

SEEDLING EMERGENCE AND GENETIC PARAMETERS OF OPEN-POLLINATED CANDEIA PROGENIES GROWN IN TREE NURSERY

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Resumo

Emergência de plântulas e parâmetros genéticos de progênies de polinização aberta de candeia em viveiro. Eremanthus erythropappus (DC.) MacLeish é uma espécie nativa, apreciada economicamente devido a sua madeira e à extração de óleo essencial, cujo princípio ativo é o alfabisabolol. Apesar da importância econômica, os estudos em melhoramento genético dessa espécie ainda são escassos. Objetivou-se determinar o percentual de emergência, parâmetros genéticos e identificar potenciais progênies de polinização aberta de *E. erythropappus* e suas respectivas matrizes. O experimento foi implantado em viveiro, em delineamento experimental inteiramente ao acaso, com 22 progênies em quatro repetições, contendo 14 mudas por parcela. Aos 30 dias após a semeadura foi avaliado o percentual de emergência, enquanto aos 100 dias foram realizadas mensurações para altura (A) e diâmetro do coleto (DC). As análises estatísticas foram realizadas por meio do método REML/BLUP. Três progênies apresentaram percentual de emergência abaixo de 50%, valor considerado limitante para o processo de produção de mudas. Verificou-se a existência de variação genética entre as progênies e correlação genética alta e positiva entre A e DC. Dessa forma, foi possível identificar progênies e matrizes superiores bem como aquelas com baixo desempenho nas características avaliadas. *Palavras-chave:* REML/BLUP; *Eremanthus erythropappus*; teste de progênies.

Abstract

Eremanthus erythropappus (DC.) MacLeish is economically appreciated due to its wood and to the extraction of its essential oil, whose active principle is alpha-bisabolol. Despite its economic importance, studies about the genetic breeding of this species remain scarce. The aim of the current study is to determine *E. erythropappus* seedling emergence rate, genetic parameters, as well as to identify potential open-pollinated progenies of this species and their respective matrices. The experiment was set up in tree nursery, based on a completely randomized design, with 22 progenies in four replicates comprising 14 plants per plot. Seedling emergence rate was evaluated 30 days after seeding, whereas plant height (H) and stem diameter (SD) were measured at 100 days. Statistical analyses were carried out based on the REML/BLUP method. Three progenies have shown seedling emergence rate lower than 50%; this value is considered limiting to seedling production process. Genetic variation was observed among progenies, as well as high and positive genetic correlation between H and SD. Thus, it was possible identifying superior progenies and matrices, as well as the ones presenting low performance in the evaluated traits.

Keywords: REML/BLUP; *Eremanthus erythropappus*; progeny test.

INTRODUCTION

Eremanthus erythropappus (DC.) MacLeish is an allogamous native tree species belonging to family Asteraceae - popularly known as candeia - which grows in shallow and low-fertility soils (SCOLFORO *et al.*, 2012). It naturally grows between forests and rupestrian fields, as well as between altitude fields and Cerrado, at altitudes ranging from 900 to 1,700 m. In addition, it is typically found in Goiás, Minas Gerais, Espírito Santo, São Paulo and Rio de Janeiro States, as well as in the Federal District (SCOLFORO *et al.*, 2012). The natural resistance of its wood to xylophagous agents enables making high-quality fence posts (SCOLFORO *et al.*, 2016). Moreover, the essential oil produced by this species is extracted from its leaves, branches and inflorescences, mainly from its stem. Alpha-bisabolol, which has dermatological, cosmetic, insecticidal, anti-inflammatory, antibacterial and antiallergic properties (KAMATOU; VILJOEN, 2009), is the main component of the species essential oil and it is widely used in the industrial sector.

Natural populations of this species - the so-called candeal - have been intensively exploited due to its economic importance (SILVA *et al.*, 2014). Its exploitation has reduced the natural occurrence area of the species.

Thus, it is necessary conducting research about silviculture and genetic improvement of candeia species to enable selecting its best provenances and progenies (MELO *et al.*, 2012). Studies focused on investigating the genetic improvement of this species have already been carried out, based on the implantation and conduction of provenance and progeny tests in Southern Minas Gerais State, most specifically in Baependi and Aiuruoca counties, where early genetic evaluations and selections are continuously carried out.

