

## Progeny evaluation and early selection for plant height in *Acacia mearnsii* improve genetic gains

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**Abstract:** *Acacia mearnsii* De Wild. is one of the most widely cultivated forest species in Brazil. Despite its importance, studies on breeding strategies for this species are scarce. Therefore, the aim of this study was to evaluate the progeny and develop early selection strategies for *A. mearnsii*. Individuals of half-sib progenies were classified according to their genotypic value and selected based on their relative performance. The additive effect was only significant for plant height. The best progenies and plants should be selected based on plant height after approximately 60 days of being in nursery and 2 years of field cultivation. This strategy eliminates the worse progenies, discards inferior genotypes in the nursery, eliminates unselected genotypes based on plant height before flowering, and intercrosses only the best plants in field cultivation. This resulted in an annual genetic gain of 3% for plant height and 2% for diameter at breast height.

**Keywords:** Forest breeding, nursery, progeny test, intercrossing, indirect gain

### INTRODUCTION

The genus *Acacia* may be found growing naturally in southeastern Australia, with approximately 120-130 species cultivated in all regions of the world, except Europe and Antarctica (Turnbull et al. 1998). *Acacia mearnsii* De Wild. has hermaphroditic flowers with either male and female reproductive organs, or only male functional reproductive organs (Grant et al. 1994). It is a crosspollinated species with partial self-compatibility (Crong and Fuller 1995), and is mainly insect-pollinated (Grant et al. 1994). Plants may start to flower at 2 years of age (Sherry 1971), with seeds being available for collection, for 2-3 weeks, only 12-14 months later (Searle 1997).


In Brazil, *A. mearnsii* was introduced in Rio Grande do Sul in 1918, where almost all of its cultivation is still carried out (Ageflor 2020). However, little information is available regarding adaptations made to germplasm introduction and evaluation for Brazilian growing conditions. *Acacia mearnsii* is cultivated in an area of approximately 75,900 hectares in Rio Grande do Sul and is used for wood production and its bark is used for tannin extraction (Ageflor 2020). Studies indicate the possibility of obtaining multiple uses from *A. mearnsii* and diversification of its products, both timber and non-timber products. In addition, this species is indicated for use in the afforestation of parks and gardens because of its ornamental characteristics (Lorenzi et al. 2018).

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Because of the growing demand for raw materials, *A. mearnsii* cultivation has become increasingly important in the forest-based industrial market, leading to an increase in the area of fast-growing homogeneous forest plantations (Machado et al. 2014). New plantations are most commonly established using seedlings, the seeds of which are collected from areas of commercial production with little or no genetic control, resulting from crossing related individuals, which promotes inbreeding depression (Chan et al. 2015). In addition, most of the seeds are collected from anthills (Chan et al. 2015), which compromises their physiological and sanitary qualities.

To improve perennial cross-fertilized species such as *A. mearnsii*, it is essential to define a selection strategy that maximizes genetic gain and reduces the time required to complete each cycle. Progeny tests are the most appropriate improvement strategy because they evaluate the performance of the selected plants based on estimated genetic values, thereby facilitating the selection of the best progenies and plants (Pires et al. 2013, Costa et al. 2015). Progeny evaluation allows for the estimation of genetic parameters for all traits of interest, as well as the definition of the best selection strategy based on the genetic gain (Kubota et al. 2015). It also enables the assessment of genetic diversity to guide management and conservation strategies (Duarte et al. 2012). Based on the definition and use of more efficient strategies, a mixed linear model analysis via restricted maximum likelihood (REML) best linear unbiased prediction (BLUP) has been used in plant breeding programs (Borges et al. 2010).

Considering the relevance of *A. mearnsii* production chains to the forestry sector, and the need for a breeding method that can maximize genetic gain, this study aimed to evaluate the progeny and develop early selection strategies for *A. mearnsii* to improve genetic gain.

