic actions affecting the populations of dispersers (Santos et al., 2015; Carvalho et al., 2017).

In assessing the population structure of the *E. edulis* in the secondary forests, rural homegardens, and sites inside a protected area, De Souza et al. (2021) observed that the highest variation in genetic diversity indexes was recorded in rural homegardens, with the presence of alleles that were not found in the protected area. Genetic diversity indexes varied less in the protected area, while the average values were higher in secondary forests.



In general, the above mentioned studies showed  $f$  values close to that observed for the population in the present study; in certain plots (as in plots 8 and 16) the studied population shows greater diversity than that of natural populations. The present findings suggest that no detriment to maintaining diversity exists for this population in its managed setting: this population presents individuals with wide genetic diversity and these individuals are in a large area with a high density of individuals. In the addition, the sustainable exploitation of these individuals (for fruit production) also could allow the action of dispersers and pollinators. Therefore, sufficient genetic variability was observed in the managed area to promote *ex situ* conservation and the selection of promising genotypes for breeding.

Regarding the analysis of genetic structure in the managed *E. edulis* population, most of the genetic diversity was observed within the plots (among the individuals of each plot), as shown by the low  $F_{ST}$  (0.088) and  $G_{ST}$  (0.083) values, which indicate moderate differentiation among the plots. These analyses reveal a sharing of alleles among individuals from various plots. However, plot 20 presented individuals divergent from the

others. Other studies of *E. edulis* diversity also note that most of the genetic variability occurs within populations, confirming the tendency for allogamous species (Cardoso et al., 2000; Seoane et al., 2005; Santos et al., 2015).

The greatest genetic differentiation detected for the plot 20 (pairwise  $F_{ST}$  and clustering analysis) and lesser differentiation among plots 1–8 and among plots 11–15 may be explained by the implementation history of the area. The plots 1–19 mainly contain plants derived from natural regeneration (over more than 30 years). In contrast, plot 20, corresponds to a more recent area (12–15 years), with the planting of seedlings propagated by seeds collected from plants in the district of Santa Marta, in the municipality of Vargem Alta, Espírito Santo about (50 km far from the studied area). In addition, the results of the STRUCTURE, with delta  $K = 5$ , also shows that the plots 1, 5, and 6 as quite different from the others.

The UPGMA clustering showed the genetic dissimilarity between individuals, which may be due to the flow of genes and alleles that are shared among the *E. edulis* plants in the study area. Thus, the analyses performed in the present study

