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ORIGINAL ARTICLE

Enhancing genetic fitness while maintaining genetic variability in *Araucaria angustifolia*

Melhorar a aptidão genética, mantendo a variabilidade genética de Araucaria angustifolia

Jose Arimateia Rabelo Machado[1], Ananda Virgina Aguiar[2], Ananias de Almeida Saraiva Pontinha[1], Bruno Marchetti Souza[3], Alexandre Magno Sebbenn[1], John Edward Hallsworth[4], Valderês Aparecida Sousa[2], Miguel Luiz Menezes Freitas[1]

[1]Instituto Florestal de São Paulo – IF, São Paulo, SP, Brasil
[2]Empresa Brasileira de Pesquisa Agropecuária – Embrapa Florestas, Colombo, PR, Brasil
[3]Faculdade de Engenharia de Ilha Solteira, Universidade Estadual Paulista "Júlio de Mesquita Filho"– UNESP, Ilha Solteira SP, Brasil
[4]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Chlorine Gardens, Belfast, United Kingdom

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Abstract

Araucaria angustifolia provides timber, edible seeds and pharmaceuticals and has a unique ecology (once shared with that of the dinosaurs), yet is now threatened with extinction. Here, we assess and reconcile the counteracting goals of enhancing genetic fitness and maintaining genetic variability during *ex-situ* conservation of *A. angustifolia* in a provenance-and-progeny test to improve growth vigor. After 33 years, survival, height, diameter at breast height, and volume of trees were determined. Sixteen selection scenarios were tested using two strategies: selection among and within progenies, and selection only within progenies, each one for eight values of selection intensity of total survival in the trial (7.6, 11.5, 15.3, 19.1, 22.9, 26.8, 30.6, and 34.4%). The variance components and genetic parameter estimates were determined using REML/BLUP. There was significant genetic variation among provenances and progenies for all traits. For all growth-phenotype traits, the heritability at mean (0.419–0.542) and within progeny level (0.186–0.207) indicate the potential for tree improvement by selection among and within progenies. The selection strategy enhanced growth phenotype whilst maintaining an effective population size of 19.1% selected individuals within progenies (three females and two males). This preserved sufficient genetic variability among and within provenances, and resulted in genetic gain, providing higher-value seed production.

Keywords: *Araucaria angustifolia*; Coniferous; Genetic conservation; Gymnosperm; Quantitative genetics; Tree breeding.

Resumo

Araucaria angustifolia fornece madeira, sementes comestíveis e fármacos e possui uma ecologia única (antes compartilhada com a dos dinossauros), mas agora está ameaçada de extinção. Aqui, avaliou-se e reconciliou-se objetivos contrários de aumentar a aptidão genética e manter a variabilidade genética durante a conservação *ex-situ* de *A. angustifolia* em um teste de procedencias e progênie para melhorar o vigor de crescimento. Após 33 anos do plantio, a altura, o diâmetro à altura do peito, volume e a sobrevivência das árvores foram medidos. Os componentes da variância e as estimativas dos parâmetros genéticos foram determinados utilizando o método REML/BLUP. Dezesseis cenários de seleção foram testados usando duas estratégias: seleção entre e dentro das progênies, e seleção apenas dentro das progênies, cada um para oito intensidades de seleção de árvores de sobrevivência total no ensaio (7,6,

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11,5, 15,3, 19,1, 22,9, 26,8, 30,6 e 34,4%). Variação genética significativa entre procedências e progênies foi detectada para todos os caracteres. Para todos os caracteres de crescimento, a herdabilidade no nível médio (0,419-0,542) e dentro das progênies (0,186-0,207) mostrou a possibilidade de melhoramento da população pela seleção entre e dentro da progênie. A estratégia de seleção mais eficaz para melhorar o fenótipo de crescimento, mantendo um tamanho efetivo da população próximo ao original foi a seleção de 19,1% dos indivíduos dentro das progênies (três fêmeas e dois machos). Isso preservou variabilidade genética suficiente entre e dentro das procedências e resultou em ganho genético, possibilitando a produção de sementes de maior valor.

Palavras-chave: *Araucaria angustifolia*; Conífera; Conservação genética; Gimnosperma; Genética quantitativa; Melhoramento florestal.