## MATERIAL AND METHODS

The experiments were conducted by the Federal University of Santa Maria, in partnership with SETA S/A Company, between December 2015 and December 2019. We evaluated half-sib progenies of 32 selected plants from a progeny test of 139 plants introduced from Australia in a nursery located in Montenegro and in a field at Encruzilhada do Sul. Both are located in Rio Grande do Sul, Brazil.

For seedling production, approximately 200 seeds of each selected plant were sown in 280 cm<sup>3</sup> polypropylene tubes filled with pine bark substrate. This was done in December 2015. The height of the seedlings was evaluated 22 days after sowing, which was measured from the level of the substrate to the tip of the last leaf. Plant height and stem diameter were measured 51, 76, and 125 days after sowing.

The best seedlings of each progeny were planted in the field on September 19, 2016. Each progeny was divided into replicates comprising three individuals. Each replicate was located at a maximum distance in the field to avoid parental crosses. Plant height was evaluated 85 days after planting, whereas height and diameter (stem diameter or diameter at breast height (DBH)) were evaluated 280, 434, 799, and 1100 days after planting. The absolute average increment in plant height was obtained because the same genotype was used in both the nursery and in field cultivation. The experiment was conducted in an incomplete block design with six replications of three plants.

The data were subjected to deviance analysis (Resende 2002), and the likelihood ratio was used to test the difference significance in the fit of different models, defined as follows:  $\lambda = 2[\text{Log}_e L_{p+1} - \text{Log}_e L_p]$ , where  $L_{p+1}$  and  $L_p$  represent peaks of the likelihood function associated with the full and reduced model, respectively.  $\lambda$  is compared with the probability density function ( $\chi^2$ ), considering the number of degrees of freedom and the error probability.

The REML method was used to estimate the variance components, and the BLUP method was used to estimate the phenotypic and genotypic values (Resende 2002) with the help of the Selegen REML/BLUP software (Resende 2016). Model 67 (progenies and individuals), based on repeated measures, was used for the selection of families, while Model 6, which considers each assessment separately, was used for the identification of superior individuals or genotypes.

Statistical model 67 corresponds to the design in incomplete blocks, expressed by  $y = Xm + Za + Wp + Qs + Tb + e$ , where  $y$  is the data vector;  $m$  is the vector of the effects of measurement-repetition combinations (fixed) added to the overall mean;  $a$  is the vector of individual additive genetic effects (random);  $p$  is the vector of plot effects (random);  $s$  is the vector of permanent (random) effects;  $b$  is the vector of block effects (random); and  $e$  is the vector of errors or (random) residuals.

Statistical model 6 corresponds to the design in incomplete blocks, expressed by  $y = Xr + Za + Wp + Tb + e$ , where  $y$  is the data vector;  $r$  is the vector of the repetition effects (fixed) added to the general mean;  $a$  is the vector of additive genetic effects (random);  $p$  is the vector of plot effects (random); repeatability  $b$  is the vector of block effects (random); and  $e$  is the vector of errors or residuals (random). Capital letters represent the incidence matrices for the effects of each statistical model.

The permanent phenotypic effects (mean components) of the 32 *A. mearnsii* progenies were obtained to classify and identify the best families. For all progenies and individuals, the genotypic value represents the general mean of the experiment, added to the genotypic effect. Furthermore, the genetic gain is the average of the predicted genetic effects of each selected genotype, which, when added to the general mean, results in the mean of the improved population. The relative performance represents the relationship between the means of the improved population and the genotype with the highest genetic value. Both progenies and genotypes that presented a relative performance greater than 80% for the genotypic value were selected.

Based on the selected individuals, the genetic gain for plant height and diameter was calculated as the difference between the original mean and the mean of the selected genotypes. The selection gains ( $G$ ) and percentages ( $G\%$ ) were calculated using the following formulas:  $G = (MS - MO)$  and  $G(\%) = \left[ \frac{(MS - MO)}{MO} \right] \times 100$ , respectively, where  $MS$  is the mean of the selected genotype and  $MO$  is the original mean.

