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Estimation Of Early Genetic Parameters In Progenies Of E. Cladocalyx And E. Botryoides In Areas With Frost Occurrence

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Abstract:
The forestry sector in Brazil grows every year; therefore, in-depth studies on certain species become indispensable. One of the main forest genera of economic importance in Brazil is Eucalyptus. However, there are few studies on species adapted to regions of intense cold, with the occurrence of frosts, and that show good production. Thus, we aim to estimate genetic parameters in three cold-tolerant Eucalyptus progenies. After 45 days from sowing, the seedlings were repotted, individualized in plastic bags, beginning the measurements of height and diameter. After about 95 days, the seedlings were planted in the field, in a completely randomized experimental design, with one plant per plot. The three treatments showed an imbalance in the number of repetitions due to differences observed in germination between species. Four evaluations were carried out: at 30 and 90 days in the nursery and at 120 and 180 days in the field, where we estimated the parameters with the help of the SELEGEN – REML/BLUP software. For diameter, the nursery evaluations showed higher heritabilities (h^2 = 0.257 and 0.478 and = 0.206 and 0.407 for 30 and 90 days, respectively) than those obtained in the field (h^2 =0.002 and 0.006 and = 0.001 and 0.004 for 120 and 180 days, respectively), probably due to the more homogeneous environmental conditions considered in the nursery. For height, the heritabilities were low in both cases (nursery and field) for all measurements. For both traits, we must consider that these are perennial species, which remain in the field for a long time, and the early period of evaluation in the field contributes to the non-significant heritability values. Mahalanobis distance was used as a measure of dissimilarity using the measurements of the traits under study, which grouped the treatments into two groups, highlighting the genetic variability among the species for the traits in question. We conclude that it was possible to estimate genetic parameters, even with low germination, being able to group the treatments into distinct groups based on quantitative traits, indicating that there is possibly variability among the species.

***Keywords**: Forest improvement, quantitative genetics, genetic variances.*

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I. Introduçtion

Eucalyptus cultivation in Brazil began over a century ago when Edmundo Navarro de Andrade brought *Eucalyptus* spp. seedlings to produce railroad sleepers. Since then, the country's forestry sector has grown significantly, with major technical and technological advancements, especially in eucalyptus silviculture (IBÁ, 2015). In 2016 alone, the timber sector generated about R$ 65 billion in the Brazilian economy (CONSUFOR, 2016).

Eucalyptus plantations occupy 5.7 million hectares of the country's planted tree area and are mainly located in Minas Gerais (24%), São Paulo (17%), and Mato Grosso do Sul (15%) (IBÁ, 2017). Considering the economic importance of the *Eucalyptus* genus and the expansion of plantations with high market demand, there is a justified need for studies on genetic improvement for productivity, targeting species that tolerate low temperatures and frost occurrences, not only for the region where the study is being developed but also for the entire southern region of the country, which also suffers from similar conditions during winter, as the topic has been little explored.

Among the *Eucalyptus* species with frost tolerance most planted in Brazil, we can mention *Eucalyptus benthamii* and *Eucalyptus dunnii*. Suitable for planting in regions with minimum temperatures of -10º C for *Eucalyptus benthami*i and -5º C for *Eucalyptus dunnii* (FAO, 1981), these species have shown good frost resistance, even in the seedling stage, less than 30 days after planting (SANTOS, 2006). In view of the good results of the studies, it becomes feasible to conduct new studies with different species of the genus, as evaluated in this experiment.

Eucalyptus cladocalyx F. Muell. is native to southern Australia, naturally occurring in the southern Flinders Ranges, northern Kangaroo Island, and western Eyre Peninsula. It can reach up to 35 meters in height under good growth conditions (FLORBANK, 2017a). It is commonly planted throughout southern Australia for wood and firewood production. It is drought-tolerant and easily adapts to different soils and climates (MARCAR, 2004). Its wood has moderate mechanical resistance, mainly used in furniture, flooring, panels, firewood, beams, poles, and general construction (BOLAND, 2016).

