Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v41i1.42602

Methods for overcoming dormancy and seed storage of *Enterolobium contortisiliquum*

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ABSTRACT. The production of forest species that present seedlings with exogenous dormancy is limited by low germination rates, which can be overcome by rupturing or weakening the tegument. This study aimed to evaluate the efficiency of low-cost methods of overcoming dormancy and determine the effect of storage on the seeds of *Enterolobium contortisiliquum*. Four storage periods were used, freshly collected seeds (0), stored for 6, 12 and 18 months, and four methods were applied, mechanical scarification and immersion in water (EI), mechanical scarification (ES), immersion in water (IM) and control (TE). We obtained the percentage values of seedling emergence (EP) and the mean emergence time (TME). Conductivity and moisture were obtained as indicators of the physical and physiological quality of seeds, respectively. Variations in moisture content and electrical conductivity during storage did not have effect on the percentage of germination. EI treatment was more efficient for overcoming dormancy than the ES was, regardless of storage time. IM and TE treatments did not lead to germination. EI was the most efficient treatment for decreasing the TME. The results indicate that alternative and low-cost methods are efficient at overcoming exogenous dormancy and thus can be used to optimize the process of seedling production.

Keywords: exogenous dormancy; immersion; mechanical scarification; leguminous plants; seedling production; pacara earpod tree.

Received on October 6, 2017.
Accepted on March 8, 2018.

Introduction

Developing a better understanding of the autoecology of forest species is one of the initial steps to producing high-quality seedling in large quantities, especially in cases where dormancy is caused by the presence of chemical substances or a tegument (Scalon et al., 2005). The seeds of leguminous plants under natural conditions present an elevated germination time, which is considered a tactic for distributing the germination period over time and space (Eira & Caldas, 2000). In terms of production, this delay is an advantage that allows seed storage. Nevertheless, methods for overcoming dormancy must be implemented to increase the seedling emergence rate.

Several studies have sought to improve the seedling production process by analyzing the methods of overcoming dormancy and increasing and homogenizing the percentage of seedling germination (Leperlier et al., 2018; Lopes & Souza, 2008). Among the methods that break the seeds physical dormancy, mechanical scarification represents one of the most frequently used methods because it is a practical, safe and low-cost technique (Hu, Baskin, Baskin, Yang, & Huang, 2017). Despite the high number of studies on the effectiveness of methods for overcoming dormancy in germination (Varela & Lizardo, 2010; Scalon et al., 2005; Alexandre, Gonçalves, Rocha, Arruda, & Lemes, 2009; Lessa, De almeida, Pinheiro, Nogueira, & Medeiros Filho, 2014), most of these studies have not provided practical information to facilitate the selection of the most appropriate methods based on their costs and benefits. Therefore, orthodox seeds (i.e., seeds that can be stored; Roberts, 1973) have been studied, especially with regard to the possible negative effects of storage time on the physical and physiological quality of the seeds (Jayasuriya, Asanga, Wijetunga, & Baskin, 2013).

The pacara earpod tree, *Enterolobium contortisiliquum* (Vell.) Morong. (Fabaceae), is a leguminous species native to Brazil, and it is characterized as deciduous during winter and represents a pioneer and hygrophytic-selective species (Souza, Melo, Halfed, & Reis, 2017). This tree has been used in the restoration of degraded areas and mixed-species plantations because of its rapid growth, which can reach five meters in less than two years (Souza et al., 2017; Araujo & Paiva Sobrinho, 2011). Nevertheless, this species presents low seedling germination because of an impermeable cover formed by macrosclereids and a high number of hydrophobic substances that induce the physical dormancy of the seeds (Baskin, Baskin, & Xiaojie, 2000). In theory, this dormancy can be overcome using treatments that rupture or weaken the tegument, thereby facilitating water absorption and, consequently, germination (Baskin et al., 2000).

This study aimed to evaluate the efficiency of low-cost methods of overcoming dormancy and determine the effect of storage on the seeds of *E. contortisiliquum*. We tested the effect of four methods of overcoming dormancy as well as the impact of storage on seedling emergence and the physical and physiological quality of seeds. Specifically, we tested the hypotheses that (i) storage has an effect on physical and physiological quality of seeds; (ii) treatments with mechanical scarification associated with immersion in water are more efficient for overcoming dormancy relative to treatments without scarification, regardless of the storage time; and (iii) treatments with scarification and immersion in water reduce the mean emergence time (TME) of seedlings. The results presented here contribute to the optimization of seedling production processes as well as the knowledge of the reproductive biology of *E. contortisiliquum*.