## RESULTS AND DISCUSSION

Deviance analysis showed that the additive and permanent effects were significant for both plant height and diameter (Table 1). These results explain most of the variation observed in the plant traits analyzed and indicate the existence of genetic variability among progenies. Knowledge of the genetic variability in populations enables the development of selection strategies that can be adopted in breeding programs (Otsubo et al. 2015). Notably, in addition to indicating the possibility of genetic gain due to selection for plant height, additive effects are heritable by subsequent generations and, therefore, should be considered a crucial parameter in the genetic improvement of populations. The random effects of plot and block (evaluations) were not significant for any of the evaluated traits (Table 1), indicating that they have little effect on the observed variation and that it is possible to perform individual analyses to identify the best time for the selection of individual plants or genotypes.

Regarding the variance components, all individual phenotypic variances for plant height were explained by residual variance (71.6%; environmental and non-additive genetics) and additive variance (16.9%) (Table 1). Regarding plant diameter, the majority of the individual phenotypic variance was explained by residual variance (59.4%) and the variance of permanent effects (33.4%). The fact that the residual variance was greater than the genotypic variance indicates that the environment (residual effect) had a significant influence on phenotypic variance.

Although both traits were greatly influenced by the environment, plant height showed an adequate value of

**Table 1.** Analysis of deviance (ANADEV), variance components, and genetic parameters for plant height (cm) and diameter (mm) of 32 *Acacia mearnsii* progenies cultivated in a nursery (three evaluations) and field (four evaluations)

Effects	Height	Diameter
Additive	22457.89**	8787.51
Plot	22443.46	8787.22
Permanent	22500.06**	9030.93**
Block	22441.09	8786.83
Full model	22441.04	8786.83
Component/Parameter <sup>1</sup>		
$V_a$	804.959152	6.647374
$V_{plot}$	67.197728	1.086394
$V_{perm}$	475.528671	36.290074
$V_{block}$	7.452678	0.066163
$V_e$	3411.500861	64.401656
$V_p$	4766.639089	108.491661
$h_a^2$	0.168874 ± 0.0472	0.061271 ± 0.0349
$c_{plot}^2$	0.014098	0.010014
$c_{perm}^2$	0.099762	0.334496
$c_{block}^2$	0.001564	0.000610
$r$	0.284296 ± 0.0307	0.406391 ± 0.0449
Overall mean	347.641679	45.971451

\*\*  $p < 0.01$  and \*  $p < 0.05$  by the  $\chi^2$  test with one degree of freedom in the Likelihood Ratio Test.

<sup>1</sup>  $V_a$ , additive genetic variance;  $V_{plot}$ , environmental variance between plots;  $V_{perm}$ , variance of permanent effects;  $V_{block}$ , environmental variance between blocks;  $V_e$ , residual variance;  $V_p$ , individual phenotypic variance;  $h_a^2$ , individual heritability in the narrow sense (additive effects);  $c_{plot}^2$ , coefficient of determination of plot effects;  $c_{perm}^2$ , coefficient of determination of permanent effects;  $c_{block}^2$ , coefficient of determination of block effects;  $r$ , individual repeatability; and the overall mean is the overall mean of the experiment.

additive variance, which consists of the heritable portion of the genotypic variance that guarantees genetic gain from selection. Therefore, the variance components and genetic parameters indicate that plant height can be used to select the best *A. mearnsii* progenies and plants. This is a relevant result, as it shows that *A. mearnsii* plants can be selected based on one of the most important silvicultural traits for yield, which favors and increases the efficiency of field work, eliminates unnecessary evaluations, and enables direct and indirect gains from selection. This is an operational advantage because the plantation evaluation process is one of the most time-consuming and costly steps in genetic improvement programs. Furthermore, as Resende et al. (1991) observed, plant height and diameter are highly correlated. This suggests that selection for one trait promotes indirect selection gains for the other. Plant height was also found to be suitable for the early selection of *Jatropha curcas* plants (Borges et al. 2014).