Eucalyptus botryoides Sm. naturally occurs on the southern coast of New South Wales, from the city of New Castle to the entrance of Lake Victoria, in southeastern Australia. It is medium to tall, reaching between 30 to 40 meters in height (BLAKELY, 1955). According to Boland et al. (2006), it grows in good quality, slightly saline soils, and is also found in sandy and saline soils. It has good resistance to wind and frost, as well as drought (BLAKELY, 1955). It produces high-density, durable wood, used in flooring, laminating, beams, boxes, structures, sleepers, and posts, as well as for ornamentation and cellulose production in some cases (CABI – Forest Compendium, 2008). The current research aims to estimate the genetic parameters for growth traits in nursery and field conditions for two cold-tolerant Eucalyptus species, as well as to investigate the genetic variability existing in the species under study.

II. Material and methods

Seed Germination

The germination of the seeds was carried out in plastic bags approximately 8 cm wide x 15 cm high, filled with a substrate based on vermiculite and pine bark. For each treatment, about 40 seeds were used, with initially six treatments (one of *E. cladocalyx* and two of *E. botryoides*). The trials were conducted at the forest nursery of the Department of Forest Engineering at UNICENTRO, Irati campus, in the state of Paraná, at an altitude of approximately 812 meters. The climate is type Cfb, according to the Köppen-Geiger classification, with precipitation of 1100 to 2000 mm, severe and frequent frosts over an average period of 10 to 25 days annually (SCHALLENBERGER et al., 2011).

Table 1: Species and progenies that went to the field after germination. Each progeny represents a treatment.

Species	Treatment
Eucalyptus cladocalyx F. Muell.	CODC8
Eucalyptus botryoides Sm.	CODB5, CODB7

Measurement of Quantitative Traits of Seedlings and Experimental Design

Forty-five days after germination, the plants were transplanted into individual containers. From then on, monthly evaluations were carried out until completing 60 days post-transplantation, evaluating the following traits: plant height (H, m) and stem diameter at ground level (COL, cm). The measurement procedure was carried out using a tape measure to assess plant height and a digital caliper with three decimal places for stem diameter.

After 105 days in the nursery, the seedlings were taken to the field for trial implementation. The experimental design used was completely randomized, with one plant per plot, three treatments with an unbalanced number of repetitions, being for CODB05 a total of 19 sample units, CODB07 with 22, and CODC08 with only 3, in a spacing of 2 x 2 meters, and a double line border made with Eucalyptus spp. at the same spacing.

Estimation of Genetic Parameters

The genotypic values of each trait analyzed were estimated by the SELEGEN – REML/BLUP software, using the statistical model:

$$y = Xu + Za + e$$

Where y is the data vector, u is the scalar referring to the overall mean (fixed effect), a is the vector of individual additive genetic effects (assumed random), and e is the vector of errors or residuals (random). The letters X and Z represent the incidence matrices for the aforementioned effects.

The estimated parameters were additive genetic variance ($\hat{\sigma}_a^2$), residual variance ($\hat{\sigma}_e^2$), phenotypic variance ($\hat{\sigma}_f^2$), individual narrow-sense heritability ($\hat{h}_a^2$), additive heritability within progeny ($\hat{h}_{ad}^2$), individual additive genetic variation coefficient $CV_{gi}(\%)$, genotypic variation coefficient among progenies $CV_{gp}(\%)$, and residual variation coefficient $CV_e(\%)$.

To group the treatments according to a measure of dissimilarity, the estimation of the generalized Mahalanobis distance (1936) was carried out using traits evaluated in the juvenile phase. The Mahalanobis distance between a group of values with mean $\mu = (\mu_1, \mu_2, \mu_3, \ldots, \mu_p)^T$ and covariance matrix S for a multivariate vector will be defined as $D_M(x) = \sqrt{(x-\mu)^T S^{-1}(x-\mu)}$. The procedure was done in the R environment (R Core Team 2016).

III. Results and Discussion

It is important to note that of the nine treatments that were initially to be implemented, only three had enough plants for field trials, due to the occurrence of low germination in some treatments, which resulted in an insufficient number of individuals. However, we continued the experiment even though it was unbalanced and with few repetitions of some treatments.

With the data collected, we obtained the genetic parameters and the average for the traits of diameter (cm) and height (m) evaluated at 30, 90, 120, and 180 days (Table 2 and 3).

Table 2: Estimation of genetic parameters for stem diameter at 30 (DAP30) and 90 (DAP90) days in the nursery and at 120 (DAP120) and 180 (DAP180) days in the field for Eucalyptus cladocalyx and *Eucalyptus botryoides*, in the municipality of Irati-PR, Brazil.