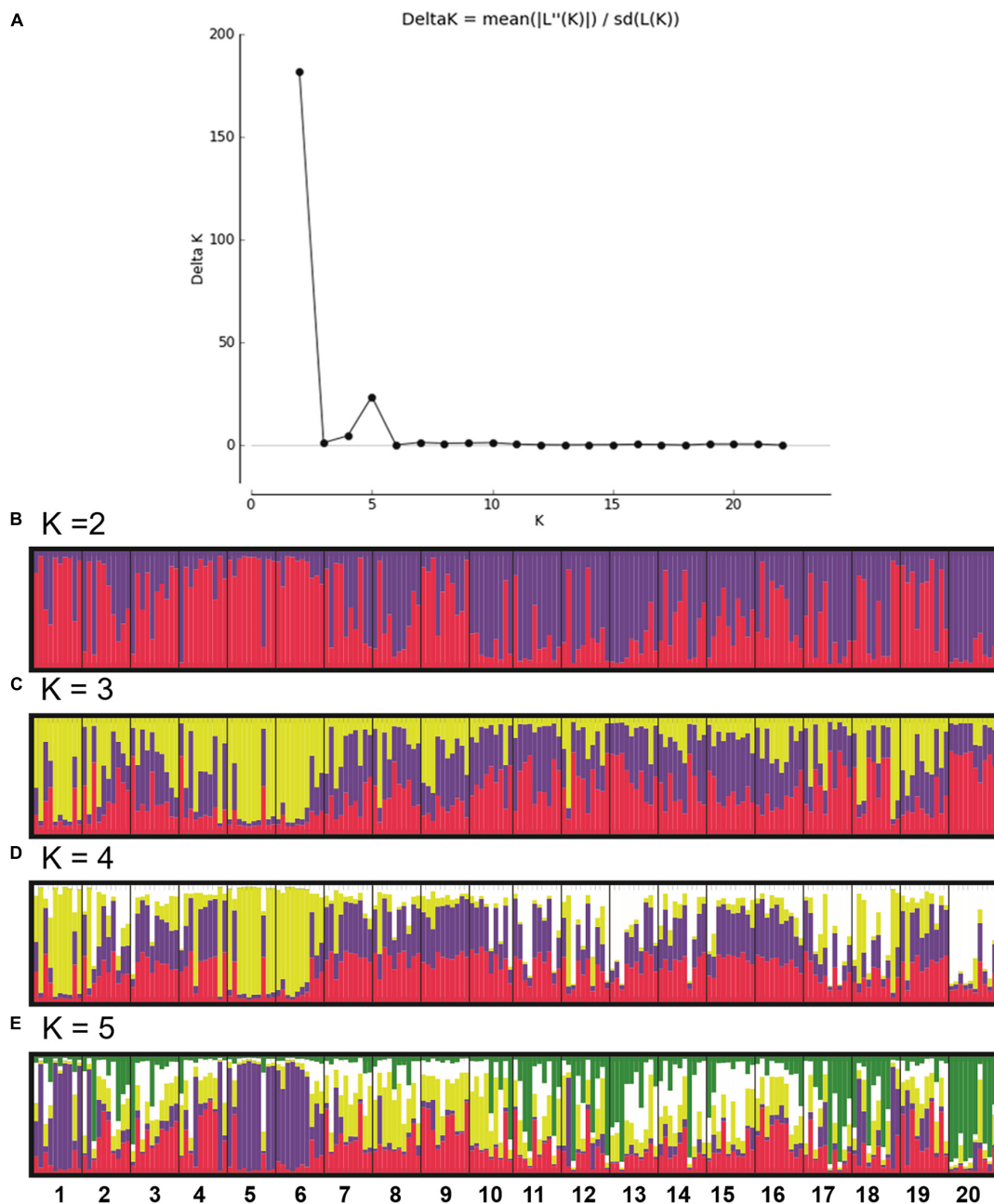


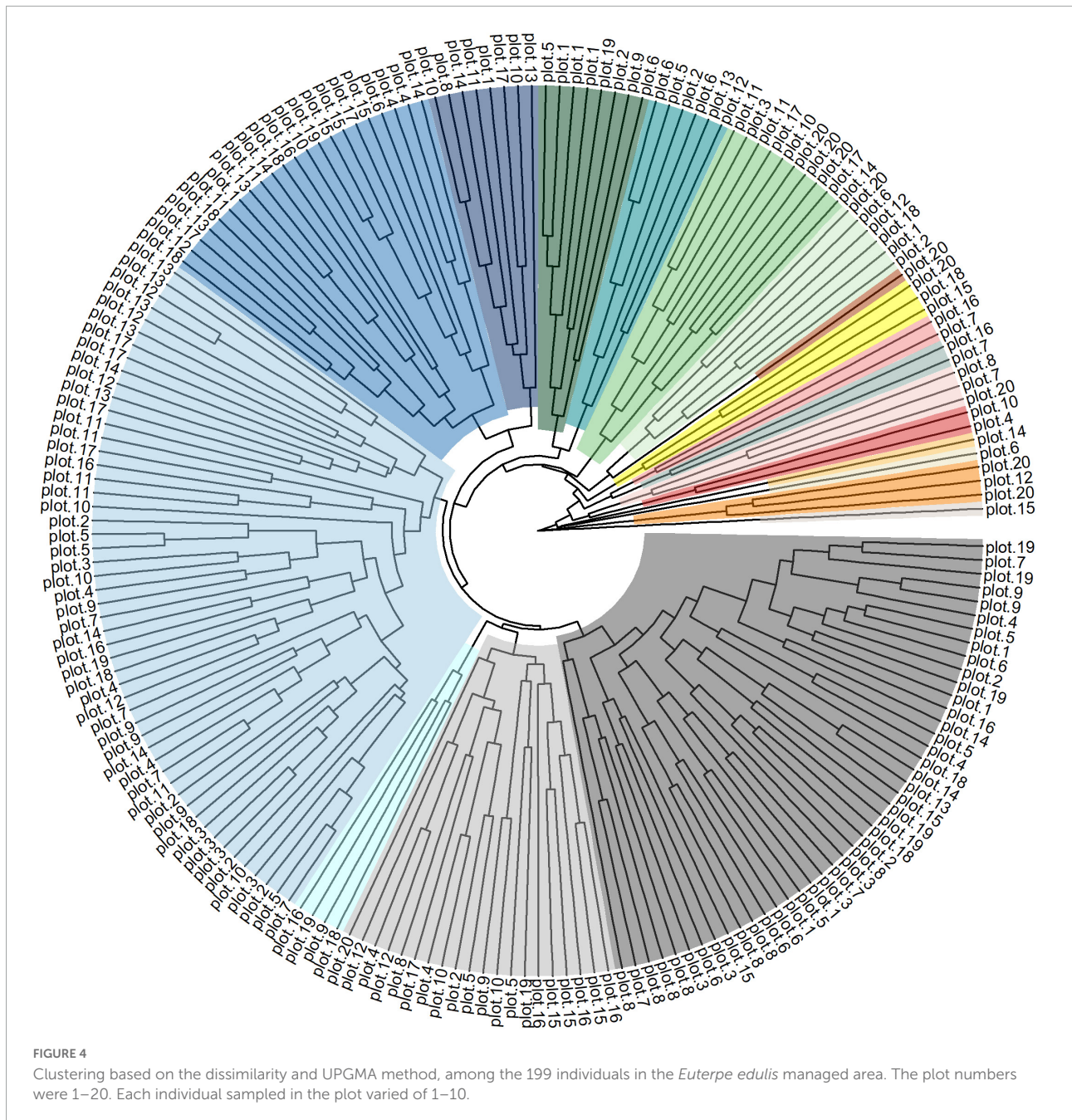
FIGURE 3

Delta K (A; Structure Harvester Application). Plot of the clustering analysis in Structure for  $K = 2, 3, 4,$  and  $5$  (B–E, respectively). 20 plots in the *Euterpe edulis* managed area (numbered from 1 to 20 and delimited by the black vertical line). Each individual is represented by a vertical line, each color is associated with a different cluster, and the length of the colored bars refers to the probability of each individual being assigned to each cluster (Structure v. 2.2 Program).

confirmed that the managed area presents a genetic divergence between individuals and that the reported inbreeding pattern resembles that described in natural populations. Thus, the study area can be assumed to present not only a conservation site but also a potential population for studying genetic improvement to maintain the incentives for the sustainable exploitation of *E. edulis*.

These satisfactory results may derive from the cultivation system used in the study area: growth over a 20-hectare area and a high density of individuals providing a large population totalling approximately 20,000 plants. For the reforestation of areas smaller than 100 hectares in populations with signs of inbreeding, the collection of seeds from 30 source plants is recommended, using plants located in one or more fragments





near the planting site (Sebbenn, 2002). However, studies of the breeding system and population structure of *E. edulis* show that the creation and management of *in situ* genetic reserves or the *ex situ* conservation and breeding of this species should include the collection of several hundred maternal pollination families from a few distant populations to maximize the genetic sampling of a larger number of pollen donors (Gaiotto et al., 2003). Still, Cerqueira et al. (2022) know that the conservation actions for *E. edulis* including selecting and replanting progenies from the forest fragments inserted in more forested landscapes, for could be a way for alternative