Environmental variance was an important component that negatively affected the heritability estimates (Table 1). Because of the high values of residual variance, the individual repeatability coefficients ( $r$ ), which represent heritability in a broad sense, are classified as low magnitude for plant height and medium magnitude for plant diameter (Resende 2016). In perennial plants such as *J. curcas*, low repeatability values are expected, mainly for productivity (Laviola et al. 2013), which corroborates the importance of its estimation. Low values for yield repeatability have also been obtained for other perennial species such as *Annona muricata* (Sánchez et al. 2017), *Poncirus trifoliata*, and *Citrus unshiu* (Imai et al. 2016).

Considering that plant height had a significant additive effect (Table 1), the 32 *A. mearnsii* progenies were ranked based on average components (individual BLUPs) (Table 2). All the evaluated progenies showed a relative performance greater than 85%, thereby confirming their superiority for having been previously selected, and indicating that all progenies should be maintained for selecting genotypes within them.

In a selection program, plants within progenies should be selected as early as possible to reduce the time needed to complete each cycle. As shown in this study, the best *A. mearnsii* progenies and plants, in terms of plant height, must be identified, and individual analysis would indicate the best time to conduct the selection process. The analysis of plant height in each evaluation showed that the additive effect was significant for all evaluations, except at cultivation days 280 and 434 in the field (Table 3). The high heritability estimates of individual additive ( $h^2_{oi} \geq 0.5$ ) and progeny mean ( $h^2_{mp} > 0.9$ ) indicate the possibility of obtaining genetic gain based on plant height during nursery cultivation. Moreover, the relative coefficients of variation were higher for nurseries than for field evaluation. The narrow-sense individual heritability coefficients adjusted for plot effects were similar between the nursery and field evaluations, but a high estimate ( $h^2_{aj} = 0.873$ ) was obtained at day 799 of field cultivation. These results indicate that plants should be selected based on plant height in the nursery 51 days after sowing and 799 days after planting in the field. These two time points for the selection of *A. mearnsii* plants corresponded to the maximum absolute average increase in plant height between the evaluations

**Table 2.** Individual additive genetic effects (a), selection gain (SG), new mean (Nm), and relative performance (RP%) estimated for plant height (cm) of 32 *Acacia mearnsii* progenies cultivated in a nursery (three evaluations) and field (four evaluations)

Progenies	a	SG	Nm	RP (%)
P060	57.4109	57.4109	405.0525	100.0
P102-2	45.9865	51.6987	399.3404	98.6
P135-2	29.7762	44.3912	392.0329	96.8
P012-1	26.5594	39.9332	387.5749	95.7
P119-2	25.1096	36.9685	384.6102	95.0
P037-1	20.158	34.1668	381.8084	94.3
P135-1	14.7054	31.3866	379.0283	93.6
P027-2	12.1788	28.9856	376.6273	93.0
P097-1	10.9559	26.9823	374.624	92.5
P011	9.0352	25.1876	372.8293	92.0
P113	5.9036	23.4345	371.0762	91.6
P032-1	5.3607	21.9284	369.57	91.2
P102-1	4.4661	20.5851	368.2268	90.9
P014-1	2.6632	19.305	366.9466	90.6
P016-1	1.7565	18.1351	365.7767	90.3
P030-1	-3.1539	16.8045	364.4462	90.0
P021	-3.6736	15.5999	363.2416	89.7
P014-2	-6.4065	14.3773	362.019	89.4
P020-1	-8.3021	13.1837	360.8254	89.1
P116-1	-8.9902	12.075	359.7167	88.8
P055-2	-9.5533	11.0451	358.6867	88.6
P133-1	-9.7257	10.1009	357.7426	88.3
P025	-10.5253	9.2041	356.8458	88.1
P027-1	-14.5025	8.2164	355.858	87.9
P140	-14.6444	7.3019	354.9436	87.6
P002-1	-18.4711	6.3107	353.9523	87.4
P119-3	-20.4015	5.3213	352.963	87.1
P119-1	-21.3619	4.3684	352.01	86.9
P055-1	-24.0517	3.3884	351.03	86.7
P001v	-24.4198	2.4614	350.1031	86.4
P075	-33.6764	1.2957	348.9374	86.1
P038	-40.1661	0	347.641	85.8