Genetic performance in breeding programs based on progenies is evaluated through genetic values, which are used to select progenies or even genetically superior individuals, based on the traits of interest (PIRES *et al.*, 2013). In addition, estimates of variance components and genetic parameters such as heritability, genetic variation coefficient and genetic correlation enable estimating the expected genetic gains resulting from the selection and monitoring of genetic variability of traits over time (OTSUBO *et al.*, 2015).

Different tree matrices can show different seed germination and seedling emergence percentages, as well as variations in initial growth traits such as plant height and stem diameter (MONTEIRO *et al.*, 2016). Therefore, it is important analyzing seedling production feasibility, based on seed germination and seedling emergence, in order to improve species selection processes (MARTINS *et al.*, 2013), since seedling production costs can have major impact on the net revenue of production forests. The investment in seeds with good genetic quality results in seedlings with good growth vigor and considerably increases the yield and profits deriving from commercial reforestation processes.

Therefore, the aim of the current study was to evaluate the seedling emergence percentage, the genetic variability, as well as to identify potential open-pollinated *E. erythropappus* progenies and their respective tree matrices, based on estimates of genetic parameters calculated at seedling stage. Based on the hypotheses of the present study, progenies present different seedling emergence percentages, as well as genetic variability between them. There is genetic correlation between plant height (H) and stem diameter (SD).

MATERIALS AND METHODS

Study site and experimental material

The experiment was carried out in a seedling production tree nursery located in Southern Minas Gerais State (latitude 21°14' S; longitude 44°59' W and altitude of 919 m), which is the natural species occurrence region where commercial *E. erythropappus* plantations have been implemented to help supplying the wood processing and oil production industry.

Seeds of open-pollinated progenies used for seedling production were collected from 22 tree matrices grown in seed orchard, deriving from provenance and progeny test applied to *E. erythropappus*, implanted in January 2005 in the rural area of Baependi County, Minas Gerais State and genotypically selected at the age of five and a half years. Trees presenting the highest height, diameter at breast height (DBH) and volume values were selected as seed collection matrices (Table 1).

Table 1. Provenance and location of matrices where seeds were collected at the provenance and progeny test, in Baependi rural area, Minas Gerais State.

Tabela 1. Procedências e localização das matrizes onde foram coletadas sementes no teste de procedências e progênies, localizado em área rural do município de Baependi, Minas Gerais.

Progenies in tree nursery	Provenance	Matrices	Block	N of tree in plot
1	Baependi	9	1	2
2	Baependi	22	6	3
3	Baependi	20	1	4
4	Baependi	20	2	4
5	Morro do Pilar	8	1	1
6	Itabirito	9	2	3
7	Baependi	22	2	2
8	Itabirito	1	1	1
9	Carrancas	25	6	1
10	Itabirito	1	6	6
11	Itabirito	15	5	2
12	Morro do Pilar	3	7	2
13	Carrancas	11	1	4
14	Delfim Moreira	2	2	2

15	Itabirito	18	1	4
16	Carrancas	25	1	6
17	Delfim Moreira	13	6	5
18	Delfim Moreira	2	1	2
19	Morro do Pilar	11	1	4
20	Carrancas	23	5	6
21	Morro do Pilar	11	6	5
22	Morro do Pilar	14	1	2

Seeds of all 22 progenies were collected in October 2014, identified, separated based on maternal origin (tree matrices), dried in oven until they reached 5% humidity and manually processed. Fruits were dried in the sun, and macerated on sieve, which had a tray placed underneath it to enable collecting the diaspores. The material in the tray was blown in order to get seed lots with greater purity and viability (SCOLFORO *et al.*, 2012). Subsequently, seeds were stored in cold chamber (at 4°C and 70% humidity), based on the methodology proposed by Tonetti *et al.* (2006) for seedling production.