Parâmetros	**Nursery**			**Field**	
	DAP30	**DAP90**		**DAP120**	**DAP180**
$\hat{\sigma}^2_a$	0.000368	0.003042		0.000041	0.000603
$\hat{\sigma}^2_e$	0.001063	0.003317		0.017704	0.093378
$\hat{\sigma}^2_f$	0.001430	0.006360		0.017744	0.093981
$\hat{h}^2_a$	0.257039 ± 0.4479	0.478369 ± 0.6110		0.002297 ± 0.0423	0.006417 ± 0.0708
$\hat{h}^2_{ad}$	0.206018	0.407512		0.001723	0.004821
$CV_{gi}(\%)$	23.296	18.416		1.193	2.303
$CV_{gp}(\%)$	11.648	9.208		0.596	1.152
$CV_e(\%)$	44.448	24.984		24.886	28.730
$Média$	0.082	0.300		0.535	1.066

Where: DAP30, DAP90, DAP120, and DAP180 are respectively the diameter trait assessed at 30, 90, 120, and 180 days; ($\hat{\sigma}^2_a$) additive genetic variance;($\hat{\sigma}^2_e$) residual variance; ($\hat{\sigma}^2_f$) phenotypic variance; ($\hat{h}^2_a$) individual narrow-sense heritability; ($\hat{h}^2_{ad}$) additive heritability within progeny; $CV_{gi}(\%)$ individual additive genetic variation coefficient; $CV_{gp}(\%)$genotypic variation coefficient among progenies and $CV_e(\%)$ residual variation coefficient.

Regarding the diameter trait, the two evaluations conducted in the nursery showed higher heritabilities, both individual narrow-sense ($\hat{h}^2_a$= 0.257 and 0.478) and additive within progenies ($\hat{h}^2_{ad}$= 0.206 and 0.407), compared to the heritabilities obtained from the field evaluations ($\hat{h}^2_a$= 0.002 and 0.006 and $\hat{h}^2_{ad}$= 0.001 and 0.004). This is likely due to the more homogeneous environmental conditions found in the nursery, such as available substrate volume and the type, quantity of water and nutrients among other factors that cannot be completely homogenized precisely in the field. When the environment is efficiently controlled, heritability tends to be higher (RAMALHO et al. 2000; RESENDE, 2007).

Table 3: Estimation of genetic parameters for plant height at 30 (ALT30) and 90 (ALT90) days in the nursery and at 120 (ALT120) and 180 (ALT180) days in the field for *Eucalyptus cladocalyx* and *Eucalyptus botryoides*, in the municipality of Irati-PR, Brazil.

Parâmetros	**Viveiro**			**Campo**	
	ALT30	**ALTP90**		**ALT120**	**ALT180**
$\hat{\sigma}^2_a$	0,000001	0,000024		0,000021	0,001240
$\hat{\sigma}^2_e$	0,000608	0,002246		0,010402	0,022710
$\hat{\sigma}^2_f$	0,000609	0,002269		0,010423	0,023949
$\hat{h}^2_a$	0,002157 ± 0,0410	0,010398 ± 0,0901		0,001994 ± 0,0395	0,051765 ± 0,2010
$\hat{h}^2_{ad}$	0,001619	0,007819		0,001496	0,039333
$CV_{gi}(\%)$	2,243	2,939		1,259	7,025
$CV_{gp}(\%)$	1,122	1,469		0,630	3,513
$CV_e(\%)$	48,287	28,782		28,188	30,677
$Média$	0,051	0,165		0,362	0,501

Where: ALT30, ALT90, ALT120, and ALT180 are respectively the height trait assessed at 30, 90, 120, and 180 days; ($\hat{\sigma}^2_a$) additive genetic variance;($\hat{\sigma}^2_e$) residual variance; ($\hat{\sigma}^2_f$) phenotypic variance; ($\hat{h}^2_a$) individual narrow-sense heritability; ($\hat{h}^2_{ad}$) additive heritability within progeny; $CV_{gi}(\%)$ individual additive genetic variation coefficient; $CV_{gp}(\%)$genotypic variation coefficient among progenies and $CV_e(\%)$ residual variation coefficient.

