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Eucalyptus citriodora, E. phaeotricha and E. propinqua seeds subjected to classification by sieves

Sementes de *Eucalyptus citriodora, E. phaeotricha e E. Propínqua* submetidas a classificação por peneiras

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Abstract

Seeds' qualities are influenced by several factors such as the density and size of seeds. The aim of this study was to evaluate the physical and physiological quality of *Corymbia citriodora*, *E. phaeotricha* and *E. propinqua* seeds that were subjected to classification by sieve. The seeds passed through sieves that retained the following sizes: C. citriodora (2.00; 1.68; 1.41; 1.19; 1.00 mm), *E. phaeotricha* (1.41; 1.19; 1.00; 0.84 mm) and *E. propinqua* (1.00; 0.84; 0.71; 0.59 e 0.50 mm). The tests of retention percentage, physical purity, germination, first count of germination, germination speed index and average germination time were performed. The design used was randomized with four replications and five treatments for C. citriodora and *E. propinqua*, and four treatments for *E. phaeotricha*, due to the number of sieves that retained seeds. The data were submitted to analysis of variance by the F test. When significant, the statistics related to the classification of seeds by sieves were compared using the Tukey test with 5% probability. The use of sieve classification is effective for the separation of seeds with high physical and physiological quality. *C. citriodora* with a seed size of 1.41 to 2.00 mm have maximum physical and physiological quality and can be pelleted. For *E. phaeotricha* and *E. propinqua*, seeds with a size of 1.19 to 1.41 mm and 0.84 to 1.00 mm, respectively, have the highest physical and physiological quality.

Additional keywords: Germination; *Myrtaceae*; purity; seed size.

Resumo

A qualidade de sementes é influenciada por vários fatores como densidade e tamanho de sementes. O objetivo desse trabalho foi avaliar a qualidade física e fisiológica de sementes de *Corymbia* citriodora*, E. phaeotricha* e *E. propinqua* submetidas a classificação por peneiras. As sementes passaram por peneiras que retinham os seguintes tamanhos: C. citriodora (2.00; 1.68; 1.41; 1.19; 1.00 mm), *E. phaeotricha* (1.41; 1.19; 1.00; 0.84 mm) and *E. propinqua* (1.00; 0.84; 0.71; 0.59 e 0.50 mm). Foram realizados os testes de porcentagem de retenção, pureza física, germinação, primeira contagem, índice de velocidade e tempo médio de germinação. O delineamento utilizado foi inteiramente casualizado com quatro repetições e cinco tratamentos para C. citriodora e *E. propinqua*, e quatro tratamentos para *E. phaeotricha* devido ao número de peneiras que retêm as sementes. Os dados foram submetidos à análise de variância pelo teste F. Quando significativas, as médias relacionadas a classificação das sementes por peneiras foram comparadas usando o teste de Tukey a 5% de probabilidade. O uso de peneiras de classificação é eficiente para a separação de sementes de alta qualidade física e fisiológica. As sementes de C. citriodora com tamanho de 1,41 a 2,00 mm apresentam máxima qualidade física e fisiológica e podem ser peletizadas. Para sementes de *E. phaeotricha* e *E. propinqua* os tamanhos de 1,19 a 1,41 mm e 0,84 a 1,00 mm, respectivamente, apresentam a máxima qualidade física e fisiológica.

Palavras-chave adicionais: Germinação; *Myrtaceae*; pureza; tamanho de sementes

Introduction

Brazil has 7.4 million hectares occupied by the Eucalyptus genus (SFB, 2018). Among the species exploited for various commercial purposes, *Corymbia citriodora* Hook stands out as it is used to produce oils for, mainly, antibacterial and antioxidant medicinal properties (Salem et al., 2018, Lin et al., 2019). *E. phaeotricha* Blakely & Mickie and *E. propinqua* Deane

& Maiden are normally used for reforestation and mainly for sawmills (Lorenzi, 2003; IPEF, 2018).

For species of the Eucalyptus genus, seed propagation is viable and adopted by small and medium nurseries for seedling production (Cetnarski Filho and Carvalho, 2009, Affonso et al., 2018, Jeromini et al., 2021). However, after harvest the eucalyptus species present lots of seeds with a high variation in terms of size, shape, mass, as well as a high percentage of impurities, especially unfertilized ovules (Nakagawa et al., 2001, Martins et al., 2014, Affonso et al., 2018, Jeromini et al., 2021).