Seedling production and experimental design

Seedlings were produced in tubes (volume of 110 cm³) filled with substrate made of 90% coconut fiber and 10% vermiculite, added with 4 kg of slow-release fertilizer (NPK 15:09:12) per m³ of substrate, based on the methodology by Melo *et al.* (2014). Five seeds were sown per container, which were taken to the shade house, where germination took place from the eighth day onwards. Cover fertilization comprising 1,000 g of monoammonium phosphate and 100 g of potassium chloride diluted in 100 L of water was performed with the aid of watering can, 15 days after sowing. Fungicide 250 SC, at concentration of 3 mL per liter of water, was applied through hand sprayer at 20 days.

Seedling emergence percentage was evaluated 30 days after sowing, by taking under consideration container with at least one emerged seedling. The first selective thinning was carried out at that time and left two seedlings per container. The second selective thinning was performed at 50 days and left only one seedling (the most central and most vigorous) per container. Next, seedlings were transferred to the area in full sun in order to keep growing and start the rustification process.

The second cover fertilization was carried out 70 days after sowing, based on the previously performed procedures. Plant density in trays was reduced by 50% at 75 days - from 432 to 216 per m² - in order to reduce plant competition for light. Plant height (H) and stem diameter (SD) were measured at 100 days. Ruler graduated in centimeters was used to measure H, whereas digital caliper (0.02 mm accuracy) graduated in millimeters was used to measure SD. The experiment has followed a completely randomized design with 22 progenies, four replications and 14 tubes per plot - it was carried out from September to December 2015.

Data analysis

The seedling emergence percentage of each progeny was estimated through the following equation:

$$E \% = 100 \left(\frac{n}{N} \right) \quad (1),$$

Wherein, n is the number of tubes with emerged seedlings and N is the total number of tubes per progeny (48). The minimum value defined for the seedling production process corresponded to emergency percentage of 50%.

Seedling variation components and genetic parameters of traits such as H and SD were estimated based on the REML/BLUP method implemented in SELEGEN software (RESENDE, 2016), by following statistical model 80:

$$Y = X\mu + Zg + Wp + e \quad (2),$$

Wherein, Y is the vector of phenotypic data, μ is the effect of the general mean, g is the vector of genotypic effects, according to which, $g \sim MVN(0, \sigma_g)$, p is the vector of plot effects assumed to be $p \sim MNV(0, \sigma_p)$, and e is the vector of errors or residuals, whose $e \sim MVN(0, \sigma_e)$. “X”, “Z” and “W” represent the incidence matrices for the referred effects (RESENDE, 2016).

Maximum likelihood ratio test (LRT) was used to check significant statistical differences in the investigated effect (progenies) - i.e., whether there was genetic variability between progenies - based on the

difference in deviances between the complete models and the one with lack of corresponding effect (progenies). The following variance components were also estimated: additive genetic variance between progenies ($\sigma_a^2 = 4\sigma_g^2$), estimates assumed the kinship of half-siblings within progenies (0.25), variance between plots (σ_p^2), environmental variance (σ_e^2), and phenotypic variance ($\sigma_f^2 = \sigma_a^2 + \sigma_p^2 + \sigma_e^2$). The estimated genetic parameters were:

Individual narrow-sense heritability:

$$h_a^2 = \frac{\sigma_a^2}{\sigma_f^2} \quad (3),$$

Coefficient to determine the effects of the plot:

$$C_p^2 = \frac{\sigma_p^2}{\sigma_f^2} \quad (4),$$

Individual additive genetic variation coefficient:

$$CV_{gi}\% = 100 \left(\frac{\sqrt{\sigma_a^2}}{x} \right) \quad (5),$$

Experimental coefficient of variation:

$$CV_e\% = 100 \left\{ \sqrt{\frac{[(0.75\sigma_a^2 + \sigma_e^2)/3 + \sigma_p^2]}{x}} \right\} \quad (6),$$

Relative coefficient of variation:

$$CV_r = \frac{CV_{gi}}{CV_e} \quad (7),$$

Selective accuracy was estimated based on Resende and Duarte (2007):

$$\widehat{r}_{gg} = \left[\frac{1}{1 + (CV_e^2/CV_{gi}^2)/r} \right]^{\frac{1}{2}} \quad (8)$$

Wherein, x is the general mean of the analyzed trait and r is the number of repetitions.