INTRODUCTION

Forest fragmentation and deforestation have caused biodiversity loss and increased the risk of local extinctions (Haddad et al., 2015). *Araucaria* forest, also known as Tropical Humid Mixed Forest, is dominated by the majestic architecture of a gymnosperm, *Araucaria angustifolia* (Bert.) O. Kuntze. The ancestors of these ancient trees once shared their ecology with that of the dinosaurs (Hummel et al., 2008). Whereas *Araucaria* forests used to occupy 20 million hectares, mostly in mountain- and plateau regions of Southern Brazil but also in pockets within highlands of Southeast Brazil, Argentina and Paraguay (Reitz & Klein, 1966; Reis et al., 2018). Now, less than 7% of the original *Araucaria* forest remains (Reis et al., 2018). The extensive and intensive deforestation of the Atlantic Forest has fragmented populations of *A. angustifolia* and this, combined with historical logging, has driven the species closer to extinction (Lacerda, 2016); it now features in the IUCN Red List as critically endangered (The World Conservation Union). *Araucaria* forest is a habitat that characteristically has endemic species and a unique and complex ecology at a range of trophic levels (Zandavalli & Dillenburg, 2015; Pereira et al., 2020). In addition, *A. angustifolia* has unique metabolites and physiology, and is valued for timber, edible seeds, and medicinal properties (Perotti et al., 2015; Peralta et al., 2016; Fonseca et al., 2020). There is, therefore, an urgent need to conserve genetic resources of this tree.

Regarding long-term conservation, the preservation of original habitat containing *in-situ* populations is best; to avoid any discontinuity of ecological and evolutionary processes (Flower et al., 2018). However, this strategy would demand the creation and expansion of legally protected areas, which implies high costs. Furthermore, considering that the remnants of the ancient *Araucaria* forest are now very small in some regions, strategies for *in-situ* conservation may no longer be sufficient to effectively protect *A. angustifolia* genetic resources (Bittencourt & Sebbenn, 2007; Medina-Macedo et al., 2016; Lacerda, 2016; Reis et al., 2018). *Ex-situ* plantations, established in provenance-and-progeny trials can be used as a complementary strategy to guarantee the genetic conservation of *A. angustifolia* (Zechini et al., 2018). Provenance-and-progeny trials also provide the biological material for breeding and seed-production programs (Aguiar et al., 2019).

Despite being one of the most-studied tree species in Brazil for *in-situ* and *ex-situ* genetic conservation, using quantitative traits (Pires et al., 1983; Sebbenn et al., 2003a, 2004; Silva et al., 2018), isozymes (Auler et al., 2002; Mantovani et al., 2006; Ferreira et al., 2012; Zechini et al., 2018), and DNA markers (Bittencourt & Sebbenn, 2009; Duarte et al., 2012; Danner et al., 2013; Sant'Anna et al., 2013; Medina-Macedo et al., 2016; Stefenon et al., 2019; Silva et al., 2020), *A. angustifolia* lacks a solid breeding program (Sousa et al., 2020).

The demand for seeds of native tree species has increased in the last decade, mainly for use in large-scale Atlantic Forest habitat-restoration projects and reforestation for commercial timber production (Schmidt et al., 2019; Urzedo et al., 2019). Provenance-and-progeny test plots can be used as an efficient and cost-effective source of high-quality seeds for this growing market (Aguiar et al., 2019). Considering the economic aspect, conservation combined with use can form the basis of more-viable public policies regarding the conservation of the species' genetic resources. Participatory plant breeding, as proposed by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and its partners (Instituto Florestal de São Paulo, universities, farmers, and forest companies), can also contribute to the conservation of this species. Participatory plant breeding is applied mainly to forest species that have economic, social, and environmental value for a given community or group of stakeholders (Reis et al., 2018). Thus, the stakeholder (typically a vendor or manufacturer of products) participates in some or all stages of the development process of new cultivars, which better meet the needs of the producers (Reis et al., 2018). This conservation-through-use strategy can reduce exploitation pressure on forest remnants, thus alleviating genetic erosion (Reis et al., 2018). *Ex-situ* provenance-and-progeny-trial plantations have also the advantage of gathering genotypes from several provenances in a single area, enabling the continuous evaluation of tree performance and the estimation of genetic parameters, as heritability and genetic gains (Aguiar et al., 2019). The estimation of genetic parameters is a necessary step for the conversion of provenance-and-progeny trials into seedling seed-orchards (Aguiar et al., 2019). The conversion is based on the identification of desirable trees by selective thinning of inferior trees (Aguiar et al., 2019; Goldringer et al., 2020) where the main challenge is to reconciling potentially conflicting objectives in the same trial, i.e., to guarantee the maintenance of genetic diversity for *ex-situ* conservation whilst at the same time achieving genetic gain for environmental reforestation.

