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Genetic variability and predicted gain in progeny tests of native Atlantic Forest timber species: *Cariniana legalis*, *Cordia trichotoma*, and *Zeyheria tuberculosa*

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Abstract Over the years, the Atlantic Forest has been one of the biomes most affected by fragmentation and despite the scarcity of studies related to their genetic and breeding aspects, native Atlantic Forest species can provide various high-quality timber products. In this context, the objectives of this work were to estimate genetic parameters and predict genetic gain with selection of provenance/progeny tests of *Cordia trichotoma*, *Zeyheria tuberculosa*, and *Cariniana legalis*, for diameter at breast height (DBH) and survival rate. Seed trees from different provenances were selected and measured, and their seeds were collected and tested in the areas of Porto Seguro - Bahia state, Brazil. All test individuals were evaluated for DBH (cm) survival rate (%) and stem straightness. The variance components and genetic parameters were estimated for DBH and survival rate. The narrow-sense individual heritability (h^2i) for DBH of the three species ranged from 0.26 to 0.72, showing moderate to high genetic control. However, based on the likelihood ratio test (LRT) there is no genetic variability among genotypes of *Z. tuberculosa*. For survival rate, high genetic control (0.54) was observed only for *C. trichotoma*, while for *C. legalis* and *Z. tuberculosa* h^2i value was 0.11 and 0.0, respectively. After genetic parameter analyses, the expected genetic gains were estimated for seed trees, potential genitors and potential clones. For seed trees, gains based on the additive genetic values (u+a) for DBH by selecting the top 10 individuals of each species per family ranged between 17.17 and 30.31%. In the case of potential genitors, by selecting the top ten individuals based on u + a value, are expected gains between 19.17 and 49.65%. The ranking of the top ten potential clones based on genotypic values (u +g) for each species showed gains between 32.43 and 56.53%. Conducting genetic breeding for *C. trichotoma* and *C. legalis* presents high potential of genetic gains and efforts should be taken into account for supporting breeding strategies for those species. In the case of *Z. tuberculosa*, the absence of genetic variability seems to be a result of the Atlantic Forest deforestation and genetic basis narrowing.

Keywords: forest breeding, genetic variation, genetic values, forward selection.

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Introduction

Brazil is considered one of the main global exporters of planted forest products (Sundstrom & Henry 2017, Cubbage et al. 2020, IBA 2021). This fact has been supported by the genetic breeding programs allied with forest management and fertilization efforts. Breeding steps, such as selection and cloning of elite genotypes, are essential tools for increasing the productivity and the quality of the produced and exported wood (Assis & Resende 2011, Beltrame et al. 2012, Oliveira et al. 2020). However, most part of these breeding programs have focused on species of the genera *Eucalyptus* and *Pinus*, both widely used in forest breeding research. Despite the gradual expansion of plantations with exotic forest species, the biodiversity of Brazilian flora allows the use of several native species for establishing commercial forests stands and for manufacturing wood products by the timber industry (Assis & Resende 2011, Batista et al. 2020). Several native species of Atlantic Forest have high-strength and durable wood potentially useful for various purposes. However, for the most of these species have not been yet developed breeding programs (Carvalho et al. 2018, Cruz et al. 2020). Genetic breeding programs should be proposed to direct this rich genetic resource for civil construction and production of floors, furniture, and ship decks, contributing to an increase in wood exports from Brazil.

In genetic parameters analyses, the potential production of a genotype may be predicted as a function of its genetic merit. Based on variability, the determination of genetic merit is fundamental for supporting individuals' selection based on traits of industry interest (Kumar et al. 2014, Santos et al. 2021a). For breeding purposes, selection based on additive genetic values (u+a) indicates the presence of favorable genes underlying traits of interest, and heritability ensures the possibility of success in breeding programs stages (Kumar et al. 2014, Santos et al. 2015). Genetic variability due to wide geographical distribution is observed in several species of Brazilian flora (Silva junior et al. 2020). In many cases, it results in different phenotypes among individuals of the same species (McKown et al. 2014, Wani et al. 2019).

In plant selection, environmental factors may mask genetic merit and induce genotypes selection based on overestimated or underestimated phenotypes (Amini et al. 2011). Especially for productivity traits, since most of them have complex genetic control and are substantially influenced by the environment (Falconer 1987, Amini et al. 2011). In general, quantitative traits such as growth and diameter at breast height (DBH), are highly influenced by environmental factors (Martins et al. 2018), and genotypes respond differently depending on the genetic control degree of these traits in a specific progeny test. To measure the influence of these variations, heritability is one of the main components to be considered, since it reflects the percentage of genetic inheritance, genetic gains, and trait susceptibility to environmental variations (Santos et al. 2015, Mihai et al. 2019). In this context, the progeny and provenance tests allow the assessment of genetic variation among and within populations and families, and estimation of the genetic parameters. However, for many Brazilian native perennial species, these parameters have not been estimated, variability within and among populations is scarce, and limited reports are available.

In this study it was evaluated the intraspecific genetic variability of three Atlantic Forest timber species. In this context, the objectives addressed were: (i) the estimation of genetic parameters for *Cariniana legalis* (Mart.) Kuntze, *Cordia trichotoma* (Vell.) Arrab. ex Steud, and *Zeyheria tuberculosa* (Vell.) Bureau ex Verl., displayed in progeny and provenance tests; (ii) the estimation of genetic gains with the selection of individuals as potential genitors based in the additive genetic values (u+a); (iii) and the estimation of genetic gains with selection of individuals as potential clones based in the genotypic value (u+g) for the three species. We hypothesized that after decades of Atlantic Forest deforestation there were no genetic variability to be used in genetic breeding programs and that the range of genetic gains with selection is low, due to hypothetical scarcity of genetic variation in Atlantic Forest timber germplasms.

Methods

Genetic material and experimental design

Provenance/progeny tests for *C. legalis* (jequitibá-rosa), *C. trichotoma* (louro-pardo or claraíba) and *Z. tuberculosa* (ipê-felpudo) were conducted in areas of the company Symbiosis Investimentos e Participações S. A. located in the municipality of Porto Seguro, in Southern Bahia, Brazil (latitude 16°34′08.29″S; longitude 39°10′09.33″W; elevation 56 m). The average temperature of the area is around 25°C, with higher temperatures occurring from December to March and lower temperatures from June to September. The annual precipitation is around 1500 mm.