Means (individual BLUP) of the two traits evaluated in the investigated progenies were predicted based on the previously defined statistical model. Seedlings with predicted mean height greater than 25 cm were defined as truncated selection criterion. Selection intensity of 55% was adopted to promote accumulated genetic gain in relation to the mean of traits, without significantly reducing genetic variability. This value corresponds to the twelve best progenies identified for each trait, based on the truncated selection criterion. Percentage gain was obtained by dividing the value recorded for the accumulated genetic gain of the selected progenies by the overall mean of the investigated trait and by multiplying it by 100. Predicted means were obtained based on the sum of the overall mean of selected progenies and the accumulated genetic gain.

Genetic correlation between H and SD was estimated through BLUPs, in the R Core Team (2018) statistical software, in order to analyze whether direct selection based on one trait can lead to indirect gains in the other one.

RESULTS

Seedling emergence

Matrices have shown differences in seedling emergence (Figure 1). Progenies 5, 15 and 17 have shown seedling emergence percentages lower than 50% - 26%, 33.4% and 24.3%, respectively. The other progenies have

shown seedling emergence percentages higher than 70% and progenies 11 and 18 have reached 100% seedling emergence.

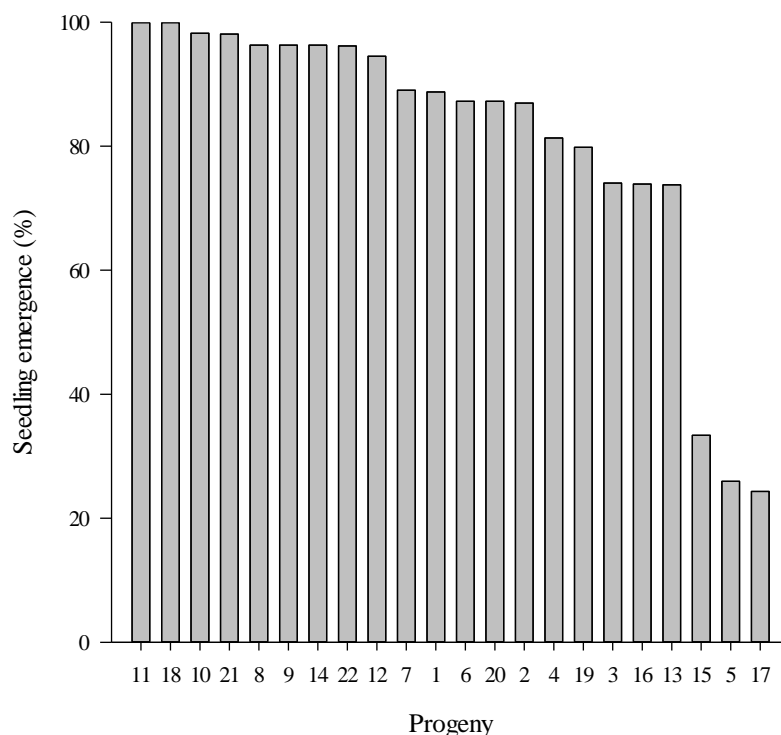


Figure 1. *Eremanthus erythropappus* seedling emergence percentage evaluated 30 days after seeding.
 Figura 1. Percentual de emergência de plântulas de *Eremanthus erythropappus*, avaliada aos 30 dias após a semeadura.

Genetic parameters

Deviance analysis has shown significant differences in traits H and SD between progenies (Table 2). The coefficient of individual narrow-sense heritability (h_a^2) was high in H (0.54) and medium in SD (0.25). The coefficient used to determine environmental effects between plots (C_p^2) has indicated low variability between plots - 10% in H and 4% in SD. The individual additive genetic variation coefficient (CV_{gi} %) has shown considerable genetic variability in both traits - 18.1% in H and 11.2% in SD - whereas the estimated selective accuracy was high in H (97.4%) and SD (97.3%).