**Table 3.** Analysis of deviance (ANADEV), variance components, and genetic parameters for plant height (cm) of 32 *Acacia mearnsii* progenies cultivated in a nursery (three evaluations) and field (four evaluations)

Effect	Plant height in nursery (cm)			Plant height in field (m)			
	51 days	76 days	125 days	280days	434 days	799 days	1100 days
Additive	1207.64**	1320.42**	1395.69**	-10.63	137.49	378.99**	428.72*
Parcel	1134.15	1227.53	1295.34	-8.8*	139.4	370.98*	422.1
Permanent	1134.33	1227.46	1295.33	-12.92	137.17	366.01	421.9
Full model	1134.13	1227.44	1295.33	-12.92	137.17	365.8	421.9
Component/ Parameter <sup>1</sup>							
$V_a$	85.558	165.179	199.080	0.085	0.051	1.091	0.699
$V_{plot}$	0.339	0.838	0.270	0.060	0.082	0.276	0.065
$V_{block}$	0.306	0.115	0.026	0.000	0.001	0.019	0.000
$V_e$	-45.278	-98.113	-114.880	0.191	0.447	0.158	0.976
$V_p$	40.925	68.019	84.496	0.336	0.580	1.545	1.741
$h_a^2$	0.500	0.537	0.539	0.173	0.102	0.290	0.218
$h_{aj}^2$	0.212	0.246	0.236	0.308	0.102	0.873	0.417
$c_{plot}^2$	0.008	0.012	0.003	0.177	0.141	0.179	0.038
$c_{block}^2$	0.007	0.002	0.000	0.001	0.001	0.013	0.000
CVgi (%)	24.484	25.767	21.760	10.092	4.601	11.344	7.065
CVgp (%)	12.242	12.884	10.880	5.046	2.301	5.672	3.533
CVe (%)	3.661	3.464	2.733	9.837	7.114	6.490	3.688
CVr	3.344	3.719	3.982	0.513	0.323	0.874	0.958
$h_{mp}^2$	0.985	0.988	0.990	0.612	0.386	0.821	0.846
$h_{ad}^2$	3.397	4.807	4.337	0.250	0.079	0.838	0.349
Acprog	0.993	0.994	0.995	0.782	0.621	0.906	0.920
Mean	37.78	49.88	64.84	2.89	4.91	9.21	11.83

