

## Selection methods to optimize the gain and genetic diversity in *Pinus caribaea* var. *caribaea*

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**Abstract:** We estimated genetic parameters of two *Pinus caribaea* var. *caribaea* progeny tests based on growth traits aiming at the identification of the best selection strategy. This study was conducted in two progeny tests located in Selvíria (MS), Brazil. Tests were set up in block design, one with 76 progenies (area 1) and the other with 99 progenies (area 2). Genetic parameters were estimated using the SELEGEN software through REML/BLUP method. To estimate the genetic gain through selection, four selection methods and ten selection intensities were tested. The intensity of selection that maximizes genetic gain and genetic diversity was calculated by the optimization point. Significant variation was observed between and with progeny for all traits in area 2 and only within plots for height in area 1. Higher values of heritability were observed in area 1. Considering the optimization of selection, the best selection method for both areas was the individual.

**Keywords:** Tree breeding, genetic parameters, REML/BLUP method, selection intensity

### INTRODUCTION

Genetic breeding is one of the tools most used in the Brazilian forestry. The selection method is one of its fundamental strategies for obtaining productive genotypes adapted to the edaphic and climatic conditions of the country (Assis and Resende 2011). Exotic species, such as *Pinus* spp. and *Eucalyptus* spp., are being increasingly cultivated to maintain the supply of timber and non-timber products to forest industries (IBÁ 2023). The Cerrado (Brazilian Savannah) biome is characterized by soils poor in nutrients and dry winters and springs. The *Pinus* species that best adapt in this region were those of tropical origin: *P. caribaea* var. *hondurensis*, *P. caribaea* var. *caribaea*, *P. caribaea* var. *bahamensis*, *P. oocarpa* and *P. kesiya* (Santos et al. 2021). *P. caribaea* is the most productive for wood and resin in tropical areas. The varieties *caribaea*, *hondurensis* and *bahamensis* are the most planted in tropical regions for economic purposes due to their fast growth, good stem shape, resin production and adaptation to the environmental conditions (Schimleck et al. 2018).

Low genetic variation has been observed in *P. caribaea* var. *caribaea* natural populations, which may be associated with their narrow distribution range

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(Rebolledo Camacho et al. 2018). Different from the *hondurensis* and *bahamensis* varieties, this variety can only be found in small areas of Cuba. Genetic variability is the base of genetic breeding. Breeding populations with low genetic variability usually cannot archive high genetic gains, leading to a waste of resources (Isik and McKeand 2019). For any species, this condition is expected in more advanced breeding generations, after several selections have already been applied. So, it is important to study strategies that can better explore the genetic potential of the species progeny tests. The strategies can focus on selection methods and intensities that maximize genetic gains in long-term genetic breeding programs (Hodge and Dvorak 2014). As the situation of the *caribaea* variety has been shown to be atypical, besides maintaining genetic variation in the population, it is important to perform crosses or infusions with new genotypes to increase the genetic variation of the breeding populations (Schmid et al. 2023). Thus, after estimating the genetic parameters, several selection proposals should be examined to prevent significant loss of genetic variability (Schmid et al. 2023).

The definition of the best method will depend on the population genetic diversity and the purpose of the selection. The selection can be practiced at the level of individuals, progenies mean, and between and within progenies (Cotterill 1986, Danusevicius and Lindgren 2002a). In this context, the objective of this study was to estimate the genetic variability in two *P. caribaea* var. *caribaea* progeny tests and to analyze the effects of different methods and intensities of selection on the genetic diversity and genetic gains after selection.

## MATERIAL AND METHODS

### Sampling and experimental design

The seeds used in the present work were from clonal orchard of *P. caribaea* var. *caribaea* of the Center for Genetic Conservation and Improvement of Tropical Pine - CCGMPT, located in Aracruz, ES. The first test (area 1) was installed in 1988, and the second (area 2) in 1989. Two *P. caribaea* var. *caribaea* progeny tests were conducted, one with 76 progenies (area 1) and the other with 99 open-pollinated progenies (area 2). Both tests were set up at the Fazenda de Ensino, Pesquisa e Extensão belonging to the State University of Engineering of Ilha Solteira - FEIS/UNESP (lat 20° 20' S, long 51° 24' W, alt 370 m asl) in municipality of Selvíria, MS, Brazil. The climates are of Aw type according to Köppen's classification, with mean annual temperature of 25.1 °C and mean annual rainfall of 1,305 mm (Capo et al. 2022). The soil of the site was classified as *Latossolo Vermelho distrófico típico* (Oxisol) with clay texture. The experimental areas are in Cerrado biome area.

