

Molecular and quantitative genetic analysis of the neotropical tree *Campomanesia xanthocarpa* (Mart.) O. Berg

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Abstract *Campomanesia xanthocarpa* is a fruit tree species of the Myrtaceae family with potential for fruit commercialization; however, studies on the genetic diversity and variability of the species are necessary to make it commercially viable. The objective of this study was to characterize the diversity and intrapopulation spatial genetic structure (SGS) of a *C. xanthocarpa* population and compare the genetic parameters of quantitative traits between and within open-pollinated progenies. For the analysis of genetic diversity, 80 individuals from a population in the Irati National Forest, Parana, Brazil, were analyzed. The observed (H_o) and expected (H_e) heterozygosity, fixation index (F), and intrapopulation spatial genetic structure (SGS) were assessed. To analyze the quantitative traits, a field progeny test was installed using a randomized block design with nine progenies, three blocks, and four plants per plot at a spacing of 9 m². Total height (H), basal diameter (d_{base}), crown length and diameter (C_{crown} and d_{crown}) at two years and nine months of age were measured. For the studied population, H_o and H_e had an average of 0.478 and 0.717, respectively, with an average fixation index of 0.333, suggesting that the population may be suffering from inbreeding. We found linkage disequilibrium for one evaluated pair of loci. SGS suggested a minimum distance of 25 to 50 m between mother trees for seed collection. For the quantitative genetic analyses, we found that average heritability ranged of 0.15 to 0.55 being considered from moderate (d_{base}) to high (H, C_{crown} and d_{crown}). The traits H and derown were particularly noteworthy and can be used to obtain superior genotypes for the other traits (d_{base} and C_{crown}), since the correlation was significant and high (> 0.80). The results provide applicable information for the implementation of breeding and conservation programs for the species.

Keywords: Guabiroba, genetic structure, heritability, kinship.

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Introduction

Assessing the genetic resources of a population is an area of research highly relevant for breeding and conservation. As such, a large number of studies have been conducted to quantify genetic diversity and understand its magnitude, characteristics, and distribution between and within populations (Cruz et al. 2011, Lima et al. 2020). The first and most important step in choosing natural populations for inclusion in breeding, management, and genetic conservation programs, is a genetic analysis of the population. Understanding the genetic structure provides essential information about the adaptive potential of a species, and the possibilities for genetic gains with the selection of superior phenotypes (Frankham et al. 2010).

Population genetics parameters that provide key information for species conservation and management include genetic diversity, intrapopulation spatial genetic structure (SGS), reproduction system, and gene flow patterns, among others (Loiselle et al. 1995). For studies on genetic diversity, estimates of allelic richness and genotypic frequency are essential, as they provide the basis for identifying changes in the structure of a population (Yeh 2000). These changes can be caused by high levels of inbreeding, resulting in a greater number of homozygous than heterozygous genotypes within the same population (Falconer & Mackay 1996).

The evaluation of SGS at the intrapopulation level is crucial for decision making about genetic resources (Kevin et al. 2004), as it is influenced by factors such as pollen and seed dispersal, clustering of isolated individuals, and mortality, among others. These factors may favor mating between related individuals and, consequently, lead to biparental inbreeding (Cavallari Neto et al. 2004). Understanding SGS is also key when developing population sampling strategies, as it enables us to determine the minimum distance between

individuals selected for seed collection, either for the purposes of conservation or genetic improvement. Such a strategy helps to avoid sampling from related mother trees and ensures a representative sampling of the population (Walsh & Lynch 2018).

It is important to highlight that the success of any breeding and/or conservation program depends on information regarding the magnitude of genetic variation in the species of interest. Thus, understanding variability is the main prerequisite for breeding and/or genetic conservation programs (Falconer & Mackay 1996).

The best way to assess the value of selected parents as potential genetic material is to assess the performance of their offspring through progeny arrays. Such analyses can also offer important information about the expression of genetic variability in the face of environmental factors and quantify the heritable traits that are economically and ecologically important (Duarte et al. 2012). Assessing genetic parameters between and within progenies, as estimated from progeny arrays, allows us to evaluate genetic variability and infer information about the species mating system. This method is widely used for preliminary genetic studies of native species (Kageyama 1990, Resende & Fernandes 1999), as well as in the selection of superior individuals as a source of seeds to established seedling seed orchards (Kageyama & Vencovsky 1983).

The fruit tree species *Campomanesia xanthocarpa* (Mart.) O. Berg, commonly known as guabiroba and from the Myrtaceae family, has significant economic potential. However, as of yet, it has not been the focus of genetic improvement and conservation programs. The species has hermaphrodite flowers (Backes & Irgang 2002), with a monoecious sexual system (Carvalho 2006), and an allogamous reproductive system with gametophytic self-incompatibility (Santos et al. 2019). However, the species has polyembryonic seeds with apomictic embryos identical to the mother plant (Silva 2016).

The fruit of the species has potential for commercialization as it can be used in several food products, such as ice cream, jellies, liquors, juices, among others, and it has a high nutritional value and low-calorie count (Vallilo et al. 2008). Its leaves have medicinal properties and have been used to treat heart disease (Klafke et al. 2016), diabetes mellitus, weight gain (Klafke et al. 2010), as well as trichomoniasis (Mehriardestani et al. 2017), skin infection, candidiasis, and generalized infection in animals (Markman et al. 2002).

Considering that *C. xanthocarpa* is a species with ecological, nutritional, and medicinal potential, genetic studies are necessary to inform the effective development of breeding, conservation, and management programs for the species. As such, the objective of this study was to characterize the diversity and SGS of a *C. xanthocarpa* population and estimate the genetic parameters for growth traits between and within progenies.