For the height trait, heritabilities can be considered low (CRUZ, 2005) in both locations (nursery and field) and evaluation periods (30, 90, 120, and 180 days). However, for both traits, it should be taken into account that they are perennial species, which remain for a long time in plantations, and that the early period of evaluation in the field contributes to these values not being significant. Ideally, productivity traits should be evaluated at ages close to the rotation period, as this will more accurately reflect the potential genetic gains. Despite this, periodic monitoring during the different stages of planting is an important tool, as it is possible to verify and infer if there are correlations, and the magnitude of this association between the juvenile period and the harvest age, allowing time to be saved and selection to be carried out more efficiently and less expensively at an early stage (FERREIRA, 2005; TAMBARUSSI, 2017).

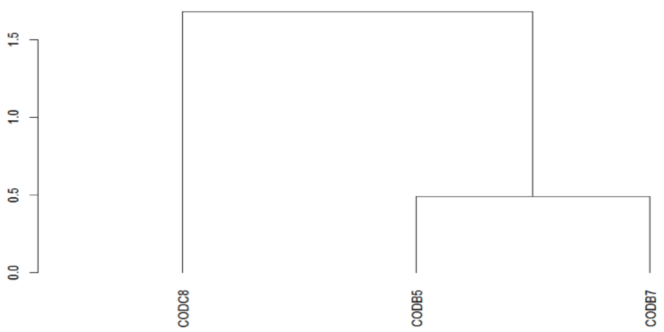
It is important to emphasize that, so far, there are no estimates in the literature of parameters for the traits studied under the conditions of the experiment for the mentioned species. However, the results obtained differed from those by Tambarussi et al. (2016), in an experiment with the species *Eucalyptus urophylla* S.T. Blake, conducted in the municipalities of Itatinga/SP (Eucatex S.A. Company) and Palma Sola/SC (Palmasola Company), both with the occurrence of frosts, and in Anhembi/SP (Anhembi Experimental Station, ESALQ/USP), used as a control, where high genetic variability was observed in individuals of the species within the local climates (Anhembi/SP and Itatinga/SP, humid temperate with dry winter and hot summer, and Palma Sola/ SC, humid mesothermic climate with hot summers and rigorous winters), resulting in a high heritability value.

Shimizu et al. (2013), in a similar study conducted with 4-year-old individuals of the species *Pinus elliottii* in Ribeirão Branco - SP, found high heritability values for height and DBH (0.39 and 0.29), which also differ from the results obtained from field evaluations in this experiment. Huber et al. (2001), also found a wide variation in the results for heritability (between 0.047 and 0.255) due to the individual x environment interaction, by submitting individuals of *Eucalyptus grandis*, in the Maravillas – COL region, with a cold climate and high altitude, evaluated at 3- and 6-years post-planting. According to results found by Kageyama and Vencovsky (1983) in studying *E. grandis*, the interaction of progenies with locations is significant for all evaluated traits (cylindrical volume, survival, and tree trunk shape), especially height and DBH, reflecting in the high reduction of heritability levels.

Klug et al. (2009) explain that low heritability values can occur due to several factors, such as the additive variance itself and the environmental variance, which directly influence heritability. If there is low influence of the additive variance, it will result in few favorable alleles being passed to the progenies, leading to low heritability. The environment can also directly interfere with heritability, such as with the occurrence of drastic temperature differences, excess or deficit of water, availability of nutrients in the soil, among other factors.

The Mahalanobis distance is a useful estimate in which quantitative variables are used to estimate the genetic distance between families (MANLY, 1994), as in the present study. From the progenies studied, two groups resulted (Figure 1), one group formed by the treatments CODB5 and CODB7, both of the species *E. botryoides*, and another group with only the treatment CODC8, which belongs to the species *E. cladocalyx*.

Figure 1: Dendrogram based on the Mahalanobis distance matrix, where the values on the abscissa represent dissimilarity.



The values of the Mahalanobis distance matrix allow for inferences about how genetic variability is distributed for a given trait, or set of traits (JOHNSON and WICHERN, 1992; CRUZ et al., 2011), among species or groups within species. The fact that treatments of the same species are grouped in the same cluster makes evolutionary and biometric sense, considering that they are species with little improvement and study, and probably, but not necessarily, have similar values for the traits of diameter and height in nature.

IV. Conclusion

There is genetic variability in the studied species and progenies for growth traits, indicating the possibility of improving them.

Given the low heritabilities observed, it is necessary to conduct the experiment for a longer time to observe possible relationships between early ages and rotation ages.

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