The experimental design of area 1 was of blocks with 80 treatments (four commercial controls and 76 progenies), four blocks and linear plots. In area 2, a block design was used, with 100 treatments (one commercial control and 99 progenies), three blocks, three replications and linear plots with ten plants. In the two areas, the spacing used was 3 × 3 m. Selective thinning of 50% were performed at 14 and 15 years after planting in area 1 and 2, respectively. Growth traits were measured at 28 and 27 years old in areas 1 and 2, respectively. The evaluated traits were: total height (H, in m); diameter at breast height (dbh, in cm); survival (SUR) and volume of wood (V, in m<sup>3</sup> tree<sup>-1</sup>). Volume (m<sup>3</sup>) was estimated according to the formula:  $V = 0.0798 + 0.3dbh_i^2 H_i$  (Lindberg and Hollaus 2012), where: V = volume; dbh<sub>i</sub> = diameter at breast height; H<sub>i</sub> = height.

Deviance analysis, estimation of variance components and genetic parameters

The REML/BLUP (restricted maximum likelihood/best linear unbiased prediction) method was used for deviance analysis, estimation of the components of variance and prediction of the genetic values. For the two areas the mixed linear model was applied as proposed by Resende (2016):  $y = Xb + Za + Wc + e$ , with: y = data vectors; b = block effects vectors (fix); a = additive genetic effects vectors (random); c = plot effects vectors (random); e = Error effects vectors (random), and X, Z and W = incidence matrices for referred effects. So, the genetic parameters were calculated: coefficient of individual additive genetic variation (CVgi(%)), and of genetic variation between progenies (CVgp(%)), experimental coefficient of variation (CVe(%)), determination coefficient of plot effects ( $\hat{C}_{plot}^2$ ), narrow-sense individual heritability ( $\hat{h}_a^2$ ), progenies mean heritability ( $\hat{h}_m^2$ ), within plot additive heritability ( $\hat{h}^2$ ), and relative coefficient of variation (CV<sub>r</sub>) being  $CV_r = \frac{CV_{gp}(\%)}{CV_e(\%)}$ .

## Selection strategies

Different selection methods and intensities were analyzed to define the best selection strategy to be applied in the trials. Therefore, the selection methods were based on dbh values due to its high  $\hat{h}_a^2$  and high positive significant correlation with volume (Guera et al. 2019). In order to estimate the expected gain with selection, individual additive values (BLUP's) of the dbh trait were considered, for both area 1 and area 2, according to methodology proposed by Resende (2016). Four selection methods (within progenies selection, progenies selection, between and within progenies selection and individual selection) and ten selection intensities (7, 12, 17, 22, 27, 32, 37, 42, 47 and 52%) were adopted, totaling 40 selection strategies for each area.

After applying the different selection methods and intensities, the genetic gain (GS%), and genetic diversity (D) were estimated. Genetic diversity (D) was calculated according to Wei and Lindgren (1991) by:  $D = N_{fe} / N_{fo}$ , where:  $0 < D \leq 1$ , being  $N_{fe}$  the effective number of selected progenies and  $N_{fo}$  the original number of progenies.  $N_{fe}$  was estimated using the equation:  $N_{fe} = (4N_f \bar{k}_f) / [\bar{k}_f + 3 + (\sigma_{kf}^2 / \bar{k}_f)]$ . Where:  $N_e$  is the effective population size;  $N_f$  is the number of selected progenies;  $k_f$  is the number of individuals selected per progeny;  $\bar{k}_f$  is the mean number of individuals selected per progeny; and  $\sigma_{kf}^2$  is the variance of the number of individuals selected per progeny.  $GS\% = \mu a 100 / \mu f$ , where:  $\mu a$  is the genotypic mean of the selected individuals; and  $\mu f$  is the phenotypic mean of the population individuals (Resende 2002). To determine the intensity of selection that maximizes both GS% and D, the values were compared using a relative scale of 0.0 - 0.1. Quadratic equations were used to estimate the regression curves for GS and D. The point of intersection of the curves was used to determine the optimal selection intensity for each method.