Material and methods

Seeds collection and processing

To obtain seeds that presented homogenous genetic and morphological aspects, the fruits were harvested from a single site (15°16'48.09"S, 49°34'2.36"W; 541 m a.s.l.) and the same seed lot was used throughout the experiment. The fruits were collected after falling off the tree to avoid harvesting before maturation, which could result in a total or partial loss of seeds. After collecting, the seeds were processed using a wood mortar and pestle. Because the seeds are extremely rigid, the mechanical shock does not damage the seeds.

Storage

After processing, the seeds were stored in transparent and impermeable plastic packages under ambient room temperature (±30°C) and without exposure to sunlight. We chose four storage periods to evaluate the viability of the seeds over time: freshly collected seeds (zero) and seeds stored for 6, 12, and 18 months.

Sampling design

The experiment was performed in a greenhouse with an automated micro-sprinkler irrigation system in a randomized complete block design (RCBD) with a 4 x 4 factorial scheme composed of the four storage periods (0, 6, 12, and 18 months) and four methods of overcoming dormancy (EI – mechanical scarification and immersion in water for 12 hours; ES – mechanical scarification; IM – immersion in water for 12 hours; and TE – control). Four repetitions of 40 seeds were performed, for a total of 2,560 seeds. The chosen treatments to overcome dormancy were as follows. For the El method, tegument scarification was performed using a double wheel bench grinder (Disma, model 350W, 3450 RPM) on the lateral portion of the seed, and then the seeds were immersed in mineral water for 12 hours. In the TE, none of the methods for overcoming dormancy were applied. The percentage values of seedling emergence (EP) and mean germination time (TME) (Labouriau & Valadares, 1976; Labouriau, 1983) were recorded starting on the second day after seeding until the 12th day.

Physical and physiological quality

The seed moisture content for the storage periods of 0, 6, 12, and 18 months of storage was evaluated using four repetitions of 25 seeds each, which were weighed and then dried in an oven at 105°C for 24 hours (Brasil, 2009). After this period, the samples were weighed again, and the difference in weight was interpreted as the moisture content, which was represented as a percentage.

To obtain the electrical conductivity values, four repetitions of 25 seeds for each storage period were tested. The seeds of each repetition were weighed using an analytical balance with 1 mg (0.001 g) resolution, and they were then placed in plastic containers with 50 mL of distilled water and retained inside a BOD chamber at 25°C for 24 hours (Vidigal, Lima, Bhering, & Dias, 2008). The electrical conductivity of the solution of each of the replicates was determined using a microprocessor-based benchtop conductivity meter (model LSDDS-W). The results were represented in μS cm^{-1} g^{-1} of seeds (Krzyzanowski, Vieira, & França Neto, 1999).

Data analysis

Initially, the EP was represented based on the percentage of emerged seedlings and the TME was performed as described by Maguire (1962). The moisture content values, conductivity, EP and TME were standardized via the square root transformation. To test the effect of storage on the physical and physiological quality of the seeds, we created one-factor linear models using the storage period as the predictor variable and the moisture content and electrical conductivity as separate response variables.

The efficiency of the treatments with mechanical scarification and immersion in water relative to that of the treatments without scarification was tested using two-factor linear models using the storage period and treatment as predictor variables and the EP and TME as response variables. We also evaluated the interactions between the predictor variables for the EP and TME separately. All models were validated through a visual evaluation of the homogeneity and normality of the residuals according to the protocol of Zuur, Leno, and Elphick (2010). The tests were performed using the function "lm" in the package "vegan" (Oksanen et al., 2016) in R software (R Core Team, 2017).

Results and discussion

The storage period had an effect on the physical and physiological quality of the seeds. The seeds in the "zero" period presented higher moisture content (mean = 18%) compared with those stored for 12 months (mean = 15.12%) and 18 months (mean = 14.69%; Figure 1A; Table 1). This relation is normal in freshly collected seeds that have not yet undergone dehydration processes promoted by storage time (Souza et al., 2016). However, the decrease in moisture during the storage periods did not reduce the seed viability because this class of orthodox seeds supports dehydration until the moisture content reaches 2-5% (Roberts 1973). Despite the decreasing moisture tendency, the moisture content of the seeds stored for six months did not differ from that of the seeds stored for other lengths of time (mean = 17.13%; Figure 1A).