Materials and Methods

Description of the Study Area

The *C. xanthocarpa* population used in this study is located in permanent plots (25 ha) installed in the Irati National Forest (Flona), within the Araucaria Forest biome. The Flona is located between the municipalities of Fernandes Pinheiro and Teixeira Soares, Parana state, Brazil (50°33'44.889" W longitude and 25°20'24.818" S latitude; ICMBIO 2013; Figure 1). The climate of the region is characterized as Cfb, subtropical, humid, mesothermal, with cool summers, the occurrence of severe and frequent frosts, and no dry season (ICMBIO 2013). The temperature ranges from -5°C to 38°C. Rainfall occurs most frequently from September to February, with an average monthly precipitation of 193.97 mm and monthly average relative humidity of 79.58% (IBGE 2014).

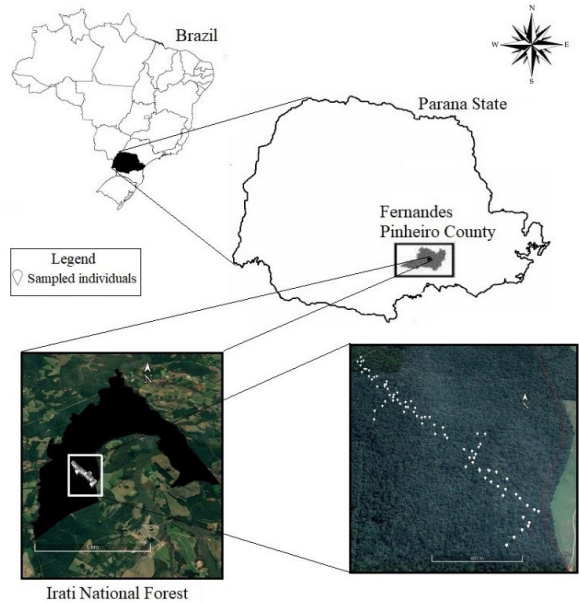


Figure 1 Location of *Campomanesia xanthocarpa* mother trees in the Irati National Forest, Parana state, Brazil.

The soils in the permanent plots are predominantly classified as Cambisol and are made up of three types (Typical Dystrophic Haplic; Leptic Dystrophic; and Typical Alitic) with small inclusions of Typical Dystrophic Red Latosol (Figueiredo Filho et al. 2011). For this study, 80 mother trees distributed throughout the study area (permanent plots) were selected with diameter at breast height (DBH; at 1.3 m from the ground) greater than or equal to 10 cm. Based on the identification and location of all individuals of the species in the 2017 forest inventory, twenty-six individuals were included in the DBH class of 10-20 cm, 22 in the 20-30 cm class, 16 in the 30-40 cm class, and 16 in the 40-50 cm DBH class.

During the forest inventory, branches with reproductive structures were collected and exsiccates included in the UNICENTRO Herbarium (HUCO), registration numbers 4787 to 4801. The nomenclatures adopted follow the pattern suggested by the Angiosperm Phylogeny Group IV (APG 2016).

Genetic diversity

Microsatellite analysis

For the genetic analysis, leaf samples were collected using a slingshot from 80 open-pollinated mother trees in the study area (Figure 1). The leaf samples were packed in airtight plastic containers containing silica gel, individually identified, and stored until DNA extraction.

For DNA extraction, the samples were macerated in liquid nitrogen to obtain a very fine powder. After maceration, samples were placed in 1.5 mL Eppendorf® tubes and kept on ice to avoid DNA degradation. For DNA extraction, the CTAB 2% method was used, based on the protocol described by Doyle & Doyle (1987).

Eight microsatellite loci (SSR) were used for the analysis. These markers were tested and evaluated by Silva (2012), who developed them for species of the *Eucalyptus* (EMBRA 14, 69, 123, 166 and 210) and *Eugenia* (EUN 5, 7 and 11) genera, both of which are from the Myrtaceae family (Table 1). DNA quality was assessed by electrophoresis on 1.8% agarose gel, and 5 μL^{-1} was subsequently diluted in 45 μL^{-1} of ultrapure water and stored in a freezer (-18 °C). The loci were subjected to *C. xanthocarpa* DNA amplification tests via Polymerase Chain Reaction (PCR). The PCR reagents were prepared to a final volume of 10 μL , containing: approximately 1 ng μL^{-1} of

genomic DNA (10 ng μL^{-1}); 1X 10X Enzyme Buffer; 0.3 mM MgCl_2 (50 mM); 1 mM dNTPs (2.5 mM); 0.2 mM Initiator (forward + reverse); 0.1 U of Taq DNA Polymerase (1 U μL); and 6.2 μL of ultrapure water.

The PCR protocol was conducted as follows: (1) DNA denaturation at 94°C for 5 minutes; (2) 94°C for 45 s; (3) specific annealing temperature of each primer (Table 1) for 1 min; (4) amplification with Taq DNA polymerase at 72°C for 90 s; (5) 35 cycles of the 2nd to 4th step; (6) a final extension for 5 min at 72°C; and cooling until reaching 4°C.

After performing the PCR reaction, the amplification products were stained with 10 μL of BlueJuice (solution of Bromophenol Blue, Xylene cyanol, glycerin and ultrapure water), and prepared on 6% non-denaturing polyacrylamide gels, in a 45 cm vertical vat for 4.5 hours at 600 volts, with a 1X TBE buffer solution. Staining was performed with silver nitrate, following the protocol by Creste et al. (2001). After drying the plates, the size of the alleles was determined by comparison with a standard molecular weight marker with a 100 base pair interval.

Analysis of genetic diversity and Hardy-Weinberg equilibrium

Genetic diversity was estimated using the following indices: total number of alleles per locus (K); allelic richness (R); rare alleles (A_r); observed heterozygosity (H_o); and expected heterozygosity (H_e) under Hardy-Weinberg

Table 1 Nuclear microsatellite loci, motif, variation in number of alleles in base pairs (amplitude) for the primer and the species, annealing temperature in degrees Celsius (T_a in °C), and references for the primers used in the genetic analysis of a *Campomanesia xanthocarpa* population in the Irati National Forest, Parana state, Brazil.