Species evaluated in the tests were chosen according to their good silvicultural performance and high wood economic value. First, the seed trees of the three selected species were selected in the natural rainforests of southeastern Brazil and then their circumferences at breast height were measured. The seed trees of *C. legalis* were selected in five different municipalities of Espírito Santo state and for *C. trichotoma* the collection occurred in 12 municipalities in the states of Minas Gerais, Rio de Janeiro and Espírito Santo. For *Z. tuberculosa* the seed trees were selected in the states of Minas Gerais and Espírito Santo in 12 different municipalities. Subsequently, the seed trees were phenotypically selected and had their seed collected to conduct progeny/provenance tests.

The progeny/provenance tests were conducted using different numbers of seed trees for each of the three species (21, 23, and 30 for *C. legalis*, *C. trichotoma*, and *Z. tuberculosa*, respectively). In each test, all families were represented by 10 individuals. Plantings of *C. trichotoma*, *Z. tuberculosa*, and *C. legalis* were carried out in May 2014, November 2013, and April 2014, respectively. The statistical arrangement used was a randomized block design with 10 blocks, single-tree plots, and plants displayed in 4 m × 4 m grid.

Before planting, the area destined to assembling the tests was subsoiled to a depth of 60 cm. At the time of planting, each plant was fertilized with 200 grams of simple superphosphate. After that the seedlings did not receive additional fertilization. There was also no pruning until the moment of data collection. Seedlings were crowned once in the first year and weeds were controlled with herbicide (glyphosate + Clorimurom-ethyl) twice a year.

Trait measurements

The tests for *C. trichotoma* and *C. legalis* were evaluated at 35 months (2.9 years) and those for *Z. tuberculosa* were evaluated at 42 months (3.5 years) of age. All individuals were evaluated for the following traits: circumference at breast height (CBH, cm), survival rate (%) and stem straightness. CBH was determined with the aid of a tape measure, and the other two traits were determined by the observations of a single researcher. The diameters at breast height (DBH, cm) were obtained by dividing the CBH values by π (the ratio between the circumference and diameter of a circle estimated as 3.14159). For the stem straightness, grades 1, 2, 3, and 4 were assigned for the trees, where 1 represents a straight stem and grades 2, 3 and 4 represents more elevated degrees of tortuosity. The stem straightness scale was used in association with the additive genetic value (u + a) for the selection of potential genitors with straight stem and high values of u+a.

Quantitative genetic analyses and selection of superior genotypes

In order to estimate the genetic parameters of the studied traits, the mixed model used for this experimental network analysis containing different families, provenances, and individuals arranged in random blocks and single-tree plots was given by the equation y = Xr + Za + Ts + e, where y is the data vector, r is the vector of block effects (assumed to be fixed) plus the overall average, a is the vector of individual additive genetic effects (assumed to be random), s is the vector of population or provenance effects (random), and e is the vector of errors or residuals (random). The incidence matrices of these effects are represented by capital letters (Resende 2016).

The mixed model equations were defined as:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + A^{-1}\lambda_1 & Z'W \\ W'X & W'Z & W'W + I\lambda_2 \end{bmatrix} \begin{bmatrix} \hat{r} \\ \hat{a} \\ \hat{s} \end{bmatrix} \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix}$$

where X, Z and W: incidence matrices of the effects of blocks, additive genetic values and provenances, respectively; A^{-1}: kinship matrix among half siblings; I: identity matrix;

$$\lambda_1 = \frac{\hat{\sigma}_e^2}{\hat{\sigma}_a^2} = \frac{1 - hi^2 - c^2prov}{hi^2}; \lambda_2 = \frac{\hat{\sigma}_e^2}{\hat{\sigma}_c^2} = \frac{1 - hi^2 - c^2prov}{c^2prov}$$

σ_e^2 = residual variance

σ_a^2 = additive genetic variance

$h_i^2 = (\sigma_a^2)/(\sigma_f^2)$ narrow-sense individual heritability

$\sigma_f^2 = \sigma_a^2 + \sigma_{prov}^2 + \sigma_e^2$ = phenotypic variance

σ_{prov}^2 = variance among provenances

$c^2 prov = (\sigma_{prov}^2)/(\sigma_f^2)$ = determination coefficient of provenances effects

σ_g^2=variance among progenies, which is ¼ of additive genetic variance

$$CVgp\% = 100 \times \left(\frac{\sqrt{\sigma_g^2}}{\text{general mean}}\right) = \text{ coefficient of genetic variation among progenies}$$

$$CV_{gi} = 100 \times \left(\frac{\sqrt{\hat{\sigma}_a^2}}{\text{general mean}}\right) = \text{ individual genetic coefficient of variation}$$

$$CVe\% = 100 \times \left(\frac{\sqrt{\hat{\sigma}_e^2}}{\text{general mean}}\right) = \text{ coefficient of environmental variation}$$

$$CVr = \left(\frac{CVgi\%}{CVe\%}\right) = \text{ relative coefficient of variation}$$

$$C^2proc = \frac{\sigma^2 proc}{\sigma^2 y} = \text{coefficient of variation for provenance effects}$$

$$r_{\hat{a}a} = \frac{\sum_{i=1}^{n_p}\left(\sqrt{\frac{1 - PEV_i}{\sigma_g^2}}\right)}{n_p} = \text{accuracy;}$$

n_p = number of progenies;

PEV_i = variance of the prediction error of each progeny;

gain(%) = (((u+a)-u))/u×100 = potential genitors gain

gain(%) = (((u+g)-u))/u×100 = potential clones gain

u+a = additive genetic value

u+g = u+a+d= genotypic value

u = general mean of the experiment

d = dominance effect

a = additive effect

For *C. trichotoma* and *Z. tuberculosa*, the genetic parameters of DBH and survival rate were determined based on the above model, considering open pollination of the seed trees. For *C. legalis*, the model was the same, but 40% of self-pollination was considered, according to previous quantitative genetic tests elaborated to determine the ideal self-pollination rate. All data were analyzed using the mixed model methodology and the Restricted Maximum Likelihood/Best Linear Unbiased Predictor (REML/BLUP) method in the Selegen REML/BLUP software (Resende 2016).