genotypes to raise the levels of alleles and thus avoid the collapse of these populations in fragments inserted in deforested landscapes. Thus, a managed area for production of fruit and pulp, once established according to the cultivation system of the studied area, can effectively assist in the conservation of *E. edulis* and in the sustainable generation of income. Additionally, the diversification of crops with *E. edulis* seedlings obtained from genetically divergent source plants and selected for morphological characters of interest in relation to the fruit can establish productive and profitable managed areas that help expand the genetic diversity of the species. The present research

results emphasize that the studied area shows great potential for conservation and for genetic material sufficiently divergent to be used for the improvement of the species.

## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

## Author contributions

LM designed research, data analysis, and wrote the manuscript. GC coordinated sampling. MC and MF helped with the performance of molecular assays and assisted with microsatellite genotyping. MF and AF assisted with the conception and design of the project, designed research, wrote the manuscript, and secured funding. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2022.859081/full#supplementary-material>

### SUPPLEMENTARY FIGURE 1

Neighbor join clustering for twenty sampling plots of *Euterpe edulis* in the commercial studied population.

### SUPPLEMENTARY FIGURE 2

Principal coordinate analysis based on the genetic distances among the 199 individuals in the *Euterpe edulis* managed area. The numbers correspond to each individual sampled. Groups of individuals represented with the same colors correspond to each of the 20 plots: plot 1 = individuals 1–10, 2 = 11–20, 3 = 21–30, 4 = 31–40, 5 = 41–50, 6 = 51–60, 7 = 61–70, 8 = 71–80, 9 = 81–90, 10 = 91–99, 11 = 100–109, 12 = 110–119, 13 = 120–129, 14 = 130–139, 15 = 140–149, 16 = 150–159, 17 = 160–169, 18 = 170–179, 19 = 180–189, and 20 = 190–199. Axis eigenvalue 1 = 7.35%, and axis 2 = 5.87 (DarWin v. 6 Program).

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