Table 2. Deviance analysis (ANADEV) and genetic parameters linked to plant height (H) and stem diameter (SD) of *Eremanthus erythropappus* progenies evaluated 100 days after seeding.

Tabela 2. Análise de deviance (ANADEV) e parâmetros genéticos referentes à altura (A) e ao diâmetro do coleto (DC) de mudas de progênies de *Eremanthus erythropappus*, avaliadas aos 100 dias após a semeadura.

Parameter	H (cm)	SD (mm)
Maximum likelihood ratio test (LRT)	124.31*	4.98*
Additive genetic variance: σ_a^2	17.02	0.11
Variance between plots: σ_p^2	3.27	0.02
Environmental variance: σ_e^2	11.27	0.31
Phenotypic variance: σ_f^2	31.56	0.43
Individual narrow-sense heritability: $h_a^2 \pm$ standard deviation	0.54 \pm 0.13	0.25 \pm 0.09
Coefficient used to determine the effects of the plot: C_p^2	0.10	0.04

Individual additive genetic variation coefficient: CV_{gi} %	18.1	11.2
Experimental coefficient of variation: CV_e %	8.4	5.3
Relative coefficient of variation: CV_r	2.15	2.11
General mean	22.82	2.91

*Significant at 5% probability of error. Tabulated Chi-square: 3.84 at 5% significance level.

*Significativo a 5% de probabilidade de erro. Qui-quadrado tabelado: 3,84 para os níveis de significância de 5%.

Table 3. Mean components (individual BLUP) of the 12 best (55% of total) *Eremanthus erythropappus* progenies selected based on traits such as plant height (cm) and stem diameter (mm).

Tabela 3. Componentes de média (BLUP individual) das 12 melhores progênies (55% do total) de *Eremanthus erythropappus*, selecionadas de acordo com os caracteres altura (cm) e diâmetro do coleto (mm).

Order	Plant height (cm)				Stem diameter (mm)			
	Progeny	Additive effect (a)	Gain	Predicted mean (cm)	Progeny	Additive effect (a)	Gain	Predicted mean (mm)
1	21	7.04	7.04	29.86	13	0.52	0.52	3.42
2	8	5.86	6.45	29.27	12	0.40	0.46	3.37
3	13	5.36	6.09	28.91	4	0.40	0.44	3.35
4	12	2.24	5.12	27.94	8	0.18	0.38	3.28
5	11	1.80	4.46	27.28	1	0.15	0.33	3.24
6	7	1.75	4.01	26.83	11	0.13	0.30	3.21
7	14	1.31	3.62	26.44	9	0.10	0.27	3.18
8	9	1.07	3.30	26.12	18	0.10	0.25	3.16
9	2	1.00	3.05	25.87	14	0.08	0.23	3.14
10	20	0.66	2.81	25.63	21	0.04	0.21	3.12
11	4	0.49	2.60	25.42	2	0.04	0.20	3.10
12	18	0.46	2.42	25.24	7	0.04	0.18	3.09
General mean				22.82	2.91			

The selection of the 12 best progenies enabled gain in H and SD by 10.6% and 6.2%, respectively, in relation to the general mean (Table 3). In addition, 11 (2, 4, 7, 8, 9, 11, 12, 13, 14, 18 and 21) of the 12 progenies selected based on H have also shown greater gain in SD.

Progeny 5 recorded the lowest values for additive effects, which are inheritable, i.e., they are passed on from parents to progeny (-7.66 for H, and -0.55 for SD). This progeny has also presented one of the lowest seedling emergence percentages. Similar outcome was observed for progenies 15 and 17, which indicates their low performance in the analyzed traits, as well as their difficulty in producing seedlings due to their low emergence, in comparison to other progenies. There was high genetic correlation between plant height and stem diameter 100 days after sowing (0.72).

DISCUSSION

Seedling emergence

Some progenies can be ruled out if one takes into consideration that the percentage of 50% was set as minimum value for seedling production. Therefore, despite the high genotypic value presented by progenies 5, 15 and 17, it is not recommended using them because their seedling production is highly costly, and they present low seedling emergence percentage. The other progenies presented seedling emergence percentages higher than 70%; thus, they can be indicated for selection processes due to their high seedling emergence percentage, which, in association with growth in H and SD, helps optimizing the production process and makes its seedlings suitable for planting (LISBOA *et al.*, 2018; MELO *et al.*, 2018).