Subsequently, the individuals belonging to the families with positive additive effects were selected according to their additive genetic values (u + a). For selecting individual as potential clones, it was considered the genotypic values (u + g). Beyond additive genetic values the grade for stem straightness was also addressed before indicating individuals as potential genitor. In that case, only individuals with both favorable additive genetic values as potential genitor and grade 1 for stem straightness were indicated for selection. Considering the recommendations for future crosses among the best genotypes, only one best-ranked individual per family was selected as a potential genitor of each of the top 10 families. These top 10 best-ranked potential genitors from each species will be the target of our future studies on early flowering and controlled pollination. Moreover, using the results from the mixed model, a comparison between the 10 best-ranked seed trees based on the predicted genetic values (u+a) and their phenotypic measurements taken in field was conducted for three tree species. The comparison resulted in a coincidence table. A likelihood ratio test (LRT) was set in order to verify the significance of individual genetic variability and provenance effects.

Results

Genetic parameters

The degree of genetic control on the phenotypes of the *C. trichotoma*, *Z. tuberculosa*, and *C. legalis* individuals might be inferred from their individual heritability values (Table 1). In the case of C. trichotoma, the narrow-sense individual heritability (h^2i) indicates moderate genetic control for DBH and high for survival rate. For *C. legalis*, a high h^2i value resulted for DBH and a low inheritance rate for survival rate. Like *C. trichotoma*, the h^2i values observed for the DBH of *Z. tuberculosa* was moderate (0.26), however an approximately null value was observed for survival rate. Although the favorable condition for breeding programs based on predicted heritability for DBH, the likelihood ratio test (LRT) of 5% of significance demonstrated the absence of genetic variability among genotypes for *Z. tuberculosa.*

In general, the three species presented low values of coefficient of variation for provenance effects (c^2proc) for both traits, DBH and survival rate. Among the three species, *C. trichotoma* showed the highest c^2proc values for DBH (0.20), and in the case of *C. legalis* the observed value was null. Considering the individual genetic variation coefficients (CVgi) and the genetic variation among progenies (CVgp) for DBH, both parameters followed the same pattern of narrow-sense individual heritability and *C. legalis* exhibited the highest values (29.89 % for CVgi and 14.98 % for CVgp), followed by C. trichotoma (24.05 % for CVgi and 12.02 % for CVgp) and *Z. tuberculosa* (19.99% for CVgi and 10.0% for CVgp). For survival rate, *C. trichotoma* presented the highest CVgi (28.69%) and CVgp (14.35%) among the three species (Table 1).

The residual coefficient of variation (CVe%) suggests that environmental conditions is an important factor to be considered, as the percentage of this parameter ranged between 21% and 55% for both analyzed traits. Comparing the three species, *Z. tuberculosa* and *C. legalis* presented similar higher magnitudes of CVe for DBH (32.77% and 33.79%, respectively), and for survival rate the experimental heterogeneity for both species were even higher (54.97% for *Z. tuberculosa* and 48.63% for *C. legalis*). In the case of the relative coefficient of variation (CVr), the observed value was greater than 1 only for *C. trichotoma*, both for DBH and survival rate. Although *Z. tuberculosa* and *C. legalis* showed CVr values lower than 1 for DBH, the presented values rather high. Considering the survival rate, unlike *C. trichotoma* low CVr values were observed for *Z. tuberculosa* (0.07) and *C. legalis* (0.27). (Table 1).

Table 1 Estimation of genetic parameters for diameters at breast height (DBH, cm) and survival rate for *Cordia trichotoma*, *Zeyheria tuberculosa*, and *Cariniana legalis*.

	Cordia trichotoma		***Zeyheria tuberculosa***		***Cariniana legalis***	
Effect	**DBH**	**Survival rate**	**DBH**	**Survival rate**	**DBH**	**Survival rate**
h^2i	0.45*	0.54	0.26	~0.00	0.72*	0.11
c^2prov	0.20*	~0.00	0.05	0.03	~0.00	~0.00
CVgi%	24.05	28.69	19.99	3.645	29.89	13.08
CVgp%	12.02	14.35	10.0	1.82	14.95	6.54
CVe%	21.42	26.56	32.77	54.97	33.79	48.63
CVr	1.12	1.08	0.61	0.07	0.89	0.27
$r_{\hat{a}a}$	0.62	0.76	0.54	0.08	0.90	0.63
u	**9.91**	**0.87**	**7.83**	**0.74**	**4.09**	**0.80**

Notes: h^2i: narrow-sense individual heritability; c^2proc: coefficient of variation for provenance effects; CVgi%: individual genetic coefficient of variation coefficient of individual additive genotypic variation; CVgp%: coefficient of genetic variation among progenies; CVe%: coefficient of environmental variation; CVr: relative coefficient of variation; râa: accuracy; u: overall average.; *5% of significance by likelihood ratio test (LRT).

Representing the reliability of the experiment, the accuracy (râa) of the predicted genetic values was higher for DBH than for survival rate. Comparing the accuracy of DBH among the three species, the difference of values was smaller between *C. trichotoma* and *Z. tuberculosa* and the higher accuracy value was observed for *C. legalis* (0.90). For survival rate, similar values were obtained for *C. legalis* (0.63) and *C. trichotoma* (0.76), whereas *Z. tuberculosa* showed the lowest accuracy (0.08) (Table 1).

Selection of seed trees, potential genitors, and potential clones

Seed trees

The percentage of predicted gains indicates breeding success by selecting seed trees based on their additive genetic values (u + a). The genetic gains for DBH, in relation to the general means obtained by selecting the top 10 individuals of each species, ranged from 7.71% to 35.64% for *C. trichotoma* (Figure 1 – G1) and 6.29% to 32.34% for *Z. tuberculosa* (Figure 1 – G2). In the case of *C. legalis*, for the best nine families, the range of genetic gains included a minimum of 10% and a maximum of 45.04% (Figure 1 – G3), being higher than the range of gains predicted for the other two species. In the case of this last species, the seed tree of the family 19 demonstrated a negative gains perspective for DBH, and as a result, the individuals from this family are not indicated in future breeding strategy, according DBH (Figure 1 – G3).

Coincidence between seed trees genetic values and phenotypic measurements

Coincidence indices based on the predicted genetic values and phenotypic measurements taken in the field for seed trees showed that 40% of the top 10 seed trees ranked in the genetic analyses of *C. trichotoma* and *Z. tuberculosa*, matched with the best seed trees observed in the field. For *C. legalis*, the match was smaller (30%) (Table 2). The correspondence between the genetic values of seed trees and phenotypic measures resulted in low-correlation values for *Z. tuberculosa* (0.15) and *C. trichotoma* (0.14). The discrepancy among the DBH values for the *C. legalis* seed trees and the predicted genetic values resulted in a negative correlation for this species (-0.50).