## RESULTS AND DISCUSSION

After thinning, 729 and 1,384 plants remained in areas 1 and 2, corresponding to a survival rate of 54.3% and 93.1%, respectively. The low mortality rate observed in area 2 is due to random natural causes and attacks of leaf-cutting ants observed in the field. After 11 years, the survival rate of this area remained high and the thinning had no negative effect on the survival of the plants. In contrast, the high mortality rate observed in area 1 is due to the presence of an understory with a large number of lianas. In search of light, the lianas can reach the top of the trees, causing strangulation. Therefore, management of invasive vegetation in pine plantations, even in adult phase, is essential to maintain high survival rates. Studies with *P. caribaea* var. *hondurensis*, in a location with similar characteristics to those of this study area, also found high survival rates. Moraes et al. (2007) report a 90.4% survival rate two years after selective thinning of 40%. The authors concluded that there was good conduction of the experiment regarding the management directed to the production of favorable conditions to the adaptation of the species to the study region.

The mean values for the traits were lower in area 1 than in area 2: 30.8 and 31.92 cm (dbh), 26.62 and 28.81 m (H) 1.06 and 1.26 m<sup>3</sup> tree<sup>-1</sup> (VOL), respectively. The species has good growth potential in the region when compared to other varieties of *Pinus caribaea*. In the same region of this study, at 14 years old, progenies of *P. caribaea* var. *hondurensis* reached similar average values of 23.2 cm (dbh), 25.04 m (H) and 0.75 m<sup>3</sup> tree<sup>-1</sup> (V), considering the proportional growth over the years (Moraes et al. 2007). In areas 1 and 2, 51.3% and 34% of the progenies have better growth in dbh than the control, respectively. At the individual level, area 1 had 33.2% of the individuals with the best performance than the control, and area 2 had 7%.

$CV_e(\%)$  was low in both areas for all traits. The  $CV_e(\%)$  for dbh, height and volume in areas 1 and 2 ranged from 3.3% to 16.4% (Table 1). This indicates low environmental variability among plots within blocks, except for height, which had the highest  $\hat{C}_{plot}^2$  effects (22%) (Table 1). According to Pimentel-Gomes and Garcia (2002),  $CV_e(\%)$  values between 10 and 20% are considered low for experiments where there is competition between plants. Low values of  $CV_e(\%)$  indicate good accuracy of the method used to estimate genetic parameters (Oliveira et al. 2014). Similar values were found for a 13-year-old *P. caribaea* var. *bahamensis* progeny test also in Selvíria (Silva et al. 2011). The good environmental control can be confirmed from the  $\hat{C}_{plot}^2$ , which were low for the studied areas (<10%). According to the classification presented in Mistro et al. (2019), the height and volume in area 2 had the lowest values of accuracy. So, the dbh should be the prioritized trait in the selection for wood production because it has better precision in the prediction of genetic values and high correlation with height and volume.

**Table 1.** Estimates of genetic parameters and Deviance analysis (ANADEV) for silvicultural traits in two areas of *P. caribaea* var. *cari-baea* at 27 and 28 years old, respectively, in Selvíria, MS, Brazil

Parameter	Area 1			Area 2		
	dbh	Height	Volume	dbh	Height	Volume
$\mu$	30.48	26.62	1.06	31.92	28.81	1.26
$CV_{gi}$ (%)	6.21	5.52	12.57	4.66	1.55	7.09
$CV_{gp}$ (%)	3.1	2.76	6.28	2.33	0.77	3.55
$CV_e$ (%)	3.27	6.73	13.94	7.97	6.17	16.36
$\hat{C}_{plot}^2$	0.07	0.22	0.05	0.12	0.21	0.09
$CV_r$	0.69	0.63	0.67	0.45	0.21	0.35
$\hat{h}_o^2$	0.15±0.08	0.20±0.09	0.11±0.07	0.07±0.04	0.02±0.02	0.03±0.03
$\hat{h}_m^2$	0.48	0.4	0.45	0.2	0.05	0.12
$\hat{h}^2$	0.13	0.2	0.09	0.06	0.02	0.024
<b>Effect</b>	<b>LRT</b>					
Progeny	2.43	2.43	1.55	0.86	0.05	0.20
Plot	2.76	22.89**	1.06	25.75**	64.44**	13.85**