Loci	Motif	Amplitude (bp)	Amplitude in species (bp)	T_a	Reference
EMBRA14	(AG) ₈ AAC(AG) ₂₅	100/200	95-125	49 °C	Brondani et al. 1998
EMBRA69	(AG) ₅ TT(AG) ₁₉	200/300	110-112	50 °C	Brondani et al. 2002
EMBRA123	(TC) ₂₁	100/200	132-150	50 °C	Brondani et al. 2006
EMBRA166	(GA) ₁₉	100/200	132-162	50 °C	Brondani et al. 2006
EMBRA210	(TC) ₂₅	190/210	190-213	50 °C	Brondani et al. 2006
EUN 5	(AG) ₂₂	150	32-167	54 °C	Ferreira-Ramos et al. 2008
EUN 7	(CA) ₉	100/200	177-197	52 °C	Ferreira-Ramos et al. 2008
EUN 11	(AG) ₂₀	200	128-180	52 °C	Ferreira-Ramos et al. 2008

equilibrium. The inbreeding coefficient was estimated from the fixation index (F) at the level of loci and as an average across loci (Weir 1996). The standard error of F was estimated by bootstrap permutations over loci. All analyses were carried out with the FSTAT software (Goudet 2002). Statistical significance was tested using the Monte Carlo permutation method (1,000 replications) (alleles between individuals) and a Bonferroni correction (95%, $\alpha = 0.05$).

For each locus, the average frequency of null alleles (na) was estimated using the maximum likelihood method described by Dempster et al. (1977), in the FreeNA software (Chapuis & Estoup 2007), accepting a frequency $n > 0.20$ as indicative of the presence of null alleles. The statistical significance of na was verified using the permutation method (10,000 repetitions) and a Bonferroni correction ($\alpha \leq 0.05$). This analysis allowed us to identify genotyping errors, or verify the presence of alleles that did not amplify even if they do exist (null alleles).

Linkage disequilibrium

The linkage disequilibrium was performed using 1,000 Monte Carlo permutations (alleles between individuals) and a Bonferroni correction (95%, $\alpha = 0.05$), in the FSTAT software (Goudet 2002). The tested null hypothesis was that the genotypes of one locus are independent of the genotypes of other loci. This hypothesis was tested using the chi-square test (χ^2) as described by Weir & Cockerham (1979).

Intrapopulation Spatial Genetic Structure and Coancestry

The intrapopulation spatial genetic structure (SGS) refers to the non-random distribution of genotypes within a population (Vekemans & Hardy 2004), in this case among the sampled seed trees. The analysis of SGS was performed based on the coancestry coefficient (θ_{xy}) between pairs of trees, using the method described by Loiselle et al. (1995).

Seven 50 m distance classes (from 25 to

525 m) were plotted. To test whether the mean values were significantly different from zero, the 95% confidence interval was estimated for each value observed in each distance class, using 1,000 permutations of individuals between different classes. The confidence interval was used to construct the correlogram. These analyses were conducted using the SPAGeDi software, version 1.3 (Hardy & Vekemans 2002).

To check the number of genetically distinct groups (K), a Bayesian analysis was performed with the software STRUCTURE 2.3.4 (Pritchard et al. 2000, Falush et al. 2007). The analyses were carried out with 10 independent simulations for $K = 1 - 7$. In each simulation, 100,000 Markov Chain Monte Carlo (MCMC) interactions with an initial burn in of 100,000 were considered. The analysis was performed using a mixed model and correlated allele frequencies. The most likely number of clusters (K) was determined by the method described by Evanno et al. (2005), using the online application STRUCTURE Harvester (Earl & Von Holdt 2012).

Genetic variability

In November 2016, fruits were collected from 10 reproductive seed trees (individuals not previously sampled for the DNA analysis) within the permanent plots in the Flona. Fifty fruits were collected per seed tree and stored in individually identified airtight containers. For seed extraction, the pulp of the fruit was placed in a sieve and kept under running water. Subsequently, the seeds were placed on absorbent paper and maintained moist for 24 hours until sowing.

Seedlings were grown in 100 cm³ rigid plastic tubes, with commercial substrate of bio-stabilized pine bark and 4 g Kg⁻¹ of Osmocote® 14-14-14 Classic (a controlled release fertilizer), considering each seed tree as a treatment. The seedlings were kept in a greenhouse for 90 days, at a temperature of $25 \pm 4^\circ\text{C}$ and relative air humidity $\geq 80\%$, with controlled irrigation. The

seedlings were then acclimatized in 50% shade conditions. At 180 days they were transplanted into polypropylene plastic bags (15 cm wide, 25 cm high, and 0.15 microns), with substrate containing a mixture of soil (60%), sand (40%), and 100 g of Osmocote® per 18 liters, and left in full sun until one year and three months of age. Seedlings were then planted at the Irati Campus of the Centre-west State University, UNICENTRO, in Irati, Parana (50°39'53.36 "W latitude and 25°32'24.49 "S longitude).

Before planting, the soil was prepared by plowing and tilling. The seedlings were planted in circular pits (20 cm diameter and 20 cm height), at a spacing of 3 m x 3 m, with a simple border line. The seedlings used to surround the progeny test were made up of the same genetic material as the progenies from the selected matrices. The progeny test had a total area of 1,422 m². It is important to note that progenies from seed tree 7 had a mortality of 100%, leaving only the progenies of nine seed trees in the test.