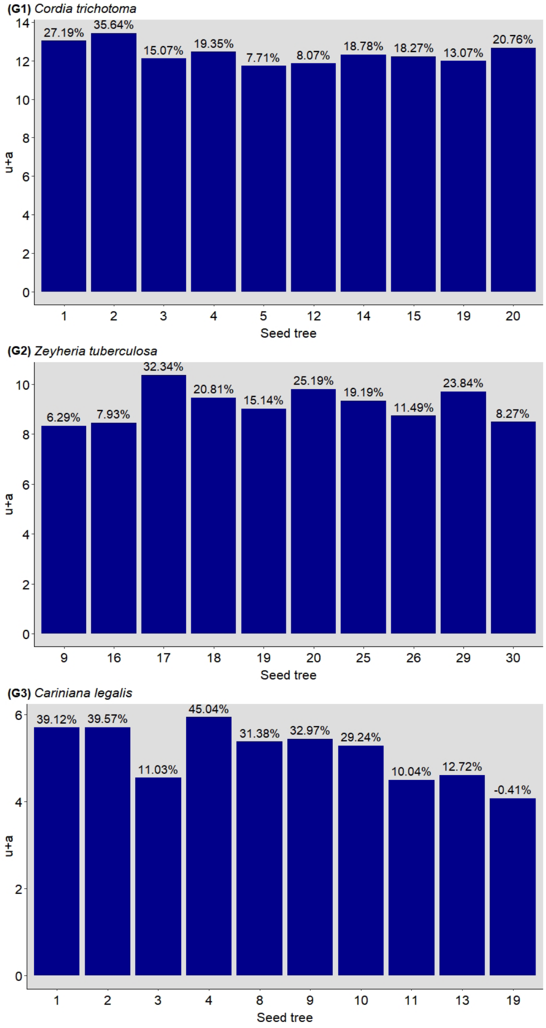

Figure 1 The selected seed trees and gains based on the additive genetic values (u + a) for DBH (diameter at breast height) for *Cordia trichotoma*, *Zeyheria tuberculosa*, and *Cariniana legalis*.

Table 2 Ranking of coincidence index based on the genotypic values and field phenotypic measurements of DBH for the top ten seed trees of *Cordia trichotoma*, *Zeyheria tuberculosa* and *Carianiana legalis*.

Cordia trichotoma						Zeyheria tuberculosa						Cariniana legalis					
Genetic ranking			Phenotypic measurement			Genetic ranking			Phenotypic measurement			Genetic ranking			Phenotypic measurement		
O	Tree	GV	O	Tree	Pm	O	Tree	GV	O	Tree	Pm	O	Tree	GV	O	Tree	Pm
1	20	11.13	1	16	54.46	1	20	5.93	1	25	39.17	1	4	5.93	1	12	128.98
2	12	10.88	2	5	50.64	2	17	5.70	2	20	37.58	2	2	5.70	2	17	114.65
3	2	10.67	3	14	48.73	3	29	5.69	3	26	36.62	3	1	5.69	3	5	90.76
4	11	10.65	4	12	43.95	4	25	5.44	4	14	36.31	4	9	5.44	4	3	89.17
5	10	10.56	5	4	42.68	5	18	5.37	5	23	35.35	5	8	5.37	5	19	89.17
6	4	10.41	6	24	40.76	6	9	5.28	6	7	34.08	6	10	5.28	6	18	82.80
7	3	10.30	7	11	38.22	7	4	4.62	7	17	34.08	7	13	4.62	7	16	76.43
8	1	10.25	8	17	37.90	8	26	4.54	8	8	33.12	8	3	4.54	8	13	74.84
9	14	10.25	9	15	34.08	9	30	4.50	9	12	30.57	9	11	4.50	9	15	73.25
10	19	10.23	10	27	34.08	10	19	4.07	10	21	29.62	10	19	4.07	10	21	70.38

Notes: O: order; GV: genetic value; Pm: Phenotypic measurement

Potential genitors

Potential genitors (test individuals) with high genetic merit and high possibility of transmitting favorable genes to their offspring were selected (Table 3). Based on the predicted additive genetic values (u + a), the selection of potential genitors for the three species resulted in high genetic gains with selection in relation to the general mean of the experiment (Table 3). The best *C. trichotoma* potential genitors were represented by family two and family five with predicted gains of 37.79% and 30.68%, respectively. For *Z. tuberculosa* 33.98% and 29.46% of gains could be obtained by selecting individuals from families 17 and 19. Although the best families (family 4 and 9) of *C. legalis* presented their additive genetic values below the genetic values of the other two species, the gain percentages for its potential genitors were higher, with 65.33% for the first and 63.64 % for the second mentioned populations.

Potential clone selection

Individuals with high genotypic values for DBH were selected as potential clones (Table 4). Based on the predicted genotypic values (u + g), the minimum genetic gains were up to 25% for the three species, because of their dominance deviation and genetic values. *Cariniana legalis* was the species with the highest potential genetic gain (between 49.03% and 65.34%). In this case, family 4 was the most suitable for expressing genotypic values of *C. legalis* (Table 4). The potential clone genetic gains of *C. trichotoma* ranged from 36.60% to 47.07%, with gains above 40% for the first six families. For *Z. tuberculosa*, the selection of the best-ranked potential clone genetic gains was lower than the gains obtained by *C. trichotoma*; however, the selection of the first two families (family 17 and 19) might result in genetic gains above 40%.

Table 3 Ranking of potential genitors based on the additive genetic values for DBH (diameter at breast height) and stem shape for *Cordia trichotoma*, *Zeyheria tuberculosa* and *Carianiana legalis*.