Due to their small size and because they have very similar physical characteristics, like color, size, format or weight it is difficult to obtain lots of seeds with a high percentage of purity through the seed processing commonly used in eucalyptus seed production systems (Santos, 2016).

Therefore, classifying them by size using classifying sieves has been suggested, which aims to obtain purer seeds, uniform classes and an easy handling of seeds (Melo et al., 2016, Bernardi et al., 2019). In addition, classification by sieves can enable the adoption of techniques that aim to mechanize sowing more precisely, such as the pelletizing process, an activity that consists of covering the seeds to increase their size and plantability (Walker et al., 2011; Santos, 2016).

Physiological quality, or germination and vigor in other words, is influenced by several factors such as the density and size of seeds (Bernardi et al., 2019; Jeromini et al., 2019). Studies have shown that for *E. urophyla* S.T. Blake, *E. saligna* Sm and *E. maculata* Hook, seed germination speed is affected by seed size more than by other factors such as temperature, light and maturation (Aguiar et al., 1979; Aguiar and Mardegan, 1987; Silva et al., 1994;). As for E. *grandis*, Hill ex Maiden, the size of the seed influences the germination potential (Nakagawa et al., 2001; Jeromini et al., 2021) and the seedlings survival in a nursery (Naidu and Jones, 2010).

Nevertheless, for C. citriodora, *E. phaeotricha* and *E. propinqua*, no research was carried out to obtain this information. So, the aim of this study was to evaluate the physical and physiological quality of C. citriodora, *E. phaeotricha* and *E. propinqua* seeds that were subjected to classification by sieve.

Material and methods

Corymbia citriodora, *Eucalyptus phaeotricha* and *E. propinqua* seeds from the Seed Production Area (APS - F1) located in Anhembi - SP, were sent to the Seed Analysis Laboratory and the following tests were conducted:

Retention in sieves - for each species of eucalyptus, two repetitions of 100g of commercial seeds were used, placed on the upper sieve of a set of six wire mesh sieves, fitted one on top of the other, in decreasing order of mesh size, from top to bottom. A non-perforated bottom was fitted under the overlapping sieves and this set was stirred for three minutes. In this way, the commercial seeds were separated in the following sizes: *C. citriodora* (2,00; 1,68; 1,41; 1,19; 1,00 mm), *E. phaeotricha* (1,41; 1,19; 1,00; 0,84 mm) and *E. propinqua* (1,00; 0,84; 0,71; 0,59 and 0,50 mm).

The commercial seeds retained by each sieve, and which passed through the sieve immediately above, were separated, weighed and had their retention percentage calculated. This test was carried out following the methodology prescribed in the Rules for Seed Analysis (Brasil, 2013), in an adapted way due to the greater number of sieves. The average percentage of the two repetitions was presented as the result, in whole numbers (Brasil, 2013). After classification, the different sized commercial seeds were subjected to the following tests.

Physical purity - determined by two 0.50 g subsamples, which were weigh on a precision scale (0.001 g) and the inert material was removed before manual separation with the aid of tweezers and a magnifying glass. The results were expressed as a percentage (Brasil, 2013).

Germination - conducted with four subsamples of 100 seeds for each treatment and placed on filter paper previously moistened with distilled water in an amount of 2.5 times the mass of the dry paper. The seeds were placed in transparent plastic boxes measuring 11.0 x 11.0 x 3.5 cm with a lid and kept at a temperature of 25˚C with a photoperiod of 8 hours.

Normal seedlings (with a developed radicle and aerial part) and abnormal (radicle and / or aerial part with some damage that prevented their development in the field) were counted and the results were expressed as a percentage (Brasil, 2013):

First count - conducted along with the germination test, recording normal seedlings on the fifth day after sowing and with the results expressed as a percentage (Brasil, 2013).

Average germination time - conducted along with the germination test, calculated based on the daily count of normal seedlings until the final test date and applying the formula established by Labouriau and Valadares (1976) to the data with results expressed in days.

Germination speed index - conducted along with the germination test, calculated based on the daily count of normal seedlings until the final test date. The formula used by Maguire (1962) was applied to the data.

Statistical analysis - The design used was randomized with four replications and five treatments for *C. citriodora* and *E. propinqua*, and four treatments for *E. phaeotricha*, due to the number of sieves that retained seeds. The data were submitted to analysis of variance by the F test. When significant, the statistics related to the classification of seeds by sieves were compared using the Tukey test with 5% probability.