To estimate genetic parameters, the progeny test was installed with a randomized block design, with nine progenies, three blocks, and four plants per block, for a total of 108 seedlings. The progenies were measured for the following traits: total height (H, cm); crown diameter (d_{crown} , cm); crown length (C_{crown} , cm); and basal diameter (d_{base} , mm). The traits were measured at 17 months after planting in the field, when the seedlings were two years and nine months of age.

The genetic parameters were estimated with the restricted maximum likelihood/best linear unbiased predictor (REML/BLUP) method, in the software R 4.0.3 (R Core Team 2020) and the statistical R Package *sommer* (Covarrubias-Pazaran 2016). The analysis of quantitative traits was performed considering the effects of the block as fixed and the effects of progenies as random, using a mixed statistical model (1):

$$y = X\mu + Zb + Wp + Ts + e \quad (1)$$

where: y = phenotypic vector of the evaluated

trait; μ = experiment average; b = vector of fixed effects associated with the block; p = vector of random effects associated with the seed trees (genotypes); s = vector of interaction between progeny and block (error between progenies); e = vector of residuals (error within progenies); and X , Z , W and T are the incidence matrices of the respective effects.

For the mixed model, we assumed that the evaluated progenies were open pollinated and the degree of kinship was half-sib (0.25). We also considered the estimated degree of kinship for the population as 0.40, as obtained through the coancestry coefficient (see section 2.2.4). The adjustment quality of the models was verified using the *lmerTest* Package (Kuznetsova et al. 2017), through which the random and fixed effects and their significance were tested by the chi-square test (χ^2) at 5% probability of error using the Likelihood Ratio Test (LRT).

The estimated variance components were obtained using the *\$varcomp* procedure in the *sommer* package, including: σ_a^2 = additive genetic variance; σ_e^2 = environmental variance; σ_f^2 = phenotypic variance; and σ_d^2 = variance within progenies. From this, the following equations were used to estimate the genetic parameters (Vencovsky & Barriga 1992): narrow-sense heritability at the individual plant level (\hat{h}_i^2) (2); heritability within progenies (\hat{h}_d^2) (3); and mean heritability at the family level (\hat{h}_m^2) (4).

$$\hat{h}_i^2 = \frac{\sigma_a^2}{\sigma_f^2} \quad (2)$$

$$\hat{h}_d^2 = \frac{0.75\sigma_a^2}{\sigma_d^2} \quad (3)$$

$$\hat{h}_m^2 = \frac{0.25\sigma_a^2}{0.25\sigma_a^2 + \frac{\sigma_e^2}{b} + \frac{\sigma_d^2}{nb}} \quad (4)$$

where: σ_a^2 is the additive genetic variance; σ_e^2 is the environmental variance; σ_f^2 is the phenotypic variance; σ_d^2 is the variance within progenies; n is the number of individuals/plot; b is the number of repetitions/block.

Individual and progeny level heritabilities were adjusted for the estimated kinship matrix of $r_{xy} = 0.40$. Thus, individual (h_i^2) (5) and within progeny (h_d^2) (6) heritability were corrected and estimated with the following equations:

$$\hat{h}_{i_adjusted}^2 = \frac{\sigma_p^2/r_{xy}}{\sigma_f^2} \quad (5)$$

$$\hat{h}_{d_adjusted}^2 = \frac{(1-r_{xy})\sigma_a^2}{\sigma_d^2} \quad (6)$$

The standard deviations of narrow-sense heritability (h_i^2 and $\hat{h}_{i_adjusted}^2$) were obtained using the *vpredict* function of the *sommer* package.

The estimated coefficients of variation were: individual additive genetic coefficient of variation ($C\hat{V}_{gi}(\%)$) (7); genetic coefficient of variation between progenies ($C\hat{V}_{gp}(\%)$) (8); environmental coefficient of variation ($C\hat{V}_e(\%)$) (9); relative coefficient of variation (\hat{b}) (10). To support the selection of the best progenies, we also estimated selection accuracy (\hat{r}_{aa}) (11), according to Vencovsky & Barriga (1992).

$$C\hat{V}_{gi}(\%) = \frac{\sqrt{\sigma_a^2}}{\bar{m}} \cdot 100 \quad (7)$$

$$C\hat{V}_{gp}(\%) = \frac{\sqrt{0.25 \sigma_a^2}}{\bar{m}} \cdot 100 \quad (8)$$

$$C\hat{V}_e(\%) = \frac{\sqrt{\sigma_e^2}}{\bar{m}} \cdot 100 \quad (9)$$

$$\hat{b} = \frac{C\hat{V}_{gp}}{C\hat{V}_e} \quad (10)$$

$$\hat{r}_{aa} = \sqrt{\hat{h}_i^2} e \sqrt{\hat{h}_{i_adjusted}^2} \quad (11)$$

where: \bar{m} is the average of progenies.

The genetic correlation (\hat{r}_{gxy}) (12) between the evaluated traits was analyzed as:

$$\hat{r}_{gxy} = \frac{\sigma_{gxy}}{\sqrt{\sigma_{gx}^2 \sigma_{gy}^2}} \quad (12)$$

where: r_{gxy} is the genotypic correlation coefficient, σ_{gxy} is the average genotypic product between the traits x and y, estimated from covariance analysis; and σ_{gx}^2 , σ_{gy}^2 are the genotypic variances of the traits x and y, respectively.

The value of the effective population size (N_e) of the progeny test was estimated based on Sebbenn (2003) as:

$$N_e = \frac{0.5}{\theta \left(\frac{n-1}{n} \right) + \frac{1+F}{2n}} \quad (13)$$

where: n = is the total number of progenies per seed tree; θ is the kinship coefficient; F is the inbreeding coefficient (assumed as zero for estimation purposes).