Cordia trichotoma					*Zeyheria tuberculosa*					*Cariniana legalis*				
I	Block	Family	u+a	Gain	I	Block	Family	u+a	Gain	I	Block	Family	u+a	Gain
1	3	2	13.66	37.79	1	6	17	10.50	33.98	1	3	4	6.77	65.33
2	1	5	12.95	30.68	2	6	19	10.15	29.46	2	2	9	6.70	63.64
3	4	14	12.86	29.73	3	3	20	9.80	25.04	3	3	1	6.70	63.57
4	2	3	12.78	28.90	4	8	18	9.61	22.55	4	8	2	6.64	62.21
5	1	1	12.71	28.26	5	6	25	9.49	21.00	5	3	10	6.13	49.65
6	10	15	12.62	27.28	6	4	29	9.20	17.33	6	6	8	6.10	49.03
7	7	20	12.58	26.96	7	10	28	9.11	16.22	7	1	13	5.88	43.70
8	2	10	12.52	26.28	8	4	26	9.06	15.55	8	10	11	5.37	31.12
9	10	4	12.48	25.91	9	2	4	9.01	14.98	9	7	3	5.03	22.82
10	4	19	12.47	25.82	10	1	9	8.97	14.46					
Average Gain (%)				**27.77**	**Average Gain (%)**				**19.17**	**Average Gain (%)**				**49.65**

Notes: I: individual; u+a: additive genetic values; Gain: genetic gain (%).

Table 4 Ranking of individuals selected as potential clones based on the predicted genotypic values of DBH (diameter at breast height) for *Cordia trichotoma*, *Zeyheria tuberculosa* and *Carianiana legalis*.

Cordia trichotoma					*Zeyheria tuberculosa*					*Cariniana legalis*				
I	**Block**	**Family**	**u+a**	**Gain**	**I**	**Block**	**Family**	**u+a**	**Gain**	**I**	**Block**	**Family**	**u+a**	**Gain**
1	2	10	14.58	47.07	1	6	17	11.13	42.02	1	3	4	6.77	65.34
2	6	17	14.52	46.53	2	6	19	10.99	40.23	2	2	9	6.70	63.64
3	1	5	14.50	46.33	3	5	17	10.63	35.65	3	3	1	6.70	63.57
4	7	16	14.44	45.69	4	4	17	10.46	33.39	4	8	2	6.64	62.21
5	3	2	14.31	44.35	5	3	20	10.43	33.10	5	5	2	6.48	58.27
6	2	3	13.95	40.76	6	1	17	10.39	31.76	6	10	2	6.34	54.79
7	4	14	13.81	39.31	7	6	20	10.28	31.15	7	5	4	6.29	53.54
8	7	20	13.80	39.28	8	8	18	9.94	26.81	8	10	1	6.23	52.22
9	5	17	13.68	38.02	9	6	25	9.91	26.40	9	3	10	6.13	49.65
10	5	2	13.54	36.60	10	2	4	9.85	25.64	10	6	8	6.10	49.03
Average Gain (%)				**42.56**	**Average Gain (%)**				**32.43**	**Average Gain (%)**				**56.53**

Notes:I: individual; u+g: predicted genotypic values; Gain: genetic gain in relation to general mean of the experiment (%).

Discussion

In this study, high genetic control expressed by the narrow-sense individual heritabilities of *C. trichotoma* and *C. legalis* indicates that there is genetic control for DBH e possibility of achieving genetic gains by conducting intraspecific crosses. Comparing the result of *Z. tuberculosa* with the heritability scale established by Resende (2002), good genetic gains would be expected for this species as a result of the moderate genetic control for DBH. However, the absence of genetic variability among genotypes makes the long-term breeding program with that species not sustainable once there is not variability to be exploited over the breeding stages in progeny/provenance test evaluated in this study.

Beyond genetic factors, environmental conditions can also influence genotype behavior resulting in different phenotypic expressions according to the intrinsic occurrence area (Lopes et al. 2018, Santos et al. 2018a, Wani et al. 2019, Ishibashi et al. 2020). Provenance effect might even be pronounced when populations come from areas with distinct soil, climate conditions and vegetative characteristics, factors that can induce variation among populations (Hodge & Dvorak 2015, Silva et al. 2018). The occurrence of values closes to zero for *Z. tuberculosa* and *C. legalis* demonstrate no significant genetic differences based on the provenance for DBH of both species, as observed by Martins et al. (2018) for *Myracrodruon urundeuva* Allemão. Corroborating these results, populations of *Handroanthus vellosoi* Toledo also showed absence of significant variation among populations for DBH and survival rate (Batista et al. 2012).

Genotype selection for breeding purposes should be conducted in such a way as to provide maximum genetic gain and without reducing genetic diversity. In this case, the quantification of the variation coefficients may help to outline strategies to be adopted and ensure the success of selection (Weng et al. 2015, Santos et al. 2018b, Riva et al. 2020). The way that genetic variations are distributed is correlated with the genetic structure of the population (Schoville et al. 2012) and serves to guide the process of selection. In this study, the occurrence of higher CVgi values than the CVgp values for DBH demonstrates that the potential genetic gains are promising for all three species when selecting individuals within progenies. Considering that the prospective gain is directly correlated with the existence and magnitude of genetic variation (Bush et al. 2013), among the three species the selection of *C. legalis* genotypes may allow better genetic gains for DHB. Likewise, higher coefficients of variation within progenies than among families were observed in the provenance/progeny tests of *Handroanthus vellosoi* (Batista et al. 2012) and *Balfourodendron riedelianum* (Engl.) Engl. (Kubota et al. 2015). Zaruma et al. (2015) and Silva et al. (2018) also observed higher coefficients of variation within progenies than among families when evaluated the existence of genetic variability for DBH in populations of *Dipteryx alata* Vog. (Zaruma et al. 2015).

Owing to the relationship between the genotypic and experimental coefficients of variation, CVr values close to 1 indicate the level of genetic variance in progeny/provenance test (Resende 2002). For *C. trichotoma* both CVr values were higher than 1, indicating that both DBH and survival rate might be used in the selection process. Silva et al. (2012) suggested that when different traits enable the same level of precision, we should always conduct selection based on the simplest and most economical measurement traits.

Notwithstanding CVr values, the most appropriate parameter for inferring experimental quality and confidence in the selection of genetic materials is accuracy, once this parameter takes into account both the number of repetitions of genotypes in the experiment and the genetic variability available in the population (Resende 2002). In general, CVr vary from 0 to 1; however, for tests whose main objective is genotype selection for breeding purpose, the desired accuracy values should be above 0.70 (Resende & Duarte 2007), as observed in this study for DBH of C. legalis. Accuracy above 0.70 were also observed for *M. urundeuva*, *Astronium fraxinifolium* Schott., *Terminalia argentea* Mart. (Otsubo et al. 2015), and *Peltophorum dubium* Spreng. (Senna et al. 2012) when evaluating DBH. In contrast to the high accuracy value observed by Ostubo et al. (2015) for DBH of *M. urundeuva*, Riva et al. (2020) observed a low accuracy value for DBH of this same species. In the case of *Z. tuberculosa* low accuracy value demonstrates that the selection based on survival rate does not provide adequate precision for breeding purposes. Resende and Duarte (2007) report that increases in accuracy might be obtained by increasing test repetition numbers for quantitative traits.