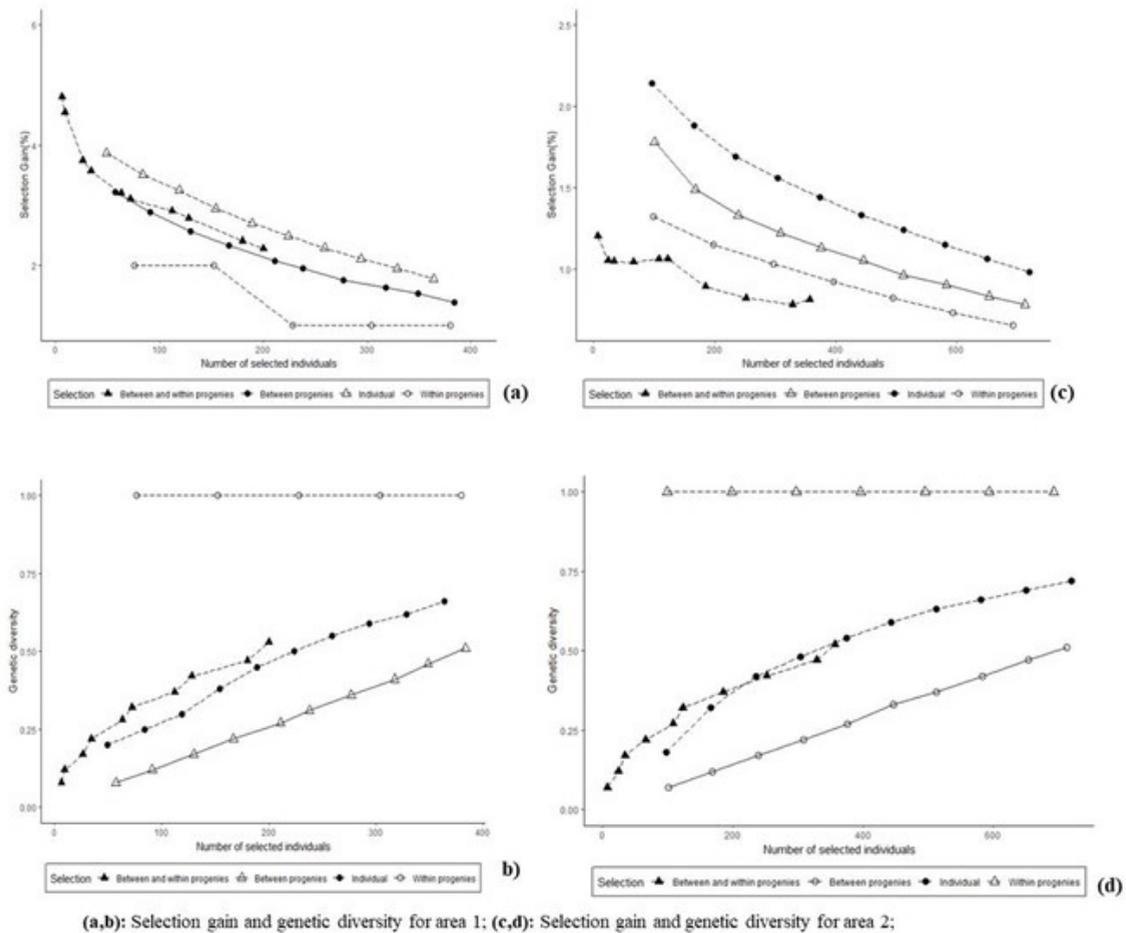
dbh: diameter at breast height (in cm); Height (in m); Volume (in m<sup>3</sup> tree<sup>-1</sup>);  $\mu$ : phenotypical mean;  $CV_{gi}$ : coefficient of individual additive genetic variation;  $CV_{gp}$ : coefficient of genetic variation between progenies;  $CV_e$ : experimental variation coefficient;  $\hat{C}_{plot}^2$ : determination coefficient of plot effects;  $\hat{h}_o^2$ : narrow-sense individual heritability;  $\hat{h}_m^2$ : progenies mean heritability;  $\hat{h}^2$ : within plot additive heritability;  $CV_r$ : relative coefficient of variation; \*\* Significant at 99% of probability with 1 degree of freedom.

