Semina: Ciências Agrárias
ISSN: 1676-546X
semina.agrarias@uel.br
Universidade Estadual de Londrina
Brasil

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Semina: Ciências Agrárias, vol. 33, núm. 1, 2012, pp. 3003-3008
Universidade Estadual de Londrina
Londrina, Brasil

DOI: 10.5433/1679-0359.2012v33Supl1p3003

X-ray test for the evaluation of the internal morphology of the seeds of seafortia palm trees

Teste de raios X para avaliação da morfologia interna de sementes da palmeira seafórtia

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Abstract

The seafortia palm tree (*Archontophoenix cunninghamii*) is originally from Australia and is widely used in ornamental gardens in Brazil. Due to the lack of information on the process of obtaining quality seedlings and the market demand for ornamental palms, the objective of this study was to use an x-ray test to evaluate the internal morphology of the seeds of *A. cunninghamii*. A total of 8 replicates of 25 seeds were submitted to x-ray analysis using the Raicenter RC 300 Plus T equipment; several radiation intensities and times were used. According to the internal morphology observed on the radiographs, the seeds were classified into 4 categories: perfect seeds, seeds with little damage (less than 50% of tissues damaged), seeds with substantial damage (more than 50% of tissues damaged), and embryos not visualized. After the radiography analysis, the seeds were submitted to a germination test for 160 days at a temperature of 30ºC using sphagnum as the substrate. Radiographic analysis at an intensity of 37 kVp and x-ray exposure for 0.25 s was sufficient to evaluate to the internal morphology of the seafortia seeds. The results obtained were as follows: 78% perfect seeds, 15% seeds with little damage, 4% seeds with substantial damage, and 3% seeds in which embryos were not observed. Seafortia palm seeds classified as perfect showed a higher percentage of germination, whereas those that had some level of damage tended to show a low physiological quality that was inimical to germination.
Key words: *Archontophoenix cunninghamii*, image analysis, germination, Australian royal palm

Resumo

A palmeira seafórtia (*Archontophoenix cunninghamii*) é originária da Austrália e é muito utilizada na ornamentação nos jardins brasileiros. Devido à carência de informações para o processo de produção de mudas de qualidade e que atendam às demandas do mercado de palmeiras ornamentais, objetivou-se com esta pesquisa utilizar o teste de raios X na avaliação da morfologia interna de sementes da palmeira *A. cunninghamii*. Foram utilizadas 08 repetições de 25 sementes submetidas ao teste de raios X no equipamento *Raicenter RC 300 Plus T* por vários tempos e intensidades de radiação. De acordo com a morfologia interna visualizada nas radiografias, as sementes foram classificadas em quatro categorias: sementes perfeitas, sementes com pequenos danos (menos de 50% dos tecidos danificados), sementes com danos substancias (mais de 50% dos tecidos danificados), e embrião não visualizado. Após serem radiografadas, as sementes foram submetidas ao teste de germinação pelo período de 160 dias na temperatura de 30 ºC com o substrato esfagno. A radiografia com intensidade de 37 kVp e exposição por 0,25 segundos aos raios X, foi o suficiente para a avaliação da morfologia interna das sementes de palmeira

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Recebido para publicação 07/06/11 Aprovado em 31/10/12

seafórtia. Os resultados obtidos foram: 78% sementes perfeitas, 15% sementes com pequenos danos, 4% sementes com danos substancias e 3% embrião não visualizado. Sementes de palmeira seafórtia, classificadas como perfeitas, apresentaram maior percentual de germinação, enquanto aquelas sementes que continham algum nível de dano, tenderam a apresentar menor qualidade fisiológica à germinação.
Palavras-chave: *Archontophoenix cunninghamii*, análise de imagens, germinação, palmeira-real-australiana

In Brazil, native palm trees have a great potential to be used as ornamental plants. However, exotic palm trees are presently the most widely disseminated in the country. The seafortia palm tree (*Archontophoenix cunninghamii* H. Wendl. & Drude) is an exotic species that has been successfully used to ornament Brazilian gardens. This palm is originally from Australia and produces a high-quality heart of palm. There are few studies on palm trees, probably because of the logistical difficulties, since they are tall perennial plants with long life cycles. Therefore, information on the physiology, anatomy, morphology, conservation, and seeds germination of the seafortia palm tree is needed in order to develop methods to improve the seed production (LUZ, 2008).

Palm trees mainly propagate through seed germination, which, in most species, is slow, irregular, and often low-yielding. In addition, the seeds rapidly lose viability when dehydrated. Studies need to be performed to provide information on the technology of propagation and cultivation of both native and exotic ornamental palm trees. Hence, identifying new ways to subsidize the development and facilitate the domestication, selection, cultivation, handling, and use of palms trees is necessary, as emphasized by Jardim and Cunha (1998).