Results

Genetic diversity

Of the 80 genotyped individuals, only four did not amplify for any of the loci. A total of 47 alleles were detected, and the number of alleles per locus ranged from two (EMBRA69 and EUN 7) to nine (EMBRA210 and EUN 5), with an average of six alleles per locus. The allelic richness ($R = 43$) was very similar to the number of total alleles ($K = 47$). The number of alleles with a frequency below 5% (rare alleles) within the population was 15. The presence of null alleles was found for three loci (EMBRA14, EMBRA166, and EUN 7) with $n > 0.20$, that is, alleles that did not amplify even if they do exist (Table 2).

The observed heterozygosity (H_o) ranged from 0.150 (EUN 7) to 0.702 (EMBRA123), with an average equal to 0.478, and the expected heterozygosity (H_e) ranged from 0.448 (EUN 7) to 0.871 (EUN 5) with an average of 0.717, considering all loci. The value of H_o was greater than H_e only for the EMBRA69. For the other loci, H_e was greater than H_o (Table 2).

The estimated population fixation index was $F = 0.333$ with deviation from the Hardy-Weinberg equilibrium, indicating that the population has a large proportion of homozygous loci. The F showed a deviation from Hardy-Weinberg equilibrium only for the loci EMBRA69 (-0.126), EMBRA123 (0.165), and EUN 11 (0.116). There was an indication of null alleles for the EMBRA14 and 166 and EUN 7 (Table 2).

We found linkage disequilibrium between one pair of loci (EMBRA69 and EMBRA166; Table 3), rejecting the null hypothesis that the genotypes of one locus are independent of the genotypes of other loci. Thus, there is a non-random association of the alleles of the locus EMBRA69 with alleles of locus EMBRA166.

The SGS was significant up to 50 m (Figure 2); therefore, up to this distance, the individuals are more likely to be related. We observed a decrease to negative θ_{xy} values at distances greater than 400 m. The levels of θ_{xy} were significantly lower than the lower limit of the 95% CI for distance classes beyond 500 m, indicating that with greater distances, the coancestry coefficient among the sampled individuals decreased (Figure 2).

The Bayesian cluster analysis to determine the genetic patterns of differentiation between individuals in the population, showed that according

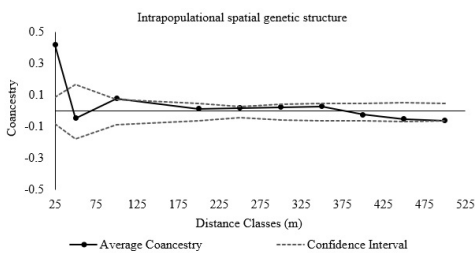


Figure 2 Correlogram of the mean coancestry coefficient between pairs of individuals for seven distance classes in a *Campomanesia xanthocarpa* population sampled in the Irati National Forest, Parana state, Brazil.

to the ΔK method, the most likely number of clusters was $K = 2$ (Figure 3). The graphical representation of the population structure allowed for easy identification of two groups.

Table 2 Genetic diversity and genetic parameters estimated for eight microsatellite loci analyzed for 76 *Campomanesia xanthocarpa* trees located in the Irati National Forest, Parana state, Brazil.

<i>Campomanesia xanthocarpa</i> (n= 76)							
Loci	K	R	A_r	na	H_o	H_e	F
EMBRA14	5	5	0	0.2549	0.3228	0.8010	0.5970*
EMBRA69	2	2	0	0.0000	0.5574	0.4950	-0.1260 ^{ns}
EMBRA123	7	7	2	0.0812	0.7022	0.8410	0.1650 ^{ns}
EMBRA166	7	5	4	0.2148	0.3168	0.6770	0.5320*
EMBRA210	9	7	4	0.1498	0.5323	0.8090	0.3420*
EUN 5	9	9	3	0.1754	0.5444	0.8710	0.3750*
EUN 7	2	2	0	0.2088	0.1501	0.4480	0.6650*
EUN 11	6	6	2	0.0326	0.7001	0.7920	0.1160 ^{ns}
Mean	6	5	2	0.1397	0.4783	0.7168	0.3333*
Total	47	43	15				

Notes: n: Sample size of the population; K: Total number of alleles per locus; R: allelic richness; A_r : rare alleles; na: average frequency of null alleles; H_o : observed heterozygosity; H_e : expected heterozygosity; F: fixation index; *: significant deviation from Hardy-Weinberg equilibrium with p-value < 0.05; ^{ns}: non-significant deviation from Hardy-Weinberg equilibrium with p-value > 0.05.

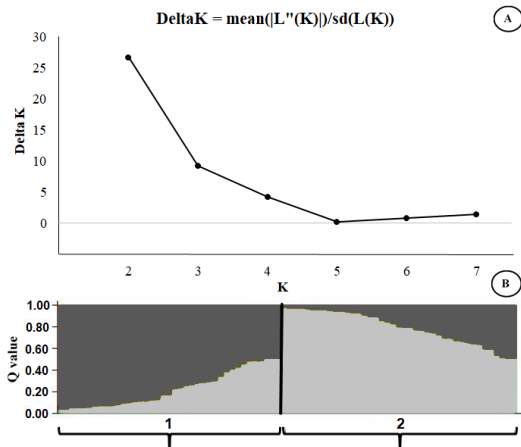


Figure 3 Genetic structure of the *Campomanesia xanthocarpa* population sampled in the Irati National Forest, Parana state, Brazil. (A) Graph based on the number of Delta-K ($K=2$) for the 76 genotyped individuals, according to Evanno et al. (2005); (B) Analysis of the Bayesian population genetic structure, in which the dark colored areas correspond to the distinct genetic clusters.

Table 3 Linkage disequilibrium between pairs of loci for *Campomanesia xanthocarpa* in the Irati National Forest, Parana state, Brazil. The limit value of significance after Bonferroni correction is 0.0018.