$CV_{gi}$ (%) was higher than  $CV_{gp}$ (%) for dbh, height and volume. The higher the  $CV_{gi}$ (%), the greater the probability to obtain superior individuals that will provide significant genetic gains through selection (Moreira et al. 2014). This value was expected, since significant differences were observed only among individuals within progenies. In general, the  $CV_{gi}$ (%) in the two areas were low compared to other progeny tests of the genus *Pinus* (Moraes et al. 2007, Souza et al. 2022). The  $CV_{gi}$ (%) were lower than those observed in *P. caribaea* var. *bahamensis* test with 119 progenies in Selvíria, MS (Missio et al. 2004), and lower than the ones found in a test with 16 progenies of *P. caribaea* var. *bahamensis* at 21 years old set up in São Paulo state (Pires et al. 2013). However, the values observed were similar to or even slightly higher (for area 2) than those observed by Tambarussi et al. (2010) for the same traits in a progeny test of *P. caribaea* var. *hondurensis*. The  $CV_r$  reveals the individual genetic potential without the environmental effect, resulting in greater reliability and genetic gain. The  $CV_r$  with estimate values close to 1 suggests that the genetic control of the characteristic is high, being slightly influenced by the environment (Resende et al. 2016).

There was no significant difference at 1% of probability among the progenies for the evaluated traits in both areas (Table 1). Significant differences ( $P < 0.01$ ) were only detected among plots for H in area 1 and for all the traits in area 2. Although no significant difference was detected by the likelihood ratio test (LRT), the variation of the individual heritability was significantly higher than zero, indicating genetic variation for the studied traits. As discussed previously, the progenies from two areas were originated from a restricted area of Cuba. Possibly, this contributed to a reduction in genetic and phenotypic variation and a high degree of kinship between individuals of these islands. However, significant genetic variation between progenies for growth traits has been observed by authors for the *bahamensis* and *hondurensis* varieties (Pires et al. 2013, Silva Souza et al. 2017). This confirms the need to conduct more careful selection in those *P. caribaea* var. *caribaea* areas.

The  $\hat{h}_o^2$  and  $\hat{h}_m^2$  were moderate in area 1 and low in area 2 for growth traits (Table 1). The individual, mean progeny, and within plot heritabilities were higher in area 1 than in area 2. Silva et al. (2011) observed  $\hat{h}_o^2$  varying from 0.10 to 0.56 for dbh and height in a study carried out with *P. caribaea* in the same region. This information allows concluding that the heritabilities found in area 1 agree with this variation and are lower in area 2, especially if compared to the estimates found in other works with the *Pinus* genus (Li et al. 2017).

The GS% for area 1 ranged from 1% to 4.8% in the four methods and ten selection intensities (6 to 384 individuals selected) (Figure 1a). The genetic diversity ( $D$ ) ranged from 0.08 to 1 in all selection strategies (Figure 1b). For area 1, the selection strategy that provided the highest gain (4.8%) was the between and within progenies selection with selection intensity of 7%. However, this method had the lowest  $D$  with only 6 individuals. Individual selection was the method