The x-ray test, standardized by the International Seed Testing Association (ISTA, 1999) allows observation of the position, form, and deformations of the embryonic axis of seeds. Being a quick and non-destructive method of image analysis complementary to the germination test, the x-ray test can be used to detect filled, empty, and mechanically or insect-damaged seeds (BINO; AARTSE; van der BURG, 1993).

This technique has been used in seed research since the 1950s, when Simak and Gustafsson first used this test to evaluate the quality of *Pinus sylvestris* L seeds. Cícero et al. (1998) evaluated the mechanical damages in corn seeds by using radiographic analysis, and Sturião, Landgaf, and Rosa (2012) identified the internal morphology of the seeds of *Syagrus romanzoffiana* by using the x-ray test.

Image analysis provides information about digitized images on the basis of features such as color and texture. It is a non-destructive method and allows the visualization of seed germination and establishment of the relationships between internal mechanical damages or alterations and germination losses (CÍCERO et al., 1998; TONETTI; DAVIDE; SILVA, 2006).

The present study aimed to use the x-ray test to evaluate the internal morphology of the seeds of *A. cunninghamii*.

Seeds were collected from 20 seafortia palm trees from Alfenas, MG. Mature seeds were hand-collected depending on the fruit color and natural shedding from the matrix plant. The seeds were transferred to the Laboratory of Seed Analysis, School of Agronomy, University *José do Rosário Vellano* – UNIFENAS, for evaluation. The equipment for conducting the x-ray test was provided by the UNIFENAS Veterinary Hospital, School of Veterinary Medicine.

After harvest, the fruits were placed on trays and immersed in water for 24 h. The water was changed at every 3 hours to avoid the development of slime and growth of microorganisms. The immersion facilitated the extraction of the bark and seeds from the pulp by using a knife. The diaspores were rinsed in tap water and dried in shade for 2 days.

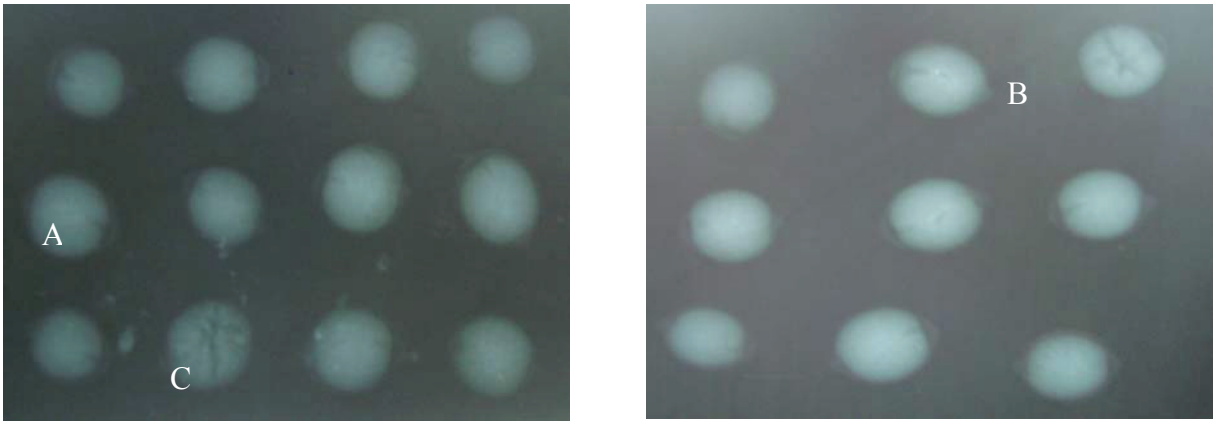
After natural drying, 5 samples containing 20 seeds each were placed in a chamber at 105 ± 2°C for 24 h to allow water content determination, according to the Seed Analysis Rules of the Brazilian Ministry of Agriculture.

Before performing the x-ray test, we conducted a preliminary test to select the radiation times and intensities necessary to visualize the internal morphology of the seeds on the basis of the results of previous studies on other species and respective visual radiographs of the seeds. The test conditions vary across different species. Eventually, 4 radiation intensities (15, 20, 25, and 30–40 kVp) at various radiation exposure times (0.1–10 s) were selected. The x-ray equipment, a Raicenter RC 300 Plus T machine, was used to analyze 8 replicates of 25 seeds on a 30 × 40 cm specific plaque; the radiographs were developed using MR 2000-1 Kodak film. The study was performed in a complete randomized design.

From the beginning of the x-ray test, the seeds were individually monitored and evaluated until the germination stage and the early seedling stage. This allowed a comparison of the internal morphology of the seeds observed in the radiographs with that after seed germination.