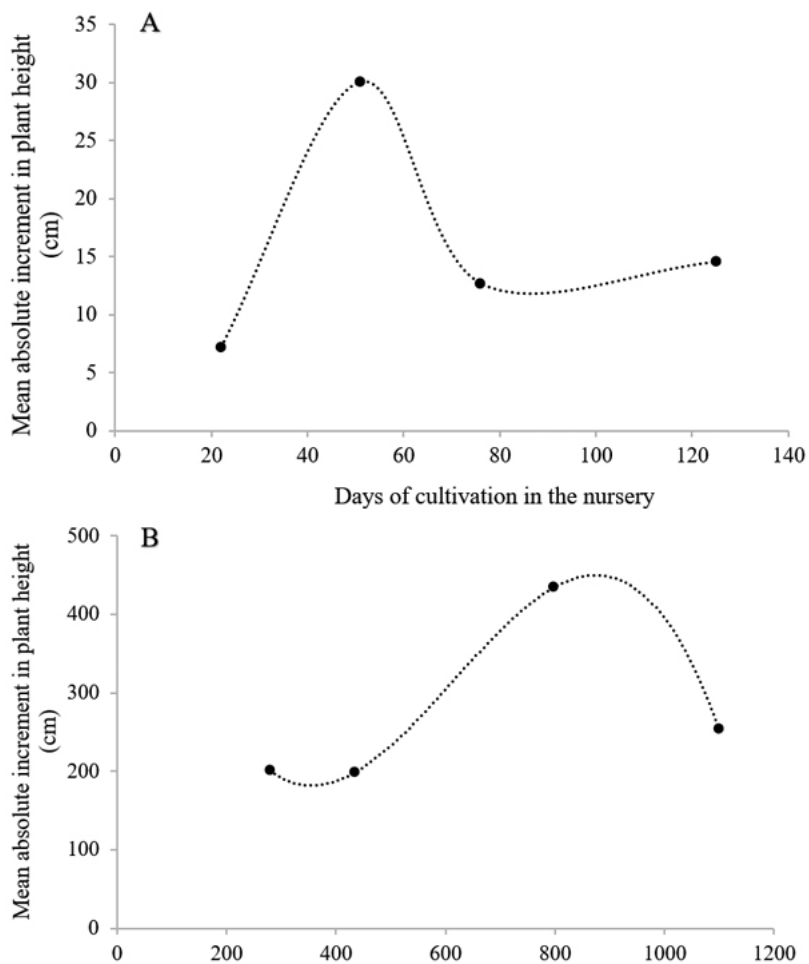
\*\*  $p < 0.01$  and \*  $p < 0.05$  by the  $\chi^2$  test with one degree of freedom in the Likelihood Ratio Test. <sup>1</sup>  $V_a$ , additive genetic variance;  $V_{plot}$ , environmental variance between plots;  $V_{perm}$ , variance of permanent effects;  $V_{block}$ , environmental variance between blocks;  $V_e$ , residual variance;  $V_p$ , individual phenotypic variance;  $h_a^2$ , individual heritability in the narrow sense (additive effects);  $h_{aj}^2$ , individual heritability in the narrow sense adjusted for plot effects;  $c_{plot}^2$ , coefficient of determination of plot effects;  $c_{block}^2$ , coefficient of determination of block effects; CVgi, individual additive genetic variation coefficient (%); CVgp, coefficient of genotypic variation between progenies (%); CVe, residual coefficient of variation (%); CVr (CVgp/CVe), relative coefficient of variation;  $h_{mp}^2$ , mean heritability of progeny assuming complete survival;  $h_{ad}^2$ , additive heritability within the plot; Acprog, accuracy of progeny selection assuming complete survival; and mean is the overall mean of the experiment.

in the nursery (Figure 1A) and field (Figure 1B), indicating that the plants should be selected approximately 60 days after sowing in the nursery and approximately 2 years after planting in the field.

Selection based on only one evaluation of plant height in the nursery and field is sufficient to facilitate breeding. At 799 days in the field, plant height showed a positive correlation ( $r = 0.571$ ,  $p < 0.001$ ) with diameter at breast height, suggesting an indirect gain from selection. These traits are easily measured and provide a growth advantage to the plants during field development (Pinto et al. 2015) because they are important traits for plantlet quality (Grossnickle and McDonald 2018). In forest species, selection is usually based on one evaluation at the earliest possibility to maximize genetic gain (Resende and Barbosa 2005). In *A. mearnsii*, unselected plants can be eliminated at 2 years old, just before they start flowering (Sherry 1971), thereby improving the gain from selection.

Similar results were obtained for *Myrocarpus frondosus*, in which plant height selected at 60 days of nursery cultivation resulted in indirect gains in the relationship between plant height and stem diameter (Bisognin et al. 2020). Early selection of eucalyptus hybrid clones for diameter at breast height can be performed after 3 years of cultivation, which led to a high correlation with diameter at breast height and plant height after 7 years of cultivation (Beltrame et al. 2012). Selection at 3 years of cultivation for diameter at breast height and plant height was indicated for *E. urophylla* clones (Pinto et al. 2014), whereas selection at 2 years of cultivation for diameter at breast height was indicated in eucalyptus clonal tests (Massaro et al. 2010).