The electrical conductivity differed among the storage periods. Freshly collected seeds and those stored for 6 months presented lower electrical conductivity values (mean = 0.84 μS cm^{-1} g^{-1} and 0.85 μS cm^{-1} g^{-1}, respectively) compared with seeds stored for 12 months (mean = 1. 10 μS cm^{-1} g^{-1}) and 18 months (mean = 1.12 μS cm^{-1} g^{-1}; Figure 1B). These results are related to the deterioration of the tegument over time, which increased the permeability of the seed to water during the test and consequently increased the electrical conductivity (Ataíde, Borges, Flores, & Castro, 2016).

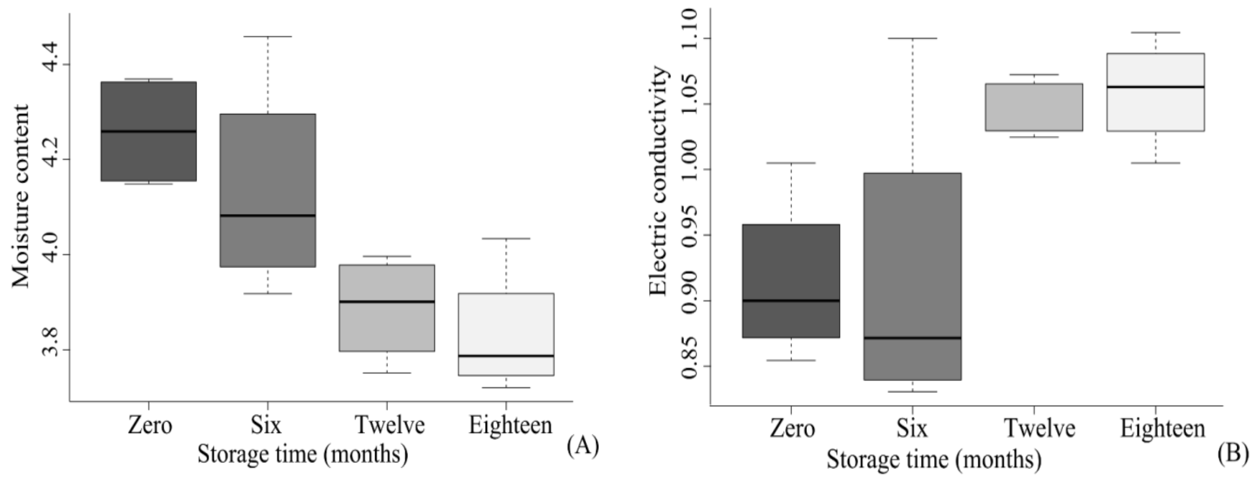

Figure 1. Variations in the moisture content (A) and electrical conductivity (B) of the seeds based on storage time.

The variations in moisture content and electrical conductivity during storage did not have an impact on the germination percentage. Although the storage period tended to have an influence on EP in the general model (Figure 2A; Table 1), the effect was only observed in the interactions among the methods for overcoming dormancy. Thus, the percentage of germination of ES in the "zero" period (without storage) was lower than that of the other methods with storage (Figure 3A; Table 2). This finding was related to the lack of immersion in water for the seeds in the ES treatment. In contrast, the results showed that immersion in water after scarification increases the moisture content in the seeds and enables the activation of metabolic processes and embryonic development (Tambelini & Perez, 1998; Thangjam & Sahoo, 2017) and that even in freshly collected seeds, the higher the concentration of hydrophobic substances is, the more difficult the germination is.