Pairs of Loci	p- value
EMBRA14 X EMBRA69	0.0536
EMBRA14 X EMBRA123	0.8786
EMBRA14 X EMBRA166	0.6625
EMBRA14 X EMBRA210	1.0000
EMBRA14 X EUN5	0.1214
EMBRA14 X EUN7	0.0304
EMBRA14 X EUN11	0.8125
EMBRA69 X EMBRA123	0.1696
EMBRA69 X EMBRA166	0.0018*
EMBRA69 X EMBRA210	0.0054
EMBRA69 X EUN5	0.7482
EMBRA69 X EUN7	0.2554
EMBRA69 X EUN11	0.7571
EMBRA123 X EMBRA166	0.7929
EMBRA123 X EMBRA210	0.9607
EMBRA123 X EUN5	0.8143
EMBRA123 X EUN7	0.7089
EMBRA123 X EUN11	1.0000
EMBRA166 X EMBRA210	0.8071
EMBRA166 X EUN5	0.5982
EMBRA166 X EUN7	0.0554
EMBRA166 X EUN11	0.8607
EMBRA210 X EUN5	0.3714
EMBRA210 X EUN7	0.8696
EMBRA210 X EUN11	1.0000
EUN5 X EUN7	0.1286
EUN5 X EUN11	0.5357
EUN7 X EUN11	0.3143

Notes: *: significant with p-value < 0.05.

Genetic variability

Deviance for the effects of progeny on the evaluated traits was significant, demonstrating that there is genetic variability between the progenies. At two years and nine months, the seedlings had an average total height (H) of 43.42 cm, basal diameter (d_{base}) of 11.84 mm and length and diameter of crown (C_{crown} and d_{crown}) of 33.83 cm and 16.53 cm, respectively (Table 4). We also observed that from the beginning of the experiment until seedlings were measured (at two years and nine months), there was an average seedling mortality of 25.93%, with no difference in mortality between the progeny.

When comparing the results obtained using the half-sib kinship matrix (0.25) and those obtained using the molecular kinship (0.40), the estimated genetic parameters were 20 to 75% lower when based on molecular kinship. The parameters that showed a drastic reduction were narrow-sense heritability (\hat{h}_1^2 and $\hat{h}_{1,\text{adjusted}}^2$) and within progeny heritability (\hat{h}_d^2 and $\hat{h}_{d,\text{adjusted}}^2$) which were moderate to high magnitude (0.16 to 0.65) for half-sib kinship and low to medium magnitude (0.04 to 0.25) for molecular kinship (Table 4).

The average heritability ($\hat{h}_m^2 > 0.50$) was high magnitude for the traits H, C_{crown} and d_{crown} , indicating the possibility of selecting progenies for these traits. The trait d_{base} presented low magnitude \hat{h}_m^2 with values of 0.23 and 0.15, considering half-sib and molecular kinship, respectively (Table 4).

The coefficients of genetic variation for individuals ($C\hat{V}_{\text{gi}}(\%)$) and between progeny ($C\hat{V}_{\text{gp}}(\%)$) were considered high, with values above 7% for all evaluated traits, except for dbase in relation to $C\hat{V}_{\text{gp}}(\%)$. The highest values for $C\hat{V}_{\text{gi}}(\%)$ and $C\hat{V}_{\text{gp}}(\%)$ were observed for crown diameter with values of 25.27% and 12.63%, respectively, considering half-sib kinship, and 19.98% and 9.99% considering molecular kinship (Table 4).

The coefficient of relative variation (\hat{b}) was low to moderate in magnitude, ranging from 0.27 to 0.47, considering half-sib kinship, and from 0.20 to 0.32 considering molecular kinship. Accuracy (\hat{r}_{aa}) was considered moderate, with values ranging from 0.40 to 0.68, for half-sib kinship, but low (0.20 to 0.34) when using molecular kinship (Table 4).

The estimated genetic correlation was high and significant between the evaluated traits, ranging from 0.81 to 0.99. These results suggest the possibility of obtaining genetic gains when selection is based on only one of the traits. Thus, the gains obtained when selecting for the trait H, may also result in gains in d_{base} , C_{crown} and d_{crown} as the correlation was 0.99, 0.94, and 0.81, respectively (Table 5).

Table 4 Estimates of genetic parameters for the traits total height (H, cm), basal diameter (d_{base}, mm), crown length (C_{crown}, cm), and crown diameter (d_{crown}, cm) of *Campomanesia xanthocarpa* progeny, evaluated in a progeny test at two years and nine months of age, installed in Irati, Parana state, Brazil.

Genetic Parameters	Matrix considering kinship of half-sibs				Matrix considering molecular kinship			
	H (cm)	d _{base} (mm)	C _{crown} (cm)	d _{crown} (cm)	H (cm)	d _{base} (mm)	C _{crown} (cm)	d _{crown} (cm)
σ ² _a	90.81	2.55	71.57	17.45	56.76	1.59	44.73	10.91
σ ² _e	0.00	4.30	0.00	0.07	0.00	4.30	0.00	0.07
σ ² _d	106.18	8.87	94.46	20.10	140.23	9.82	121.30	26.65
σ ² _f	196.99	15.72	166.03	37.62	196.99	15.72	166.03	37.62
h ² _i	0.46±0.49	0.16±0.56	0.43±0.48	0.46±0.49	-	-	-	-
h ² _{i adjusted}	-	-	-	-	0.12±0.31	0.04±0.35	0.11±0.30	0.12±0.31
h ² _d	0.64	0.22	0.57	0.65	-	-	-	-
h ² _{d adjusted}	-	-	-	-	0.24	0.10	0.22	0.25
h ² _m	0.72	0.23	0.69	0.72	0.55	0.15	0.53	0.55
f _{aa}	0.68	0.40	0.66	0.68	0.34	0.20	0.33	0.34
CV _{gi} (%)	21.95	13.47	25.01	25.27	17.35	10.65	19.77	19.98
CV _{gp} (%)	10.97	6.74	12.50	12.63	8.68	5.33	9.88	9.99
CV _e (%)	23.73	25.14	28.73	27.12	27.27	26.46	32.55	31.23
b	0.46	0.27	0.44	0.47	0.32	0.20	0.30	0.32
Phenotypic average	43.42	11.84	33.83	16.53	43.42	11.84	33.83	16.53