For breeding programs, genetic parameter quantification enables the selection of individuals and populations that can be used for recombination (Resende 2002, Santos et al. 2018b, Castro et al. 2019, Santos et al. 2021b). In this sense, selecting good genitors is crucial for obtaining new elite genotypes (Matias et al. 2020) and for optimizing high-yield forest plantations (Rocha et al. 2007). Besides predicting gain, the choice of the families to be crossed must be carefully addressed for optimizing genetic gain. The expected gain calculated based on the ranking of the top 10 individuals for additive genetic values demonstrate that the best genetic gain was shown by *C. legalis*, followed by *C. trichotoma* and *Z. tuberculosa* (Figure 1). Although the potential genetic gain for the 10 genitors was higher for *C. legalis* than for the other two species, the real gains for this species depend on the combination of families to be conducted in the crosses, once the seed tree of the family 19 of this species had negative genetic gain potentials and should be excluded from forward breeding strategy.

The first five families of *C. trichotoma* and *Z. tuberculosa* presented higher percentages than the general mean, showing to be the most promising individuals for selection and crossing. Increases in mean genetic gain percentages might be obtained by reducing the number of selected genitors. In many cases, increasing the intensity of selection by reducing the number of genitors results in higher percentage of gains (Sato et al. 2010). For *C. trichotoma* and *Z. tuberculosa*, reducing the number of selected genotypes might increase the mean genetic gains; however, a genetic base bottleneck might produce the cumulative effect of inbreeding across breeding generations (Sturion & Resende 2010, Ishibashi et al. 2021, Santos et al. 2021b).

Besides bottleneck, increases in pressure selection may also increases the probability of coincidence when comparing genetic and the phenotypic rankings. Comparing the genetic and the phenotypic rankings, *C. legalis* was the species with the lowest number of coincident seed trees with three coincident individuals among the top 10 ranked. For *C. trichotoma* and *Z. tuberculosa*, the number of matching classification was higher than *C. legalis*, with four coincident individuals for each species. For breeding purposes, these results might support the optimization of genitor selection and the obtainment of superior genotypes in a short time.

Among the three species analyzed in this study, further genetic progress in selecting genitor is expected for *C. legalis*, once for this species the prospects of high magnitude gain could be optimized by establishing controlled-crossing orchards. In the case of potential *C. trichotoma* genitors, the difference between gains of the first individual in the ranking and the last was only 11.97%, demonstrating that genetic gain prospects are similar for all selected individuals of this species and that the crossing is favorable between any genotype from the ranking. For *Z. tuberculosa*, as a result of the non-existence of variability among genotypes, breeding programs would be only favorable if a source of genetic variability is found in other remain areas of its natural occurrence. Besides breeding programs, the absence of genetic variation may also compromise in the long-term survival rate of *Z. tuberculosa* populations from the areas where the seeds had been collected for conducting the progeny/provenance tests.

As a breeding strategy, cloning may result in more productive crops (Oliveira et al. 2020), once unlike the selection for recombination, which is based on additive genetic values, selection for cloning is based on additive and dominance values, resulting in higher genetic gains (Castro et al. 2019), as predicted in this study. Although the predicted gain percentage with the selection of *C. legalis* clones was lower than for other two species, the observed value still indicates the possibility of genetic gain. Supporting these results, the repeated occurrence of specific families in the ranking of potential clones, as occurred for family 2 of *C. trichotoma*, for families 17 and 20 of *Z. tuberculosa*, and for families 1, 2 and 4 of *C. legalis*, confirms the favorable potential for cloning these families. In those cases, besides selecting individuals from the family with the highest perspective of gain, priority in selection for cloning must also be gave to the genotypes of the repeat-occurring families. This kind of information helps to support clonal testing and to indicate the genetic resource most appropriate to the establishment of plantations through cloning.

Conclusion

Although the intense and constant fragmentation of Forest Atlantic, the remain populations of *C. trichotoma* and *C. legalis* still have genetic diversity capable of being used for the development of genetic breeding programs. By exploiting the variability existent within and among progenies it is possible to achieve good genetic gains for DBH.

For *C. trichotoma* and *C. legalis* narrow-sense individual heritability may ensure the success of selection, crossing and the obtainment of genotypes with higher genetic merit. In the case of *C. trichotoma*, besides moderate heritability, the good prediction of CVgi and CVgp for survival rate also supports the indication of that trait for conducting breeding programs. These results reinforce the potential of those species for obtaining high productivity commercial plantations and to expand silviculture with non-commercially exploited forest species as a strategy to strengthen the forest-based sector.

In the case of *Z. tuberculosa*, the absence of genetic variability among genotypes can be a consequence of the continuous Atlantic Forest deforestation, which resulted in the reduction of the effective population size for this species and consequently in narrowing of genetic basis.

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References

Amini F., Mirlohi A., Majidi M.M., ShojaieFar S., Kölliker R., 2011. Improved polycross breeding of tall fescue through marker-based parental selection. Plant breeding, 130(6):701-707. https://doi.org/10.1111/j.1439-0523.2011.01884.x

Assis T.F., Resende M.D.V.D., 2011. Genetic improvement of forest tree species. Crop Breeding and Applied Biotechnology, 11(SPE): 44-49. https://doi.org/10.1590/S1984-70332011000500007

Batista C.M., Freitas M.L.M., Moraes M.A., Zanatto A.C.S., Santos P.C., Zanata M., Sebbenn A.M., 2012. Estimativas de parâmetros genéticos e a variabilidade em procedências e progênies de *Handroanthus vellosoi*. Pesquisa Florestal Brasileira, 32(71):269. https://doi.org/10.4336//2012.pfb.32.71.26

Batista F.G., Melo R.R., Medeiros D.T., Oliveira A.G.S., Freitas C.B.A., Silva E.D.G., Pimenta A.S., 2020. Longitudinal variation of wood quality in the five forest species from Caatinga. Revista Brasileira de Ciências Agrárias, 15(4):e8572.https://doi.org/:10.5039/agraria.v15i4a8572