The permanent phenotypic effects of the 32 *A. mearnsii* progenies were obtained for plant height to classify and identify the best genotypes in each of the evaluations that showed a significant additive effect. In this experiment,



**Figure 1.** Mean absolute increment in plant height (cm) of 32 progenies of *Acacia mearnsii* in nursery cultivation for 120 days (A) and in field cultivation for 1100 days (B). Plants were evaluated at 51, 76, 103, and 125 days of nursery cultivation and at 85, 280, 434, 799, and 1100 days of field cultivation.

repeated measurements of plant height were used for progeny selection and for identifying the best plants within each progeny for field planting. The high relative performance (> 85%) observed in all progenies (Table 2) and plants indicated that the selection of the best progenies with the best genotypes to be planted in the field constitutes an efficient strategy and should be performed approximately 60 days after sowing. As field selection should be performed 799 days after planting (approximately 2 years), the plants were evaluated for relative performance, with the lowest percentage being 73.4% (P038-15). At day 1100 of field cultivation, the lowest percentage of relative performance was 88.41% (P075-20), indicating the high growth vigor of all evaluated plants. Based on these criteria, 108 plants were selected with a relative performance above 80.2%, corresponding to 40.1% of the evaluated plants. The strategy of selecting 108 plants for intercrossing would result in a direct gain of 11.8% for plant height and an indirect gain of 8.1% for diameter at breast height (Table 4). The best 20 *E. camaldulensis* progenies ranked by BLUP resulted in a direct genetic gain of 19.6% for the diameter of breast height at 2 years (Costa et al. 2015). In contrast, a genetic gain of 10.9% was estimated in eucalyptus hybrid clones for the diameter at breast height at 3 years (Beltrame et al. 2012).

The results of this study support an early selection strategy for *A. mearnsii* progenies to identify the best progenies, and genotypes within progenies, for intercrossing. Deviance analysis, variance components, and genetic parameters indicate that plant height should be used for progeny and genotype selection. The selection of plants in the juvenile

stage reduces the number of genotypes to be evaluated in the field and maximizes the genetic gain in woody perennial species (Bisognin et al. 2020). The evaluation of plant height, which is easy to measure and positively correlated with the diameter at breast height, results in an operational advantage and enables direct and indirect genetic gains. In this early selection strategy, the best progenies and genotypes were selected based on plant height at approximately 60 days of nursery cultivation, and the best plants within the progenies were identified after 2 years of field cultivation. This strategy eliminates the worse progenies, discards inferior and/or inbreeding genotypes based on plant height during nursery cultivation, and eliminates unselected plants before flowering at 2 years of age (Sherry 1971) to facilitate intercrossing only selected plants. During interbreeding in the field, parental crosses are sidestepped based on the distance between selected plants of the same family. As *A. mearnsii* seeds are available for collection approximately 1 year after flowering (Searle 1997), a new cycle of selection can be started every 4 years. The proposed strategy allowed an annual genetic gain of approximately 3% in plant height and 2% in diameter at breast height in *A. mearnsii* plants (Table 4). This genetic gain was estimated by selecting the best 108 plants, which were sufficient to maintain high genetic variability, both for the selection of other traits and for conducting successive cycles of selection.

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**Table 4.** Genetic gain by selection of 32 *Acacia mearnsii* progenies for plant height (m) and diameter at breast height (cm) based on plant height at 799 days of field cultivation

Values	Height (m)	Diameter (cm)
Original mean	9.3	7.4
Improved mean	10.4	8.0
Selection gain	1.1	0.6
Selection gain (%)	11.8	8.1

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