The overarching goals of this study were to convert an *A. angustifolia* provenance-and-progeny test into a seedling seed-orchard, produce improved seeds for commercial reforestations, test different selection strategies, and produce a knowledge-based strategy for the genetic conservation and improvement of *A. angustifolia*. The specific objectives were to: (i) estimate the genetic parameters in a provenance-and-progeny test; (ii) identify superior trees from their growth phenotype; (iii) estimate genetic gains by comparing different combinations of selection intensities and methods; and (iv) propose an appropriate selective thinning regime to convert the provenance-and-progeny test into a seedling seed-orchard.

MATERIAL AND METHODS

Sampling and experimental design

The study was carried out in as part of a provenance-and-progeny test established in 1982 at the Itapeva Experimental Station of the São Paulo Forest Institute, Brazil (24°17' S, 48°54' W, and 930 m above sea level). Seeds were collected in 1981 from five natural populations of *A. angustifolia* (Table 1). Three of these populations were located within São Paulo state, at Campos do Jordão (CJ), Cunha (CU) and Itararé (IT), and the two other populations were located within Santa Catarina state, at São Joaquim (SJ) and Bom Jardim da Serra (BJS). Open-pollinated seeds were collected from random trees (from 14 to 26 trees per provenance) whilst respecting a minimum distance of 100 m between trees to avoid the collection of seeds from genetically related parents. The seedlings were germinated in polyethylene bags and planted in April of 1982 (Sebbenn et al., 2003b). The climate at the planting site is for the most part dry, with annual rainfall concentrated in summer. The average annual temperature is 18.6 °C, average annual rainfall is 1,300 mm, and the predominant soil types are dystrophic red latosols (Sebbenn et al., 2003b). We employed a compact family randomized block design with three blocks, five plots (provenances), 14 to 26 subplots (progenies/provenances), 10 plants per subplot, and a spacing of 3 x 2 m. To minimize any potential edge effects, two border rows were planted, using the same species (Sebbenn et al., 2003b). It was not possible to determine the percentage of female and male trees in the trial.

Table 1. Geographic, climatic and sampling progeny (n_p) information of the sampled *Araucaria angustifolia* provenances.

Provenance	n_p	**Geographical coordinates**	**Elevation (m)**	**Mean min.-max. temperatures (°C)**	**Mean annual rainfall (mm)**
Campos do Jordão, SP (CJ)	26	22° 44'S 45° 30'W	1,630	15.0–22.0	1,891
Cunha, SP (CU)	14	23° 14'S 43° 03'W	970	14.1–20.6	2,000
Itararé, SP (IT)	21	24° 30'S 49° 10'W	930	18.0–22.0	1,500
São Joaquim, SC (SJ)	24	28° 19'S 49° 52'W	1,380	9.2–17.3	1,600
Bom Jardim da Serra, SC (BJS)	25	28° 18'S 49° 32'W	800	9.1–17.3	1,600

SP is state of São Paulo; SC is state of Santa Catarina; min. and max. are minimum and maximum.

The trial was evaluated (33 years after planting) for the following traits: mean surviving trees per plot, total height (H) measured using a hypsometer, and diameter at breast height (dbh, cm) measured with tape graduated to 0.1 cm. True individual volume without bark (Vol, m^3.tree^{-1}) was calculated according to Shimizu (1999) by, $Vol = 0.01765474 + 0.3966295(dbh/100)^2 H$. The mean annual increment (MAI) was calculated for H, dbh, and Vol by: $MAI = x/a$, where x is the observed value of a trait and a y is the age of the trial.

Estimation of variance components and genetic parameters

Due to the unbalanced numbers of progeny per provenances and survival within the plot, the univariate additive mixed linear model implemented in the SELEGEN-REML/BLUP software (Resende, 2016) was used to estimate the variance components and the genetic parameters. Two linear mixed models were employed using the REML/BLUP procedure to obtain the genetic values at individual level. Deviance analyses were performed to test the significance of the model effects through the likelihood-ratio test (LRT). The first model included the effect of provenances, represented by: $y = X_b + Z_a + W_p + T_s + e$ and, the second model did not include the provenance effect: $y = X_b + Z_a + W_p + e$, where *y* is the data vector, *b* represents the block effects (fixed), *a* represents the individual additive genetic effects (random), *p* represents the plot effects (random), *s* is the provenance effects (random), and *e* is the residual (random). X, Z, W, and T represent the incidence matrices for the referred effects (Resende, 2002). The following variance components and genetic parameters were estimated: genetic variance among progenies within provenances ($\sigma^2_{p(p)}$), additive genetic variance ($\sigma^2_a = 4\sigma^2_{p(p)}$), environmental variance among plots (σ^2_c), genetic variance among provenances (σ_p^2 $\acute{o}^2_{\text{prov}}$), residual variance ($\sigma^2_e$), phenotypic variance within plot (σ^2_w), and total phenotypic variance (σ^2_{tp}). The estimated parameters were: the coefficient of determination of plot effects (C^2_{pl}), coefficient of experimental variation ($CV_e\%$), coefficient of individual additive genetic variation ($CV_{gi}\%$), coefficient of genetic variation among progenies within provenances ($CV_{p(p)}\%$), additive narrow sense heritability (h^2_a), mean progeny heritability (h^2_m), within-progeny heritability (h^2_w), accuracy for progeny selection, assuming complete survival (A), and relative coefficient of variation (CV_r),