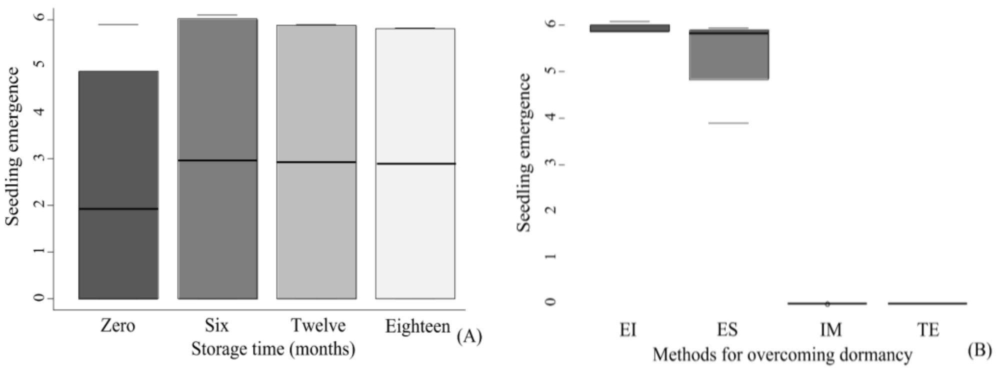

Figure 2. Variations in seedling emergence based on the storage time (A) and the methods for overcoming dormancy (B).

Treatments with mechanical scarification and immersion in water were more efficient for overcoming dormancy compared with the treatments that only used scarification, regardless of storage time (Figure 3A; Table 2). The results showed that higher EP values were observed in the EI treatment compared with the ES treatment. Moreover, the immersion-only treatment (IM) and the absence of treatment (TE) did not lead to germination (Table 1; Figure 2B). These results show that methods that do not allow for water permeation into the seed are ineffective. Similar results were also observed for the seeds of other species, such as the Jatobá or Brazilian Cherry (*Hymenaea courbaril* L.); the seeds of this tree present exogenous dormancy and an intact tegument before seeding, and these characteristics lead to a low percentage of germination (Andrade, Bruno, Oliveira, & Silva, 2010).

The TME was lower in the "zero" period compared with the six- and twelve-month periods, which did not differ from the eighteen-month period (Figure 3A; Table 1). The moisture content of the seeds was inversely related to the mean emergence time; therefore, higher humidity corresponded to a lower TME (Souza et al., 2016). The interaction between the periods and treatments only presented in the IM and TE treatments, which presented a TME of zero because germination did not occur (Figure 4B; Table 1). These results indicate that the method that included scarification combined with immersion in water is the most efficient for decreasing the mean emergence time. The emergence time is associated with the difference between the water potential of the seed and the external medium, which determines the velocity of water absorption (Li et al., 2017).

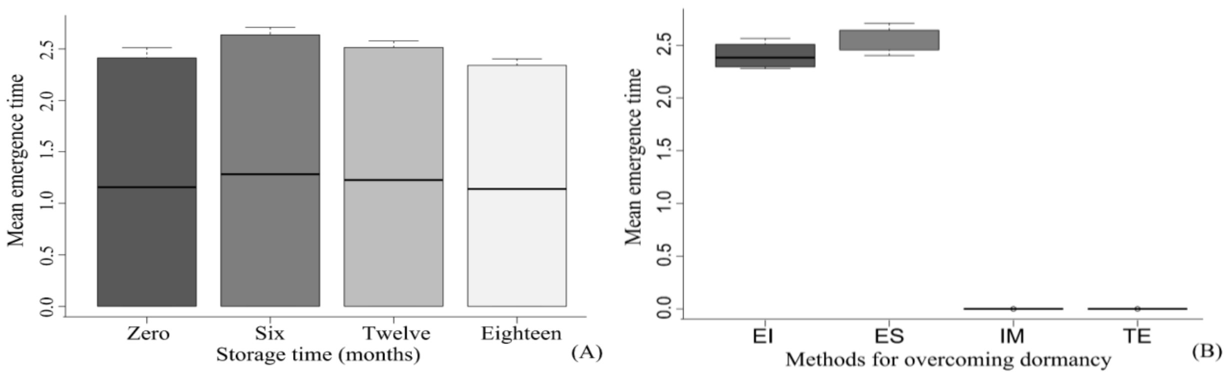

Figure 3. Variations in the mean emergence time based on the storage time (A) and the methods for overcoming dormancy (B).

Table 1. Summary of the linear model analyses between the predictors and variables of physical and physiological quality, seedling emergence and mean emergence time in the seeds of *E. contortisiliquum*.