Notes: σ²_a: additive genetic variance; σ²_e: environmental variance; σ²_d: variance within progenies; σ²_f: phenotypic variance; h²_i: additive narrow-sense heritability; h²_{i adjusted}: corrected additive narrow-sense heritability; h²_d: additive heritability within progenies; h²_{d adjusted}: corrected additive heritability within progenies; h²_m: average progeny heritability; f_{aa}: selection accuracy of progeny; CV_{gi} (%): individual additive genetic variation coefficient; CV_{gp} (%): coefficient of genetic variation between progeny; CV_e (%): coefficient of experimental variation; b: relative coefficient of variation.

Table 5 Genetic correlation for the traits total height (H, cm), basal diameter (d_{base}, mm), crown length (C_{crown}, cm), and crown diameter (d_{crown}, cm) of *Campomanesia xanthocarpa* progeny, evaluated in a progeny test, at 2 years and 9 months of age, installed in Irati, Parana state, Brazil.

r _{gxy}	H (cm)	d _{base} (mm)	C _{crown} (cm)	d _{crown} (cm)
H (cm)	1			
d _{base}	0.99**	1		
C _{crown}	0.94**	0.95**	1	
d _{crown}	0.81**	0.84**	0.96**	1

Notes: **: significant with p-value < 0.01

When evaluating the effective population size (Ne) of the progeny test, initially with half-sib kinship (0.25) and then molecular kinship (0.40), values of 3.20 and 2.22 were obtained, respectively. When multiplying this value by the number of evaluated progeny (nine), we found that of the 108 individuals in the experiment, only 28 and 19 individuals are genetically different, considering half-sib and molecular kinship, respectively.

Discussion

Genetic diversity

The genetic diversity parameters for the species showed a total number of alleles of 47 and mean number of alleles per locus of six. These results are similar to those found by Silva (2012) of a total of 43 alleles and an average of four alleles per locus for three populations of *C. xanthocarpa* using 13 loci, 10 of which are the same as those used herein. Thus, in the present study, we found higher levels of diversity in only one population than the three populations studied by Silva (2012). This difference may be due to the characteristics of each study area; herein the population occurs in a conservation area with little anthropogenic interference, whereas the populations used in the previous study were located in fragments that had been significantly affected by human intervention, one of which had already undergone genetic isolation.

Goes et al. (2019) evaluated 96 individuals from four populations using specific loci for *C. xanthocarpa* and found a total of 61 alleles with an average of four alleles per locus. Thus, the loci used in the present study are appropriate to assess the genetic diversity of the study population, even though they are not specific to the species.

The values found for observed heterozygosity (0.4778) were lower than those for expected heterozygosity (0.7168), but similar to those found for other tree species of the Myrtaceae family in the same biome (Zucchi et al. 2003, Ferreira-Ramos et al. 2008). The fixation index was high and significant (0.333), indicating that the population has more individuals with homozygous loci, which can lead to inbreeding.

The high levels of homozygosity can be attributed to crossing between related individuals, as well as the existence of facultative apomixis in the species. The latter produces polyembryonic seeds with adventitious embryony (Silva 2016) that are identical to the mother plant, which may result in the occurrence of clonal individuals within the population and thus compromise its diversity. However, for breeding purposes this behavior offers some advantages, since it is possible to develop clones at the seed level of individuals for which growth and productivity are already known (Dall'agnol & Schifino-Wittmann 2005). Nevertheless, this characteristic of *C. xanthocarpa* requires further study. It is important to note that the species has gametophytic incompatibility (Santos et al. 2019); therefore, inbreeding can only occur in a population through mating between related individuals and facultative apomixis.

The SGS suggests a pattern of isolation by distance for fruit and seed dispersal, indicating that spatially proximal plants are more likely to be related (Hardy & Vekemans 2002). The distance within which individuals

have a high degree of coancestry was 50 meters, suggesting that seed dispersal and the establishment of recruits occur close to the mother tree. This is likely due to the fruit and seed dispersal syndrome of the species; when ripe, fruits fall spontaneously (barochory), favoring primary seed dispersion via seed rain, which is usually concentrated within the canopy projection radius and can vary from 5.0 to 14.5 m (Homczinski et al. 2017). As a consequence, mating between related individuals is expected, in addition to the occurrence of clones of the mother plant through apomixis. When not affected by climatic factors, the flowering of the species has a high synchrony, which also favors mating between proximal and, consequently, related individuals (Sebbenn 2006).

A decrease in coancestry coefficient values (θ_{xy}) over distances greater than 50 m, and negative values at distances greater than 400 m, indicate secondary dispersal of fruits and seeds. According to Santos (2011), this dispersion is zoochoric, as the fruits attract birds, small mammals, fishes, and reptiles, enabling dispersal over long distances.

The studied population is made up of two populations, but these populations are not geographically separated and are undergoing an inbreeding process (due to an increase in the number of homozygous loci, $F = 0.333^*$). Thus, strategies to enrich the area are recommended, for example through the introduction of individuals from other Araucaria Forest remnants, along with the creation of ecological corridors, so as to favor increases in genetic diversity and possibly the number of heterozygous individuals in the population.

This species is not included in the Official National List of Endangered Species of Flora (Brasil, 2014). According to the National Center for Flora Conservation (CNC) (Oliveira et al. 2020), its status is considered of little concern in Brazil. However, Silva (2012) found that isolated populations

have high genetic differentiation and loss of genetic variability, probably caused by forest fragmentation, highlighting the importance of conservation and recovery programs for the species.