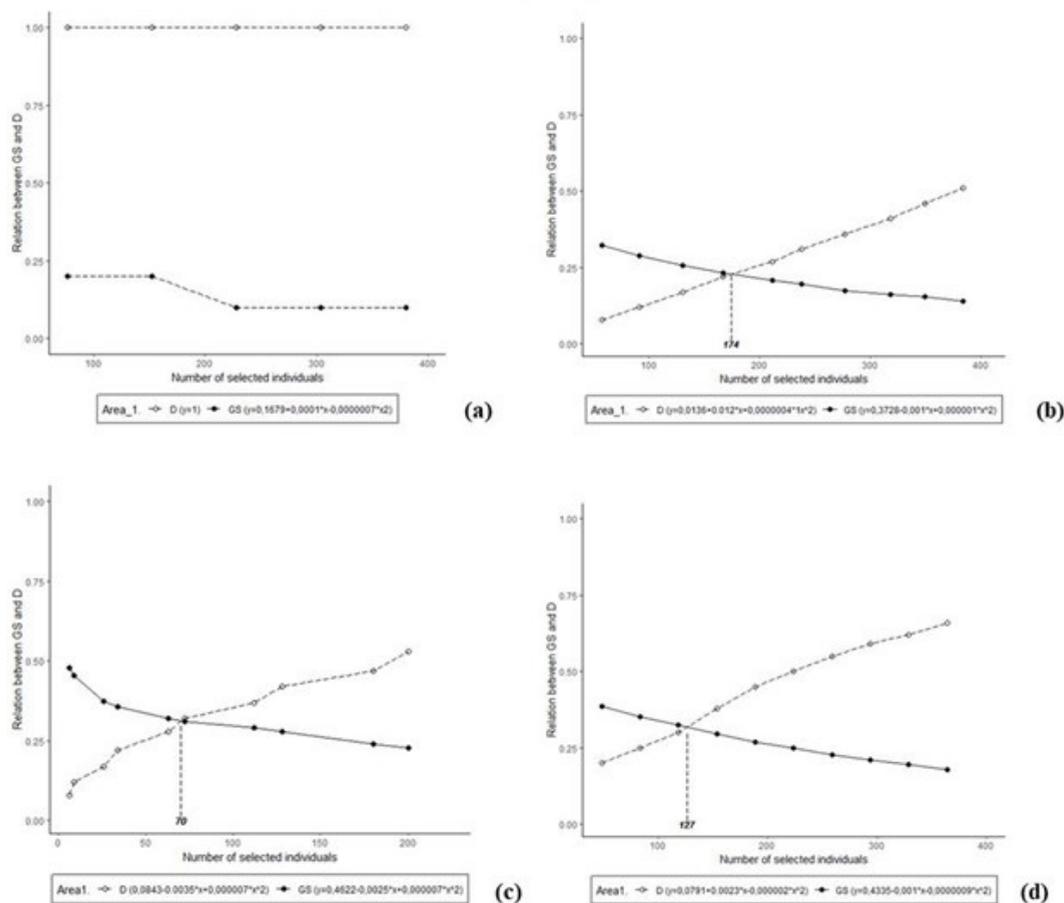


**Figure 1.** Estimated genetic gains (GS) and Genetic diversity for *P. caribaea* var. *caribaea* (area 1 and 2) considering four different selection methods and 10 intensities (7% to 52%).

that had the second highest gains, varying from 3.9% to 1.8%, and  $D$  ranging from 0.2 to 0.7. For the other selection methods, the  $GS\%$  values obtained were very low. In the within progeny selection method, the  $D$  remained at 1, since the same number of progenies will be maintained in the progeny test.

In area 2,  $GS\%$  varied from 0.6% to 2.1% (Figure 1c) and  $D$  ranged from 0.07 to 1 for all selection strategies, with a variation of 7 to 720 selected individuals (Figure 1d). In area 2, the strategy that shows higher gain was the individual selection (2.1%), with a 7% of selection intensity. However, the  $D$  (0.18) and the  $N_e$  (45.5) were very low. The same selection method with lower selection intensities provides higher  $D$  and lower  $GS\%$ . The selection among progenies was the second method with the best gains, ranging from 0.8% to 1.8%, and  $D$  ranging from 0.07 to 0.51. The lowest gains were found in the within and between, and within progenies selection methods. The optimization point corresponds to the intersection point of the gain and diversity (Figures 2 and 3). This point gives the number of individuals to select in order to optimize  $GS\%$  and  $D$  for each selection method. It was not possible to obtain a selection optimization point only with selection within progenies, in both areas. Since all progenies were kept in this method, the  $D$  values remained constant. In area 1 and area 2, the selection optimization points were 174 and 198, 127 and 112 and 70 and 20 individuals for progenies, individual and between and within progenies selection methods, respectively.