Results

The seed retention test in sieves allowed for the verification of the percentage of each seed size in the lots depending on the species that is being tested. For C. citriodora, a higher concentration of seeds was found in the two higher value sieves: 2.00 mm and 1.68 mm. These together collect 83% of commercial seeds (Fig. 1A).

For *E. phaeotricha*, there was greater retention in two smallest sieve, 1.00 mm (48%) and 0.84 mm (43%) sieves (Fig. 1B). The same situation was observed for *E. propinqua*, there was a higher concentration of seeds in the smaller sieves of ≤0.50 mm, adding up to 68% (Fig. 1C).

It was observed that the classification of seeds by sieves was significant for all variables evaluated in the three species (Tab. 1).

The purity of C. citriodora and *E. phaeotricha* seeds was higher in the sieves with larger holes, Tylers 9 and 10 (2.00 and 1.68 mm) for C. citriodora and Tylers 12 (1.41 mm) for *E. phaeotricha*, being 98.7; 99.1 and 83.3%, respectively (Tab. 2).

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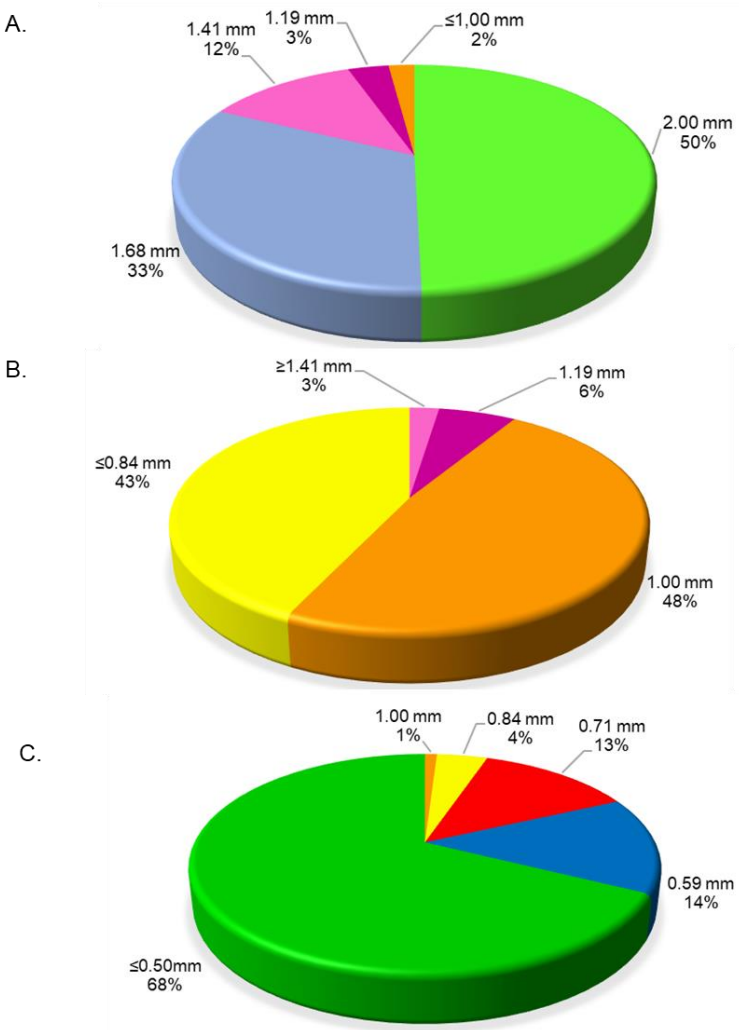

Figure 1. Percentage distribution by weight of *Corymbia citriodora* (A), *Eucalyptus phaeotricha* (B) and *E. propinqua* (C) seeds in size classes after retention test in sieves.

Table 1 - Summary of the analysis of variance for purity test, germination, first count, abnormal seedlings, average germination time and germination speed index as a function of sieve seed classification for a *Corymbia* citriodora, *Eucalyptus phaeotricha* and *E. propinqua* seed lot.