Beltrame R., Bisognin D.A., Mattos B.D., Cargnelutti Filho A., Haselein C.R., Gatto D.A., Santos, G.A.D., 2012. Desempenho silvicultural e seleção precoce de clones de híbridos de eucalipto. Pesquisa Agropecuária Brasileira, 47(6):791-796. https://doi.org/10.1590/S0100-204X2012000600009

Bush D., Marcar N., Arnold R., Crawford D., 2013. Assessing genetic variation within *Eucalyptus camaldulensis* for survival and growth on two spatially variable saline sites in southern Australia. Forest Ecology and Management, 306:68-78. https://doi.org/10.1016/j.foreco.2013.06.008

Carvalho A.M. et al., 2018. Avaliações de qualidade da madeira em espécies nativas de plantios florestais. In: Rolim S. G., Piotto D. (2018). Silvicultura e tecnologia de espécies da mata atlântica. Belo Horizonte: Ed. Rona. p. 45-52.

Castro C.A.O., Nunes A.C.P., Roque J.V., Teófilo R.F., Santos O.P., Santos G.A., Resende M.D.V., 2019. Optimization of *Eucalyptus benthamii* progeny test based on Near-Infrared Spectroscopy approach and volumetric production. Industrial Crops and Products, 141:111786. https://doi.org/10.1016/j.indcrop.2019.111786

Cubbage F., Kanieski B., Rubilar R., Bussoni A., Olmos V.M., Balmelli G., Donagh P.M., Lord R., Hernández C., Zhang P., Huang J., Korhonen J., Yao R., Hall P., Torre R.D., Diaz-Balteiro L., Carrero O., Monges E., Thu H.T.P., Frey G., Howard M., Chavet M., Mochan S., Hoeflich V.A., Chudy R., Maass D., Chizmar S., Abt R., 2020. Global timber investments, 2005 to 2017. Forest Policy and Economics, 112. https://doi.org/10.1016/j.forpol.2019.102082

Cruz S.L., Pedrozo CA., Oliveira V.X.A., Silva A.M., Resende M.D.V., Gonçalves D.A., 2020. Parâmetros genéticos e seleção inicial de procedências e progênies de taxi-branco (*Tachigali vulgaris*) em Roraima. Ciência Florestal, 30(1):258-269. https://doi.org/10.5902/1980509831631

Falconer D.S., 1987. Introduction to quantitative genetics. Viçosa, Minas Gerais.

Hodge G.R., Dvorak W.S., 2015. Provenance variation and within-provenance genetic parameters in *Eucalyptus urophylla* across 125 test sites in Brazil, Colombia, Mexico, South Africa and Venezuela. Tree Genetics & Genomes, 11(3):57. https://doi.org/10.1007/s11295-015-0889-3

IBÀ – Indústria Brasileira de Árvores, 2021. Ibá annual report. Instituto Brasileiro de Economia. São Paulo, 93 p.

Ishibashi V., Flores Júnior P.C., Martinez D.T., Higa A.R., 2021. Genetic selection of *Pinus taeda* L. through Multi-environment trial. Curitiba, 51(1):211-219. http://dx.doi.org/10.5380/rf.v51i1.68057

Kubota T.Y.K., Moraes M.A., Silva E.C.B., Pupin S., Aguiar A.V., Moraes M.L.T., Sebbenn A.M., 2015. Variabilidade genética para caracteres silviculturais em progênies de polinização aberta de *Balfourodendron riedelianum* (Engler). Embrapa Florestas-Artigo em periódico indexado (ALICE).

Kumar N., Markar S., Kumar V., 2014. Studies on heritability and genetic advance estimates in timely sown bread wheat (*Triticum aestivum* L.). Bioscience Discovery, 5(1):64-69.

Lopes R. R., Franke L. B., Souza C. H. L. D., Bertoncelli P., Graminho L. A., Pereira É. A., 2018. Genetic parameters and predicted gains with selection of interspecific hybrids of Paspalum for seed production. Crop Breeding and Applied Biotechnology, 18(3):284-291. https://doi.org/10.1590/1984-70332018v18n3a42

Martins K., Santos W.S.D.D., Quadros T.M.C., Aguiar A.V.D., Machado J.A.R., Sebbenn A.M., Freitas M.L.M., 2018. Genetic variation and effective population size of a *Myracrodruon urundeuva* (Engler) Fr. Allem. provenance and progeny test. Journal of Forest Research, 23(4):228-236. https://doi.org/10.1080/13416979.2018.1483130

Matias F.I., Valle C.B.D., Gouveia B.T., Moro G.V., Barrios S.C.L., 2020. Using additive indices and principal components to select sexual genitors and hybrids of *Urochloa decumbens*. Crop Breeding and Applied Biotechnology, 20(2). https://doi.org/10.1590/1984-70332020v20n2a18

McKown A.D., Guy R.D., Klápště J., Geraldes A., Friedmann M., Cronk Q.C., Douglas C.J., 2014. Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. New Phytologist, 201(4):1263-1276. https://doi.org/10.1111/nph.12601

Mihai G., Alexandru A., Mirancea I., 2019. Genetic variation and early selection in *Larix decidua* Mill. from progeny test in Romania. Annals of Forest Science, 76(3):81. https://doi.org/10.1007/s13595-019-0864-5

Oliveira R.S., Ribeiro C.V.G., Neres D.F., Porto A.C.M., Ribeiro D., Siqueira L., Zauza E.A.V., Coelho A.S.G., Reis C.A.F., Alfenas A.C., Novaes E., 2020. Evaluation of genetic parameters and clonal selection of *Eucalyptus* in the Cerrado region. Crop Breeding and Applied Biotechnology, 20(3): e29982031. http://dx.doi.org/10.1590/1984-70332020v20n3a35

Otsubo H.D.C.B., Moraes M.L.T.D., Moraes M.A.D., José Neto M., Freitas M.L.M., Costa R.B.D., Sebbenn A.M., 2015. Variação genética para caracteres silviculturais em três espécies arbóreas da região do bolsão sul-mato-grossense. Cerne, 21(4):535-544. https://10.1590/01047760201521041317

Resende M. D. V. D., 2016. Software Selegen-REML/BLUP: a useful tool for plant breeding. Crop breeding and applied biotechnology, 16(4):330-339. https://doi.org/10.1590/1984-70332016v16n4a49

Resende M.D.V., 2002. Genética biométrica e estatística no melhoramento de plantas perenes. Embrapa Informação Tecnológica, Colombo: Embrapa Florestas, pp 975.