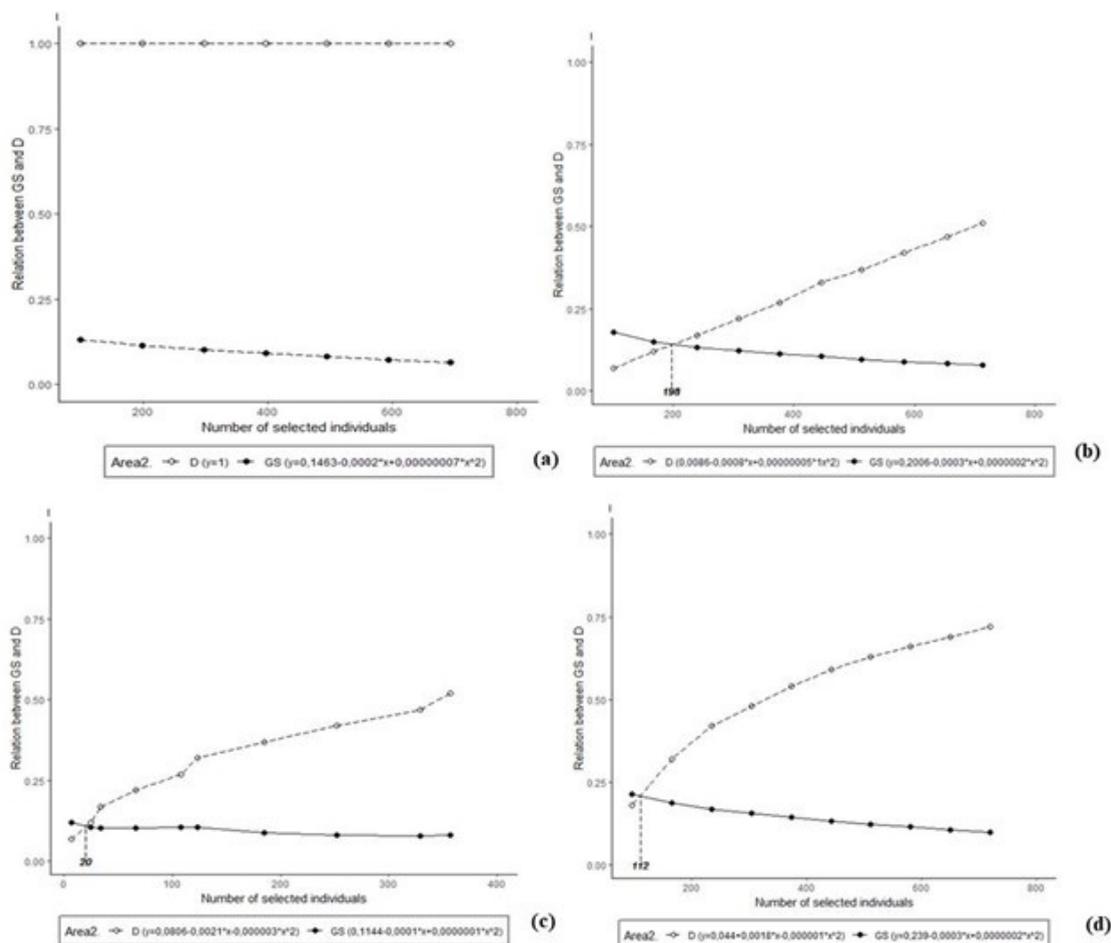
David et al. (2003) report that the  $GS\%$  for volume ranged from 4.6% to 11.8% in all selection methods and intensities, when evaluating four methods and different selection intensities in a *Pinus resinosa* seed orchard. In trying to balance



**Figure 2.** Optimization between genetic gain and genetic diversity considering four selection methods in area 1 of *P. caribaea* var. *caribaea*. (a) There was no optimization point for GS and D in selection within progeny; (b) Optimization point for GS and D in progeny selection; (c) Optimization point for GS and D in selection between and within progenies; (d) Optimization point for GS and D in individual selection.

the retention of *D* and *GS*%, they noted that combined selection would be the most efficient. Martins et al. (2015) also pointed out that combined selection ensured greater *D* for the next generation and higher *GS*%, when testing selection methods for progenies of *Eucalyptus grandis*. Other authors also report the combined selection method as the most efficient in breeding programs (Bhering et al. 2013). Meanwhile, Machado et al. (2021) focusing on the ex-situ conservation and *GS*%, pointed to the high-intensity selection within progenies strategy as the most indicated method for their purpose with a progeny test of araucaria.

After selection optimization for area 1, the selection method that obtained the *GS*% (3.2%) and *D* (0.32) was the between and within progenies with a selection intensity of 30%. The individual selection had the same values as the selection between and within progenies, but with selection intensity of 18.1% (Table 2). In area 2, the highest *GS*% and lowest *D* were observed with the individual selection with a selection intensity of 8.1%. The method of progeny selection resulted in a *GS*% of 1.4% and *D* of 0.14, with 14.1% of selection intensity (Table 2). Considering that some progenies had a higher growth than the control commercial genotypes, superior genotypes to the commercial control of these tests can be selected to compose commercial stands. In general, *GS*% tends to increase according to the reduction of number of individuals selected. High values of *GS*% were achieved by the selection strategies with higher intensity, but those led to a low *D* (Danusevicius and Lindgren 2002b). So, the between and within progenies and the individual