$$CV_e\% = 100\left(\frac{\sqrt{\sigma^2_e}}{\bar{x}}\right), \quad (1)$$

$$CV_{gi}\% = 100\left(\frac{\sqrt{\sigma_a^2}}{\bar{x}}\right), \tag{2}$$

$$CV_{p(p)}\% = 100\left(\frac{\sqrt{\sigma_{p(p)}^2}}{\bar{x}}\right), \tag{3}$$

$$h_a^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2 + \sigma_w^2}, \tag{4}$$

$$h_m^2 = \frac{0.25\sigma_a^2}{0.25\sigma_a^2 + \frac{\sigma_c^2}{r} + \frac{0.75\sigma_a^2 + \sigma_e^2}{nr}}, \tag{5}$$

$$h_w^2 = \frac{0.75\sigma_a^2}{0.75\sigma_a^2 + \sigma_e^2}, \tag{6}$$

$$A = \sqrt{h_m^2}\text{, and} \tag{7}$$

$$CV_r = \frac{CV_{p(p)}\%}{CV_e\%} \tag{8}$$

where $\bar{x}$ is the phenotypic mean of each trait. The genetic divergence (Q_{st}) among all provenances for growth traits was estimated following (Sebbenn et al., 2003b) using:

$$Q_{st} = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_{p(p)}^2 + \sigma_w^2}. \tag{9}$$

Planning of selection scenarios

The dbh trait was used for tree selection due to ease and accuracy of measurement. Sixteen selection scenarios were tested using two strategies, each one for eight values of selection intensity of total survival in the trial (7.6, 11.5, 15.3, 19.1, 22.9, 26.8, 30.6, and 34.4% of total survival trees; Table 2). Of these, eight involved selection among and within progenies (S_{aw}) and eight involved selection only within progenies (S_w). The genetic gain in the selection GS%among and within progeny ($Gs_{(aw)}\%$) and within progeny ($Gs_{(w)}\%$) were estimated according to,

$$Gs_{(aw)}\% = 100\left(\frac{i_a\sigma_m h_m^2}{\bar{x}_a} + \frac{i_w\sigma_w h_w^2}{\bar{x}_w}\right)\text{, and} \tag{10}$$

$$Gs_{(w)}\% = 100\left(\frac{i_w\sigma_w h_w^2}{\bar{x}_w}\right) \tag{11}$$

respectively, where σ_m and σ_w are mean standard deviation of genetic variation among progeny within provenance and standard deviation of phenotypic variation within progeny, respectively, $\bar{x}_a$ and $\bar{x}_w$ are the means of dbh among and within progeny in the trial before selection, respectively. The variance effective population size for selected female and male individuals (N_e) was calculated according with Bittencourt & Sebbenn (2007) using the equation,

$$N_e = \frac{0.5}{\Theta\left[\frac{n-1}{n}\right] + \frac{1+F}{2n}} \tag{12}$$

where Θ is the reproductive group of co-ancestry of selected male and female individuals, n is the total selected individuals, and F is the inbreeding in parental populations of selected individuals (assumed to be zero). The Θ values were estimated following Lindgren & Mullin (1998), assuming the co-ancestry coefficient among individuals within progeny as half-sibs ($\theta_{ij} = 0.125$) and the sexual ratio of 1:1 (1 female: 1 male) using,

$$\Theta = \frac{\sum_{i=1}^{n_f} \sum_{j=1}^{n_m} \theta_{ij} \Big/ 2 n_f n_m}{n_p} \tag{13}$$

where n_p, n_f, and n_m are the numbers of progeny, female and male trees selected within family. Finally, $Gs\%$ and N_e were plotted against the numbers of remaining individuals for different selection methods (S_{aw} and S_w). The point of intersection of $GS\%$ GS% and D D curves was considered the ideal number trees to remain on the population that would maximize both $GS\%$ GS% and D D.

Table 2. Scenarios for selection among and within progenies (S_{aw}) and within progeny (S_w), using eighth selection intensity (SI).