Resende M.D.V., Duarte J.B., 2007. Precisão e controle de qualidade em experimentos de avaliação de cultivares. Goiânia Pesq Agropec. Trop 37:182-194.

Riva L.C., Moraes M.A., Cambuim J., Zulian D.F., Sato L.M., Caldeira F.A., Panosso A. R., Moraes M.LT., 2020. Genetic control of wood quality of *Myracrodruon urundeuva* populations under anthropogenic disturbance. Crop Breeding and Applied Biotechnology, 20(4):e320920411. http://dx.doi.org/10.1590/1984-70332020v20n4a64

Rocha M.D.G.D.B., Pires I.E., Rocha R.B., Xavier A., Cruz C.D., 2007. Seleção de genitores de *Eucalyptus grandis* e de *Eucalyptus urophylla* para produção de híbridos interespecíficos utilizando REML/BLUP e informação de divergência genética. Revista Árvore, 31(6):977-987. https://doi.org/10.1590/S0100-67622007000600001

Santos E. A., Viana A. P., Freitas J.C.O., Rodrigues D.L., Tavares R.F., Paiva C.L., Souza M.M., 2015. Genotype selection by REML/BLUP methodology in a segregating population from an interspecific Passiflora spp. crossing. Euphytica, 204(1):1-11. https://doi.org/10.1007/s10681-015-1367-6

Santos T.S., Martins K., Aguiar A.V., Baptista Filho M.J., Menucelli J.R., Faria R.F.P, Moura R.D., Machado J.A.R., Longui E.L., 2021a. growth and wood quality traits in a *Dipteryx alata* Vog. (Fabaceae) progeny and provenance test. Forest Science, 67(4): 468-477. https://doi.org/10.1093/forsci/fxab010

Santos W., Aguiar A.V., Souza B.M., Araujo D., Machado C., Moraes M.L.T., Sebbenn A.M., Freitas M.L.M., 2021b. Performance and genetic variation in a provenance test of *Araucaria cunninghamii*. Pesquisa Agropecuária Brasileira, 56:e02170. https://doi.org/10.1590/S1678-3921.pab2021.v56.02170

Santos W., Aguiar A.V., Souza D.C.L., Dini D.G.T., Souza F.B., Dalastra C., Machado J.A.R., Sousa V.A., Moraes M.L.T., Freitas M.L.M., Sebbenn A.M., 2018a. Genetic variation and effective population size in *Dipteryx alata* progenies in pederneiras, São Paulo, Brazil. Revista Árvore, 42(3):e420310. http://dx.doi.org/10.1590/1806-90882018000300010

Santos W., Silva M.S.C., Deniz, L.D., Kieras, W.S., Shimizu J.Y., Sousa V.A., Aguiar A.V, 2018b. Identificação de procedências e progênies de *Pinus maximonoi* com potencial produtivo para madeira, Piracicaba. Scientia Forestalis, 46(117):127-136. http://dx.doi.org/10.18671/scifor.v46n117.12

Sato A. S., Freitas M.L.M., Lima I.L., Zimback L., Toniato M.T.Z., Sebbenn A.M., 2010. Genetic variation among and within provenances and progenies of *Corymbia maculata* (Hook.) KD Hill and LAS Johnson, in Pederneiras, SP. Cerne, 16(1):60-67.

Schoville S.D., Bonin A., François O., Lobreaux S., Melodelima C., Manel S., 2012. Adaptive genetic variation on the landscape: methods and cases. Annual Review of Ecology, Evolution, and Systematics, 43:23-43. https://doi.org/10.1146/annurev-ecolsys-110411-160248

Senna S., Freitas M.L.M., Zanatto A.C.S., Morais E., Zanata M., Moraes M.L.T., Sebbenn A.M., 2012. Variação e parâmetros genéticos em teste de progênies de polinização livre de *Peltophorum dubium* (Sprengel) taubert em Luiz Antônio-SP. Scientia Forestalis/Forest Sciences, 40:345-352.

Silva Júnior A.L., Cabral R.L.R., Sartori L., Souza L.C., Miranda F.D., Caldeira M.V.W., Moreira S.O., Godinho T.O., 2020. Evaluation of diversity and genetic structure as strategies for conservation of natural populations of *Dalbergia nigra* (Vell.) Allemão ex Benth. Cerne, 26(4):435-443.

Silva J.M., Aguiar A.V., Mori E.S., Moraes M.L.T., 2012. Divergência genética entre progênies de *Pinus caribaea var caribaea* com base em caracteres quantitativos. Pesquisa Florestal Brasileira, 32(69):69. https://doi.org/10.4336/2012.pfb.32.69.69

Silva L.G.C., Moreira J.F.L, Holanda H.B.B., Rocha E.L.B., Dias P.C., 2018. Evaluation of carnauba progenies and estimates of genetic parameters in the juvenile phase. Rev. Caatinga. Mossoró, 31(4):917-925. http://dx.doi.org/10.1590/1983-21252018v31n414rc

Sturion J.A., Resende M.D.V., 2010. Melhoramento genético da erva-mate. Colombo: Embrapa Florestas.

Sundstrom L.M., Henry L.A., 2017. Private forest governance, public policy impacts: The forest stewardship council in Russia and Brazil. Forests, 8(11), 445. https://doi.org/10.3390/f8110445.

Wani S.A., Khan P.A., Dar Z.A., 2019. Role of genetics in tree improvement. The Pharma Innovation Journal, 8(5):63-66.

Weng Y.H., Lu P., Adams G.W., Fullarton M.S., Tosh K.J., 2015. Genetic parameters of growth and stem quality traits for jack pine second-generation progeny tested in New Brunswick. Canadian Journal of Forest Research, 45(1):36-43. https://doi.org/10.1139/cjfr-2014-0106

Zaruma D.U.G., Canuto D.S.O., Pupin S., Cambuim J., Silva A.M., Mori E.S., Moraes M., 2015. Variabilidade genética em procedências e progênies de *Dipteryx alata* vogel para fins de conservação genética e produção de sementes. Scientia Forestalis, 43(107), 609-615. http://hdl.handle.net/11449/17761.