Factors of Variation	DF	Medium square	Coefficient of variation	Average	l.s.d.
			C. citriodora		
Purity	4	6,775.9**	0.36	9.30	0.63
Germination	4	14.12^{ns}	3.67	84	6.77
First Count	4	515.07**	11.26	62	15.26
Abnormal seedlings	4	3.67**	29.92	2.20	1.43
Average germination time	4	0.67^{ns}	7.10	5.45	0.85
Germination speed index	4	7.53**	5.42	6.59	1.96
Residue	15				
			E. phaeotricha		
Purity	3	1,097.76**	0.87	71.03	1.31
Germination	3	3,008.41**	14.38	38.62	11.66
First Count	3	368.33**	21.54	12.50	5.65
Abnormal seedlings	3	2.72^{ns}	84.65	1.81	3.22
Average germination time	3	1.06^{ns}	6.85	8.68	1.24
Germination speed index	3	49.79**	14.99	4.91	1.54
Residue	12				
			E. propínqua		
Purity	4	4,126.04**	1.98	50.06	2.16
Germination	4	1,904.20**	8.24	45.15	8.12
First Count	4	474.82**	8.85	22.85	4.11
Abnormal seedlings	4	2.03^{ns}	39.42	1.16	1.60
Average germination time	4	2.42**	4.65	6.80	0.69
Germination speed index	4	86.32**	10.73	8.30	1.95
Residue	15				

** and ns =significant at the 1% probability level and not significant according to the F test, respectively. DF = degree of freedom, l.s.d. = least significant difference.

Table 2 - Purity (P), germination (G), first count (FC), abnormal seedlings (AS), average germination time (AGT) and germination speed index (GSI) of *Corymbia citriodora*. *Eucalyptus phaeotricha* and *E. propinqua* seeds classified by sieve sizes.

Tratamento	P	G	FC	AS	AGT	GSI
	---------- (%) ----------				(days)	
			C. citriodora			
Tyler 9 (2.00mm)	98.7 ab	87 a	75 a	2 a	5 a	17.69 ab
Tyler 10 (1.68mm)	99.1 a	86 a	74 a	2 a	5 a	18.38 a
Tyler 12 (1.41mm)	98.5 b	85 a	50 b	2 a	6 a	16.09 bc
Tyler 14 (1.19mm)	94.4 c	83 a	54 b	3 ab	6 a	15.24 c
Tyler 16 (1.00mm)	5.7 d	83 a	57 b	4 b	6 a	15.57 c
			E. phaeotricha			
Tyler 12 (1.41mm)	83.3 a	51 b	23 a	2 a	8 a	6.92 b
Tyler 14 (1.19mm)	81.1 b	69 a	17 b	1 a	9 ab	8.66 a
Tyler 16 (1.00mm)	72.1 c	26 c	8 c	3 a	9 ab	3.16 c
Tyler 20 (0.84 mm)	47.2 d	8 d	2 d	1 a	10 b	0.89 d
			E. propinqua			
Tyler 16 (1.00 mm)	72.0 b	68 a	34 a	2 a	6 a	13.55 a
Tyler 20 (0.84 mm)	82.0 a	60 a	30 a	2 a	6 a	11.65 a
Tyler 24 (0.71 mm)	65.0 c	49 c	25 b	1 a	7 b	8.38 b
Tyler 28 (0.59 mm)	25.0 d	35 d	18 c	2 a	7 b	6.18 c
Tyler 32 (0.50 mm)	8.0 e	13 d	7 d	1 a	8 c	1.74 d

Averages followed by the same letter do not differ from each other by Tukey's test at 5% probability.

In contrast to E. *propinqua*, the highest purity (82.0%) was observed in Tyler 20 (0.84 mm) which presented one of the lowest percentages of retention, only 4%, indicating that despite high purity it is the size with the least amount of seeds in freshly harvested lots. Regarding the physiological quality, it was found that the results for the germination percentage corroborated with those obtained for physical quality, because the bigger the seed, the higher the percentage of germination for *E. propinqua*, with a maximum of 68% and for *E. phaeotricha* the maximum germination was in the Tyler 14 (1.19mm) with 69%. However, for C. citriodora, which showed no significant difference between sizes, however, presented an average of 84% germination (Tab. 2).

However, for *E. phaeotricha* and *E. propinqua*, the seeds of higher quality are in less quantity in the lot (Fig. 1B and C). Regarding seedling abnormality, only C. citriodora seeds showed a significant difference between sizes (Tab. 1 and 2).