Scenario	SI (%)	n_s	n_p	n_w	Female/male
			Among and within progeny (S_{aw})		
1	7.6	220	22	10	110/110
2	11.5	330	33	10	165/165
3	15.3	440	44	10	220/220
4	19.1	550	55	10	275/275
5	22.9	660	66	10	330/330
6	26.8	770	77	10	385/385
7	30.6	880	88	10	440/440
8	34.4	990	99	10	495/495
			Within progeny (S_w)		
9	7.6	220	110	2	110/110
10	11.5	330	110	3	220/110
11	15.3	440	110	4	220/220
12	19.1	550	110	5	330/220
13	22.9	660	110	6	330/330
14	26.8	770	110	7	440/330
15	30.6	880	110	8	440/440
16	34.4	990	110	9	550/440

Note. n_s , n_p , and n_w are the total number of selected trees, progenies, and trees within progenies, respectively.

RESULTS AND DISCUSSION

The provenance-and-progeny test presented genetic variation for all growth-phenotype traits, indicating an effective sampling strategy for the *ex-situ* conservation of genetic variation from five natural populations of *A. angustifolia*. Significant differences were detected by the

LRT among provenances and progenies for all traits, with the exception of the effect of progeny on survival (Table 3). The coefficient of determination of plot effects (C_{pl}^2 $\mathrm{C}^2_{\mathrm{plot}}$) was low for all traits (C_{pl}^2 : 0.02–0.14), as well as the coefficient of experimental variation ($CV_e\%$: 9.1–22.3%). These results indicate that the experimental design has adequate control for the environmental variation and provides accurate estimates of genetic parameters of quantitative traits.

Table 3. Mean, mean annual increment (MAI), and likelihood-ratio test (LRT) for deviance analysis and genetic parameters for growth and survival traits in the provenance-and-progeny test.

	Height (m) [MAI, m.y^{-1}]	**dbh (cm) [MAI, cm.y^{-1}]**	**Volume (m^3) [MAI, m^3.y^{-1}]**	**Survival (%)**
Mean				
Campos do Jordão (CJ)	8.68 [0.26]	12.86 [0.39]	0.090 [0.003]	91.9
Cunha (CU)	9.38 [0.28]	13.82 [0.42]	0.107 [0.003]	88.6
Itararé (IT)	8.83 [0.27]	13.55 [0.41]	0.098 [0.003]	86.3
São Joaquim (SJ)	7.82 [0.24]	11.45 [0.35]	0.070 [0.002]	83.9
Bom Jardim da Serra (BJS)	7.52 [0.23]	10.73 [0.33]	0.063 [0.002]	85.2
Overall mean	8.36 [0.25]	12.34 [0.37]	0.083 [0.002]	87.2
LRT – Provenances	48.83**	27.07**	28.84**	7.15**
LRT – Progenies	10.82**	33.73**	20.66**	2.08
Parameter				
Genetic differentiation among provenances: Q_{st}	0.152	0.083	0.088	-
Coefficient of determination of plot effect: C_{pl}^2	0.14	0.08	0.09	0.02
Coefficient of experimental variation: $CV_e\%$	9.1	10.5	22.3	13.2
Coefficient of individual genotypic variation: $CV_{gi}\%$	11.2	19.8	39.4	-
Coefficient of genetic variation among progenies: $CV_{p(p)}\%$	5.6	9.9	19.7	4.0
Narrow sense individual heritability: h_a^2	0.202	0.231	0.238	-
Progeny mean heritability: h_m^2	0.419	0.542	0.536	0.210
Additive heritability within plot: h_w^2	0.186	0.198	0.207	-
Accuracy of progeny selection: A	0.647	0.636	0.732	0.460
Coefficient of relative variation: CV_r	0.614	0.937	0.885	0.300

Note. dbh is the diameter at breast height; ***P* < 0.01 for qui-square test (x^2), with one degree of freedom.

The genetic variation among provenances, and amongst progenies within provenances, can also be exploited in breeding programs to produce improved seeds for commercial reforestation and environmental recovery. The high survival rate at 33 years (87.2%, ranging among provenances from 83.9 to 91.9%) demonstrates the overall adaptation of provenances from different geographical and climatic conditions within the trial site (Table 3). At age 18 years, the survival rate of this trial was reported as 89.7% (Sebbenn et al., 2003b), indicating a low mortality rate (< 3%) across the proceeding 15-year period (from 2000 to 2015). The IT provenance, located 50 km from the trial site, and the CJ and CU provenances located in a northerly direction from the Itapeva trial site exhibited greater heights (8.83–9.38 m), dbh values (12.86–13.82 cm), and wood volumes (0.090–0.107 m^3.tree^{-1}) than the SJ and BJS provenances which originated from the south (H: 7.52–7.82 m; dbh: 10.73–11.45 cm; Vol: 0.063–0.070 m^3.tree^{-1}). Mean annual increments (MAI) denote a decrease in the rate of growth as tree age increases. Whereas at 18 years, the MAI values were for height (0.38 m/year= 6.88 m/18 years), dbh (0.57 cm/year = 10.19 cm/18 years), and Vol (0.0021 m^3.tree^{-1}= 0.037 m^3.tree^{-1}/18 years) (Sebbenn et al., 2003b), at 33 years the values were 34.2% (=100(0.38-0.25)/0.38), 35.1% (=100(0.57-0.37)/0.57) and 4.8% (=100(0.0021-0.002)/0.0021) lower, respectively.