**Figure 3.** Optimization between genetic gain and genetic diversity considering four selection methods in area 2 of *P. caribaea* var. *caribaea*. (a) There was no optimization point for GS and D in selection within progeny; (b) Optimization point for GS and D in progeny selection. (c) Optimization point for GS and D in selection between and within progenies. (d) Optimization point for GS and D in individual selection.

selection methods should be prioritized for areas 1 and 2, respectively. Different results were achieved in tests of selection strategies with *P. caribaea*. Macedo et al. (2015) observed  $CV_{gi}(\%)$  of 18.4% for H and 9.2% for dbh in *P. caribaea* var. *hondurensis* progenies at age of 21 years. The estimates of  $\hat{h}_m^2$  for progeny means were 0.30 for height and 0.02 for dbh. To establish a seedling seed orchard, the authors suggested considering the trait height and a selection intensity of 6%, which resulted in a GS% of 2.8%. Ishibashi et al. (2022) noticed that the selection based on volume at the age of 5 years for progenies of the same species leads to higher GS%, considering selection intensities ranging from 7.8% to 6.4% and 5.4%, and from 10% to 20% and 30%. Apart from the within progeny method, the individual selection method provided the highest values of D in our study, especially with higher selection intensities.

By the analysis of all the selection strategies applied in the study, it was verified that some intensities of selection were close to the intensities of the optimization point, such as 7%, 12%, 27% and 32%. However, it was observed that even in the optimization point of selection it was not possible to obtain high GS%. The selection of only individuals in those areas is not enough to continue a breeding program. Therefore, infusion of the individuals from other breeding programs or commercial plantations should be considered. However, for the composition of commercial stands, the proposed selection strategies can be used. As GS and D vary according to the chosen method of selection and intensity,

**Table 2.** Selection intensity, number of individuals selected, genetic gain (GS) and genetic diversity (D) at the optimization point of GS and D for each selection method in two *P. caribaea* var. *caribaea* progeny tests

Selection method	Sint (%)	SI	GS(%)	D
<b>Area 1</b>				
Progenies	22.9	174	2.3	0.23
Between and within progenies	30.0	70	3.2	0.32
Individual	18.1	127	3.2	0.32
<b>Area 2</b>				
Progenies	14.1	198	1.4	0.14
Between and within progenies	10.0	20	1.1	0.10
Individual	8.1	112	2.0	0.20

Sint: Selection intensity; SI: Selected individuals; D:?

it is necessary to have a clear understanding of the objectives and limitations of the progeny test. If GS% is the priority, then selection between and within progenies or individual selection should be chosen; if D is the main priority, then progenies selection or within progenies selection would be the best selection method. And if both GS and D are equal priorities, a selection optimization, as described here, is the better way to choose the right selection methods. The test of selection strategies to check which one provides the best GS% and D for each trait of interest is fundamental for breeding programs (Souza et al. 2020). The best selection method is the one that predicts high GS%, so it can effectively guide breeding programs and make it possible to decide which selection method to use (Flôres Júnior et al. 2021).

## CONCLUSIONS

There is low genetic variability in the two progeny tests of *P. caribaea* var. *caribaea*. The expected gains with selection in Area 1 were higher than in Area 2. The selection strategy that allowed the greatest gains was selection from within progenies with 7% selection intensity in area 1. For area 2, the greatest gain was 2.14% in individual selection with 7% selection intensity. Regarding selection optimization, in area 1, 70 individuals maximize the gain and genetic diversity in selection between and within progenies, representing a selection intensity of 30%. In area 2, to optimize selection, 198 individuals must be selected in individual selection, representing a selection intensity of 14.14%. In the next generation, control crosses between genetically more distant progenies can be performed to balance the genetic variability of the breeding program, since the selection gains were low, as well as crosses between the progenies of the two progeny tests of *P. caribaea* var. *caribaea*, and crosses with other genotypes coming from other breeding programs.

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## DATA AVAILABILITY

The datasets generated and analyzed are available from the corresponding author upon reasonable request.

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## Selection methods to optimize the gain and genetic diversity in *Pinus caribaea* var. *caribaea*

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