Variables	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Physical and physiological quality (storage time influence)					
Moisture	3	0.49	0.16	6.53	<0.01
Conductivity	3	0.07	0.02	4.59	0.02
Seedling emergence					
Storage time	3	3.13	1.04	15.06	<0.01
Methods for overcoming	3	511.12	170.37	2460.88	<0.01
Storage time x methods	9	8.72	0.97	14.00	<0.01
Residuals	48	3.32	0.07		
Mean emergence time					
Storage time	3	0.2	0.07	24.09	<0.01
Methods for overcoming	3	98.25	32.75	11882.6	<0.01
Storage time x methods	9	0.21	0.02	8.34	<0.01
Residuals	48	0.13	0.00		

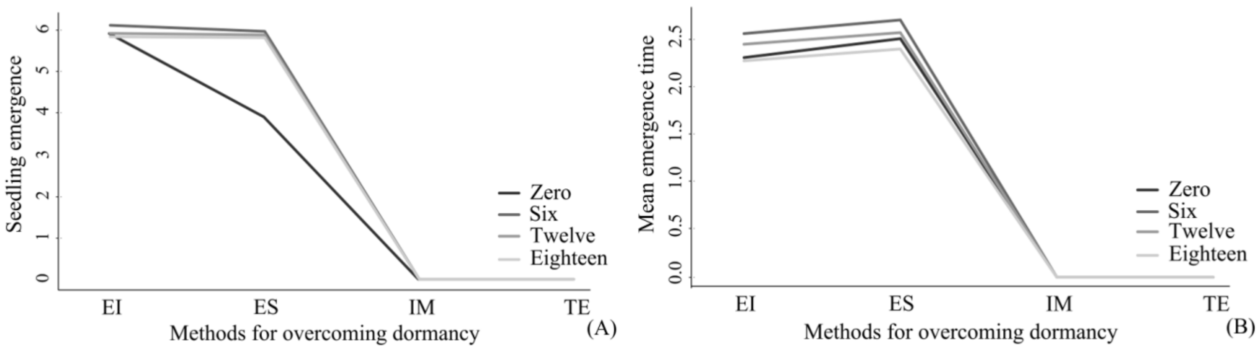

Figure 4. Effect of interactions between the storage time and methods for overcoming dormancy on the seedling emergence (A) and mean emergence time (B) in the seeds of *E. contortisiliquum.*

Table 2. Percentage of seedling emergence and mean emergence time for the storage time and methods for overcoming dormancy in the seeds of *E. contortisiliquum*. Mean values followed by the same lowercase letter in the columns and the same uppercase letter in rows were not significantly different based on the results of Tukey's test at the 5% probability level.

Storage time (months)	Methods for overcoming dormancy				Mean
Seedling emergence (%)					
	EI	ES	IM	TE	
Zero	86.87 aA	38.75 bB	0.00 aB	0.00 aB	31.40
Six	92.50 aA	88.12 aA	0.00 aB	0.00 aB	45.16
Twelve	86.87 aA	85.62 aA	0.00 aB	0.00 aB	43.12
Eighteen	84.37 aA	83.75 aA	0.00 aB	0.00 aB	42.03
Mean	86.87	74.06	0.00	0.00	-
Mean emergence time (days)					
	EI	ES	IM	TE	
Zero	5.35 cB	6.31 bA	0.00 aB	0.00 aB	2.92
Six	6.58 aB	7.33 aA	0.00 aB	0.00 aB	3.48
Twelve	6.03 bB	6.64 bA	0.00 aB	0.00 aB	3.17
Eighteen	5.19 cB	5.77 cB	0.00 aB	0.00 aB	2.74
Mean	5.79	6.52	0.00	0.00	-

Conclusion

The decreased physical and physiological quality of the seeds stored for 18 months did not affect the seed viability. Combination of scarification and immersion represented the most efficient method for seedling germination, even in freshly collected seeds, because the higher concentration of hydrophobic substances hinders germination. Water immersion and control treatments were not effective for germination. The combined method was the most efficient at reducing the mean emergence time in all storage periods. The results indicate that alternative and low-cost methods are efficient for overcoming exogenous seed dormancy and can thus be used to optimize the processes of seedling production.

Acknowledgements

We thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Apoio à Pesquisa do Estado de Goiás (FAPEG) for providing scholarship to DF; CAPES for providing scholarship to WPR; and Instituto Federal Goiano – Campus Ceres for financial support to LSRV for English review.

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