Genetic parameters

The genetic variability observed between progenies has indicated likely successful selection process (PINTO *et al.*, 2014) and good prospects for gains based on the selection of the best progenies, although they were evaluated at seedling stage. In addition, according to the heritability classification suggested by Resende (1995), the estimate of individual narrow-sense heritability (h_a^2) presented high magnitude for H (0.54) and medium

magnitude for SD (0.25). Such estimates have indicated that part of the phenotypic variation of the analyzed traits had genetic nature (RAMALHO *et al.*, 2012).

Results recorded for heritability were similar to the ones reported for *Cedrella fissilis* progenies, 102 days after sowing - 0.52 for H and 0.42 for SD (BIERNASK *et al.*, 2012). However, they were lower than the ones recorded for *Eugenia calycina* progenies 150 days after sowing - 0.76 for H and 0.87 for SD, (FREITAS; COSTA, 2015).

The greater the value recorded for coefficients of individual additive genetic variation ($CV_{gi}\%$), the greater the likelihood of getting genetic gains in the selection process, since there was genetic variability between progenies. This finding has evidenced that most of the total variation resulted from genetic causes (RESENDE, 2002). Values recorded for H (18.1%) and SD (11.2%) were considered high and corroborated values observed by Chinelato *et al.* (2014), who investigated *Schizolobium parahyba* progenies (11% for plant height at 60 days).

According to Henderson (1984), selective accuracy is the most important statistical parameter in genotypic evaluations, since it correlates the true genotypic value to the one estimated or predicted through experiments. This parameter ranges from 0 to 1; the closest values to 1 are the highest and most suitable values for it. Selective accuracy values recorded for H (97.4%) and SD (97.3%) were considered to have high magnitude, values that showed high experimental precision level (RESENDE; DUARTE, 2007). The herein recorded high precision values make selection at seedling level feasible and may lead to genetic gain in a shorter period-of-time.

The truncated selection criterion set for plant height (progenies with mean height higher than 25 cm) was defined based on predicted means (individual BLUP) recorded for the selection of the 12 best progenies in H, and of the 12 best progenies in SD. According to Melo *et al.* (2014), *E. erythropappus* seedlings without genetic improvement and grown in substrate added with coconut fiber can reach mean height higher than 20 cm, 110 days after sowing.

Thus, matrices located in the seed orchard that provided seeds of the 11 progenies recording the greatest gains in H and SD should be indicated for collection processes focused on producing seedlings the most suitable H and SD for planting, by taking into consideration 100-day production cycles. In addition, seedlings grown from these progenies could be taken to the field, depending on the appropriate edaphoclimatic conditions for the species, and implanted in progeny tests to further the investigation about forestry and genetic improvement of species *E. erythropappus*.

Genetic correlation

Genetic correlations observed between traits enabled inferring whether direct selection based on one trait can favor other traits and provide indirect gains. Seedlings presented positive and high estimated genetic correlation between H and SD (0.72) 100 days after sowing. According to Pinto *et al.* (2014), if the genetic correlation is high and positive, selection processes based on one variable has positive influence on the other variable. Thus, direct selection based on plant height can help obtaining indirect gains in stem diameter.

CONCLUSIONS

- It is not recommended producing seedlings belonging to progenies 5, 15 and 17 because they present low seedling emergence percentage because it hinders the production process.
- Genetic variability in plant height and stem diameter of *E. erythropappus* progenies at seedling stage enables successful genetic selection and allows identifying suitable matrices for seed collection purposes.
- Progenies 2, 4, 7, 8, 9, 11, 12, 13, 14, 18 and 21 must be selected to help generating larger seedlings to be planted in the field at 100 days of production, whereas their respective matrices should be used for seed collection.
- Indirect gains in stem diameter can be obtained through progenies selection based on plant height due to high and positive genetic correlation between the two traits.

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