The vigor of the seeds, characterized by germination speed, being, first count, average germination time and germination speed index, presented results like the germination test and physical quality. For the three studied species, the greater the seed, the greater the vigor, except for C. citriodora where the different seed sizes did not show significant difference for average germination time (Tab. 1 and 2).

Discussion

Classification by sieves is commonly performed for species of large crops such as beans, soybeans and corn in order to add value to the product, provide the final consumer with accurate information on the seeds he is acquiring, and to allow them to direct its use within a production process such as pelletizing (Nunes et al., 2016, Schmidt, 2019).

The purity percentage decreased as the size of C. citriodora and *E. phaeotricha* seeds retained in the sieves decreased, indicating that the largest seeds for these two species, which represent the largest fraction of the lot have the highest purity, The fractions of C. citriodora seeds from Tyler 9, 10 and 12 would be the only ones of the species evaluated that could be submitted to palletization, since, to make this covering, seed lots with a purity above 95% must be used to guarantee maximum commercialization of pure seeds (Almeida & Rocha, 2008). So, those lots of *E. phaeotricha* and *E. propinqua* seeds need the efficient processing to improve the purity.

In addition to the showing the percentage of each portion of the lot, there was possibility of improving seed commercialization, in the sense of adding value, by offering the consumer seeds of pre-established sizes, avoiding the mixing of several sizes and inert material (Fig.1).

The high purity in general in the larger sized seeds portions, may be related to the possibility of impurities passing through the sieves, since these impurities are mostly unfertilized ovules and have a smaller size than the seeds, favoring a higher concentration of them in the portion of smaller sized seeds (Martins et al., 2014, Affonso et al., 2018). This behavior was also reported for *E. maculata*, by Silva et al. (1994), E. *grandis*, E. *robusta*, and *E. urophylla* seeds (Jeromini et al., 2021)

Guaranteeing pure seeds is difficult using only a set of sieves to separate seeds of the same size, as occurred with the seed lot of *E. propinqua*, as it is possible to verify in small seed processing works. Therefore, the need to carry out more seed processing studies, to guarantee the obtaining of pure seeds for commercialization is required (Araújo et al., 2011, Caldeira et al., 2016, Melo et al., 2016, Melo et al., 2018, Bernardi et al., 2019).

In this case, the use of classification sieves in the processing of seeds of these species of eucalyptus could be adopted to obtain more uniform classes and to separate and dispose of impurities before commercialization. A study with *E. grandis*, *E. robusta*, and *E. urophylla* seeds, showed that the classification by size obtained larger and havier seeds and this seeds had the best vigor and germination rates (Jeromini et al., 2021). Thus, guaranteeing seed lots of better physical quality, as observed in seeds of grass-mombaça (Melo et al., 2018) and grass-marandú (Jeromini et al., 2019). For *E. phaeotricha* and *E. propinqua* it can be considered that the sizes from 1.00 to 1.41 mm and 0.71 to 1.00 mm, respectively, present a high standard of quality, since they have between 56% and 100% germination (Lúcio et al., 2011).

Lúcio et al. (2011) established parameters to measure the standard of quality so that seed lots of some species of eucalyptus can be marketed, and were classified as low, medium, high and very high, and in comparison, with these parameters the germination of the C. citriodora seeds, can be considered very high for all seed sizes, since they are all above a germination rate of 80%.

Thus, the smaller seeds could be removed from the seed lots to be marketed for all studied species, to increase physical and physiological quality (Tab. 2). The greater germinative potential in relation to the larger seeds were also reported by Naidu and Jones (2010) in *E. grandis* seeds and by Jeromini et al. (2021) in *E. grandis*, *E. robusta*, and *E. urophylla* seeds.

For seeds of *E. phaeotricha* and *E. propinqua*, these smaller seeds could be transferred to another set of sieves, or to a gravitational table for possible density separation as described for other species (Melo et al., 2018, Jeromini et al., 2019).

The high vigor observed in the larger sized seeds is possibly due to the greater nutrition during their formation and a greater reserve that enabled a greater physiological quality (Nakagawa et al., 2001, Jeromini et al., 2019).

Conclusions

The use of sieve to classify seeds is efficient for separating seeds of high physical and physiological quality.

The seeds of *E. phaeotricha* and *E. propinqua* with sizes from 1.19 to 1.41 mm and 0.84 to 1.00 mm, respectively, present the maximum physical and physiological quality.

The seeds of C. citriodora reteined in the largest sieves had the maximum purity and vigor.

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