Despite the fact that the study site is located in a preserved area, the population deserves special attention with regard to conservation because the species may be showing signs of inbreeding, a process that can affect adaptation to adverse conditions and survival, in addition to compromising its genetic diversity and long-term continuation in the study area.

Genetic variability

Although the species is allogamous, the use of the kinship matrix based on the molecular value is recommended for this species, as it has apomictic seeds, which favors the occurrence of clones (Tambarussi et al. 2021). By correcting the kinship matrix, the values of the estimated genetic parameters decreased, demonstrating that errors in the estimates of genetic parameters and gains with selection are possible when progenies are only considered as half-siblings.

We found a decrease in heritability estimates at the individual and within progeny level when the values were corrected for molecular kinship. Thus, we suggest the use of average heritability to select superior individuals. The average heritability was high for all characteristics, except for d_{base} , which presented low magnitude (0.23 and 0.15) for both kinship matrices.

It is important to note that according to Vencovsky & Barriga (1992), the heritability of a trait is not a fixed value, as it can vary with different genotypes, age, location, and their interaction (genotype x environment). This fact is clearly observed for this progeny test considering that in full-sun nursery conditions at 11, 13, and 15 months of age, Kampa et al. (2020) reported average heritability for height from 0.78 to 0.68 and for basal diameter collection diameter of 0.50. These values are

higher than those found in the present study based on field conditions, thus indicating the influence of the environment and the age of the progenies on these characteristics. Our results show high levels of genetic control within progeny, suggesting possible gains by selecting the best progenies as parents for seed production (Canuto et al. 2015).

Studies involving heritability estimates for native species of the Araucaria Forest are still rare, and to date no study has assessed heritability of *C. xanthocarpa* in field conditions. However, there are reports of this estimate for other native forest species, such as *Tabebuia chrysotricha* Standl (Costa et al. 2007), *Cedrela fissilis* Benth (Biernaski et al. 2012), *Handroanthus avellanadae* (Lorentz ex Griseb.) Mattos (Santos et al. 2014), *Eugenia calycina* Cambess. (Costa et al. 2016), *Dipteryx alata* Vog. (Canuto et al. 2015), and *Mimosa scabrella* Benth. (Menegatti et al. 2016). The \hat{h}^2_m in these analyses are of moderate to high magnitude, with values for height and diameter of the seedlings ranging from 0.35 to 0.96.

The values for $C\hat{V}_{gi}$ (%) were higher than $C\hat{V}_{gp}$ (%), indicating the possibility of phenotypic selection. According to Biernaski et al. (2012), $C\hat{V}_{gi}$ (%) values are generally higher than $C\hat{V}_{gp}$ (%) in progeny tests involving native trees due to their high levels of genetic variability. This has been observed in other studies on native species such as *H. avellanadae* (Santos et al. 2014), *E. calycina* (Costa et al. 2016), *Schizolobium parahyba* (Vell.) Blake (Chinelato et al. 2014), and *M. scabrella* (Menegatti et al. 2016).

The coefficient of relative variation (\hat{b}) was moderate to low magnitude depending on the kinship matrix used and the trait evaluated. This can be attributed to the high values obtained for the coefficient of experimental variation $C\hat{V}_e$ (%). According to Vencovsky & Barriga (1992), higher \hat{b} values (i.e., the closer to unity) indicate greater genetic control of the traits and less influence of environmental factors on the expression of the phenotype.

As such, the selection of progenies can be performed effectively using the traits H and d_{crown} , which present values of 0.32 with the molecular kinship matrix and 0.46 and 0.47, respectively, with the half-sib kinship matrix, which are considered low to moderate.

Because it is a non-domesticated population, without any degree of selection or improvement, the values for accuracy were moderate to low. Thus, when the best individuals are selected, \hat{h}^2_{crown} , and consequently \hat{r}^2_{aa} , will likely increase.

Therefore, we recommend that the traits H and d_{crown} are used to select the best individuals as they present high accuracy and medium heritability estimates and low to moderate \hat{b} values, in addition to presenting a strong to very strong correlation with the other traits. According to Cruz et al. (2012) and Esposito et al. (2012), genotypic correlation, which indicates associated heritable traits, can be used to guide decision-making in breeding programs.

It is important to note when this progeny test reaches the reproductive phase, it will be transformed into a seedling seed orchard, to obtain improved seeds as well as superior individuals. Breeding programs generate new base populations, this could help wild populations in any threats and / or domestication of species intended for food / medicine crops, increasing genetic diversity and the diversity of traits of interest, giving future breeding programs additional variation for climate resilience, fruit quality, etc.

Conclusion

The studied population of *C. xanthocarpa* shows increased homozygosity, suggesting that the population is experiencing a process of inbreeding, possibly caused by mating between related individuals and facultative apomixis in the species. Because this situation can compromise the genetic diversity and long-term continuation of the species in the Flona, particular attention must be paid to *in*

situ conservation strategies. The high degree of coancestry ($\theta_{xy} = 0.40$) at a distance class of less than 50 m suggests that individuals selected as seed trees and for seed collection must be located at distances greater than 50 m to avoid sampling from related individuals.

Based on the analyses performed herein, we can conclude that there is genetic variability between and within the progenies. The use of the kinship matrix as estimated through microsatellite markers enabled us to adjust the estimates of the evaluated parameters, thus making it possible to obtain more realistic results for the species. The traits H, C_{crown} and d_{crown} are particularly relevant, as they present high average heritability (\hat{h}^2_m) and coefficients of variation at the individual ($C\hat{V}_{\text{gi}}$ (%)) and progeny level ($C\hat{V}_{\text{ep}}$ (%)). Thus, these traits can be used to select superior genotypes when this progeny test is transformed into a seedling seed orchard.

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