The results indicate that great part of the genetic variation for all traits was distributed within (rather than between) provenances (Q_{st} = 0.083–0.152) (Table 3). This same pattern was also reported in *A. angustifolia* for the same traits in 18 provenances at age 23 years (Q_{st} = 0.045–0.073, Shimizu, 1999), 14 provenances at age 21 years (Q_{st} = 0.032–0.062, Sebbenn et al., 2003b), and five provenances at age 30 years (Q_{st} = 0.089–0.152, Sebbenn et al., 2004). The high genetic variation within provenances indicates the possibility of obtaining genetic gains by selection among progeny within populations and individuals within progeny. Furthermore, in general, the coefficients of individual additive genotypic variation ($CV_{gi}\%$ = 11.2–39.4%) and genotypic variation among progenies ($CV_{p(p)}\%$ = 5.6–19.7%) were generally high (> 10%, Resende, 2002), indicating that this trial presents a great potential to select plus trees within progenies and within progeny/provenances for breeding programs (Sebbenn et al., 2003a). Furthermore, the accuracy was high for growth traits (A = 0.65–0.79), indicating a strong correlation between phenotype and genotype. The most-desirable individuals from the most-divergent progenies can be selected to the development of intrapopulation hybrids aiming at timber- or seed production (Aguiar et al., 2019). The quantification of genetic divergence between provenances is important to inform tree selection for crosses made in breeding programs. Crosses between individuals from different provenances may enhance the heterosis for the quantitative traits of economic interest (Sebbenn et al., 2003b; Aguiar et al., 2019). In addition, the results of genetic differentiation between provenances will guide seed collection from the most-divergent populations for breeding and genetic conservation programs (Silva et al., 2018).

The estimates of heritability for growth-phenotype traits indicate that higher genetic gains can be obtained by selection among progenies (h_m^2: 0.419–0.524) rather than by mass selection (h_a^2: 0.202–0.348) or within-progeny selection (h_w^2: 0.186–0.207). Consistent with this, a high accuracy of progeny selection (A) and the coefficient of relative variation (CV_r > 0.6) was observed, indicating a high possibility for genetic improvement by selection among progenies. Thus, the selection based on progeny is more precise than that based on plot or mass of individual levels within the trial.

The dbh showed the highest values of heritabilities and thus presents greatest genetic control, so it is recommended as the trait for selection of trees for breeding. The optimization was the tool used to achieve the balance between N_e and genetic gain for dbh to guarantee the genetic conservation of provenances and produce seeds with a reasonable level of improvement. Of the simulated selection methods tested, the selection among and within progenies (S_{aw}) resulted in the greatest genetic gains, regardless of the selection intensity (Figure 1, Table 4). Group coancestry for female and male trees (Θ) predicts low expected inbreeding (< 0.01) in produced seeds on random mating for all scenarios of selection in the seedling seed orchard test (Table 4). As the number of selected individuals decreased with increased selection intensity, the genetic gain increased and the N_e decreased for both methods, but was more pronounced for the S_{aw} method. The expected genetic gains with selection ranged from 19.9% with 220 selected trees in the S_{aw} method to 8.6% with 990 selected trees in the S_w method. As expected, S_w was the method that maintained most of the original effective population size, with selection intensities ranging from 146.9 to 304.8, where S_{aw}, ranged from 63.1 to 283.1 (Figure 2). The different simulated intensity of selection had different impacts on the values of genetic gain ($Gs\%$) and N_e, according to the selection method (Figure 3). The optimal situation for the S_{aw} method is the selection of 660 individuals (66 progenies, each one with 10 individuals), resulting in a genetic gain of 12.7% and N_e of 188.8, where for situation for S_w method, is the selection of 550 individuals (5 individuals within each progeny), resulting in a genetic gain of 11.0% and N_e of 244.7 (Figure 3, Table 4). The S_{aw} method decreases the N_e in 50.6% in relation to the original population, where S_w method decreases about 35.9%.

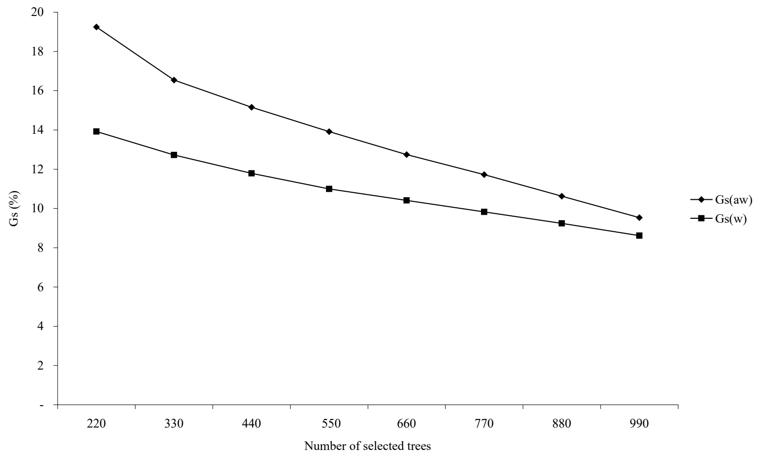

Figure 1. Genetic gain related to the number of selected individuals according to the selection method: among and within progeny ($Gs_{(aw)}$) and within progenies ($Gs_{(w)}$).

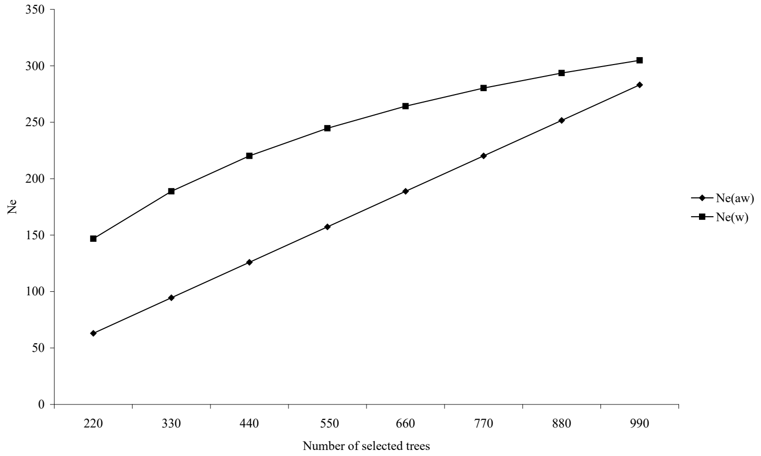

Figure 2. Relation between effective population size (N_e) related to the number of selected individuals according to the selection method: among and within progeny ($N_{e(aw)}$) and within progenies ($N_{e(w)}$).

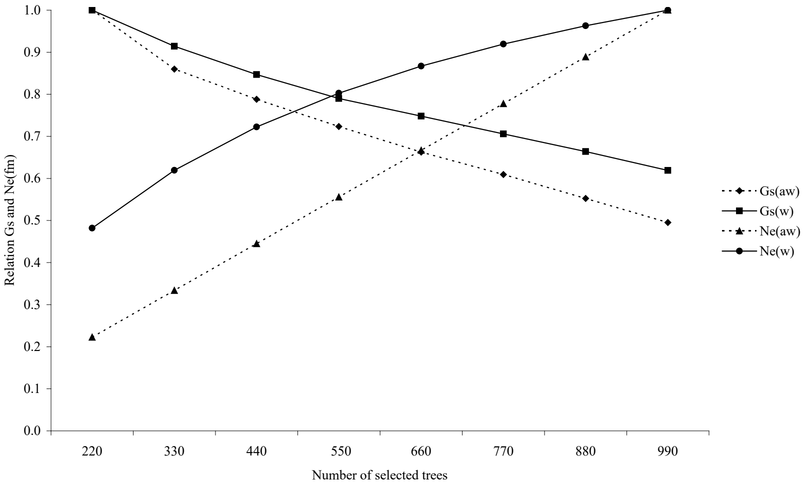

Figure 3. Optimal point (crossing the lines) to select the number of trees based on genetic gain (Gs) and effective population size (N_e) for selection among and within progeny ($Gs_{(aw)}$, $N_{e(aw)}$) and within progeny ($Gs_{(w)}$, $N_{e(w)}$).

Table 4. Genetic gain ($Gs\%$), improved mean ($\bar{x}_i$), group of coancestry among females and males (Θ), and reproductive effective population size (N_e) for diameter at breast height (dbh), according to the selection among and within progeny (S_{aw}), within progeny (S_w) and selection intensity (SI) to convert the provenance-and-progeny test in a seedling seed-orchard.

Scenario	SI **(%)**	n_s	n_p / n_w	n_f / n_m	$Gs\%$	$\bar{x}_i$ **(cm)**	Θ	N_e
				Among and within progeny (S_{aw} **)**				
1	7.6	220	22/10	110/110	19.2	14.71	0.00568	63.1
2	11.5	330	33/10	165/165	16.5	14.38	0.00379	94.5
3	15.3	440	44/10	220/220	15.2	14.21	0.00284	125.9
4	19.1	550	55/10	275/275	13.9	14.06	0.00227	157.3
5	22.9	660	66/10	330/330	12.7	13.92	0.00189	188.8
6	26.8	770	77/10	385/385	11.7	13.79	0.00162	220.2
7	30.6	880	88/19	440/440	10.6	13.66	0.00142	251.6
8	34.4	990	99/10	495/495	9.5	13.52	0.00126	283.1
				Within progeny (S_w **)**				
9	7.6	220	110/2	110/110	13.9	14.06	0.00114	146.9
10	11.5	330	110/3	220/120	12.7	13.92	0.00114	188.8
11	15.3	440	110/4	220/220	11.8	13.80	0.00114	220.3
12	19.1	550	110/5	330/220	11.0	13.70	0.00114	244.7
13	22.9	660	110/6	330/330	10.4	13.63	0.00114	220.3
14	26.8	770	110/7	440/330	9.8	13.56	0.00114	280.2
15	30.6	880	110/8	440/440	9.2	13.49	0.00114	293.6
16	34.4	990	110/9	550/440	8.6	13.41	0.00114	304.8

Population mean, $\bar{x}$ = 12.34 cm; n_s , n_p , and n_w are the total number of selected trees, progenies and trees within progeny, respectively; n_f and n_m is the number of selected female and male trees within progeny, n_f and n_m is the number of selected female and male trees within progeny, respectively; the N_e for provenance-and-progeny test is 382.

For *ex-situ* conservation of the population in the provenance-and-progeny test, the optimal point of selection was achieved for S_w with the selection of 550 individuals ($Gs\%$ = 11.0%; N_e = 244.7), three females and two males of each progeny, resulting in a seedling seed-orchard with 330 female and 220 male trees. The genetic gain predicts that random mating in the seedling seed-orchard will produce seeds that are expected reach 13.70 cm of dbh at age 33-year in plantations established in similar environmental characteristics of the Itapeva Experimental Station. The collection of seeds of all female trees will result in new forest for commercial or environmental reforestation with low inbreeding ($F = \Theta < 1\%$) and a N_e > 150. Values of N_e > 150 are expected to maintain 90% of the genetic diversity of the founder population for 10 generations (Sebbenn, 2006). The implementation of the S_w strategy will conserve the genetic variability of all five provenances and progeny within provenances, retain more than 64% (=100(244.7/382)) of the actual N_e and produce seeds with 11% of genetic gain.

Alternatively, to increase the genetic gain for commercial reforestation a clonal seed orchard could be established using vegetative propagation of selected trees in the trial based on high mass-selection intensity among and within progeny. For example, the selection of 30 of the best trees (1%), each one from a different progeny, being 15 female and 15 males will result in a genetic gain of 35% for dbh and a N_e of 30. The selection of only one tree per progeny avoids crossing between related individuals and inbreeding in the produced seeds (Sebbenn et al., 2003b; Aguiar et al., 2019).

It is a principle of biology that increased vigor, or the most-rapid growth phenotype of an organism represents optimum ecological fitness (Hallsworth, 2018). During the 33-year period, each value for mean annual dbh increase became progressively less, showing clear signs of the need of performing a selective thinning. The number of individuals is still very high. The selective thinning based on the optimal point of selection within progeny will provide a seedling seed-orchard composed of more productive trees with relevant genetic variability. Lastly, as the species is dioecious; the sex ratio of remaining trees should be considered to ensure a favorable supply of high-quality seeds. We recommend keeping the same number of males and females for each progeny.

CONCLUSIONS

Provenances and progenies within provenances present genetic differences for growth traits, which can be used to obtain genetic gains by selection among progeny and individuals within progeny. Conversion of this trial into a seedling seed-orchard is recommended to provide high-quality improved seeds for growth traits to be used in reforestations and concomitantly ensure the *ex-situ* conservation of the populations. For the purposes of combined *ex-situ* conservation and tree-improvement strategies, the most-successful selection method is high-intensity selection within progenies. This must be carried out only by selection of three females and two males within progeny. Thus, the genetic variation among *A. angustifolia* provenances and progenies within provenances and the effective population size will be maintained, and a substantial genetic gain (11%) can be obtained, providing a higher quality seed production.

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