According to the internal morphology of the seeds as revealed on the radiographs, they were classified into 4 categories: perfect seeds (no damage); seeds with little damage (less than 50% of tissues damaged); seeds with substantial damage (more than 50% of tissues damaged); and seeds without an observable embryo (Figure 1). Subsequently, the seeds were submitted to the germination test and sown into 11 × 11 × 3 cm transparent plastic boxes with lids ("gerbox" type) containing sphagnum. The boxes were placed in a BOD-type germinator maintained at 25°C with humidity at the level of field capacity and photoperiod of 8 h light and 16 h dark (LUZ, 2008) to verify the results of the radiographic analysis of the seeds and determine their actual physiological behavior.

Figure 1. Low-resolution radiographs of *Archontophoenix cunninghamii* seeds illustrating the approximate model of the classification performed: A- Perfect Seeds; B- Seeds with little damage (less than 50% of tissues damaged); C- Seeds with substantial damage (more than 50% of tissues damaged); D- embryo not visualized. (Figure merely illustrative.)



Source: Elaboration of the authors.

After the seeds were germinated for a trial period of 160 days, the tetrazolium test was performed for the seeds that did not germinate in that period. The method used conformed to the Seed Analysis Rules of the Brazilian Ministry of Agriculture. For this, longitudinal sections of the seeds were immersed in tetrazolium solution (0.5%) at 30°C for 6 h in the dark to facilitate exposure of the embryos. Subsequently, the embryos were evaluated by digital photography to determine the seed color according to peroxide activity: living tissues were stained red.

The data collected from the evaluations and seed classifications were statistically analyzed using Sisvar software.

The seafortia palm tree seeds had a water content of 19%, favoring clear internal vision with the x-ray test, as was shown for *Cecropia pachystachya* (CÍCERO et al., 2008) and *Syagrus romanzoffiana* (STURIÃO; LANDGRAF; ROSA, 2012). A radiation exposure for 0.25 s at an intensity of 37 kVp was used as suggested by these authors on the basis of the regulation of the x-ray equipments according to ISTA (1999).

Table 1 presents the results of the radiographic analysis of the internal morphology and seed germination of the seafortia palm, as described by Bino, Aartse, and van der Burg (1993). The radiographic analysis revealed that 78% of the seeds were perfect and had no visible morphological deformation that could indicate problems with the germination process. Of the perfect seeds, 56% germinated and developed perfect seedlings without any anomalies, and 22% did not germinate. Luz (2008) conducted trails for determining the optimal conditions for seed germination and achieved 79.86% seed germination in *A. cunninghamii*, indicating that the potential for germination of palm seeds varies depending on the genotypic and phenotypic conditions.

Table 1. Results of the radiographic analysis of 200 seafortia palm tree seeds (*Archontophoenix cunninghamii*) and the test results of seed germination after passing through the x-ray test- Germination Percentage (G) and Non-Germination (NG) – and Viability NG Seeds through tetrazolium test.

Category	Mean units	Total	%	G (%)	NG (%)	Viability of NG
Perfect seeds	19.5 ± 2.77	156	78	56 ± 11.90	22 ± 5.65	0
Small damages	3.75 ± 2.91	30	15	09 ± 5.13	06 ± 8.00	0
Great damages	1.00 ± 1.60	08	04	02 ± 3.02	02 ± 3.70	0
Embryo not observed	0.75 ± 0.70	06	03	1.5 ± 2.07	1.5 ± 2.98	0
Total		200	100	68.50	31.5	0

Source: Elaboration of the authors.

Only 3% of the seeds evaluated developed embryos that could not be identified in the radiographs due to radiopacity, and half of these seeds germinated into normal seedlings. In addition, 19% of seeds had visible damage, with 15% having less damage and 50% having internal tissue damage. Even with this identification, 9% of the seeds with little damage germinated, whereas half of those with substantial damage also germinated and developed perfect seedlings. The identification process was as suggested by Cícero et al. (2008) for *Cecropia pachystachya* seeds, and by Sturião, Landgraf, and Rosa (2012) for *Syagrus romanzoffiana*. Oliveira, Carvalho, and Davide (2003) suggested that *Peltophorum dubium* seeds with little damage could germinate, but those with damage to over 50% of tissue could not. Tonetti, Davide, and Silva (2006) evaluated the physical and physiological qualities of *Eremanthus erythropappus* seeds and reported that the germination and development of normal

seedlings is maximized from the seeds classified as perfect.

This indicates that interpretation of the internal morphology of the seeds does not indicate an exact relationship with the physiological processes (CÍCERO et al., 2008), but such an analysis is potentially useful for determining the seeds classified as perfect that can result in more than 50% of germination and perfect seedlings. This suggests that the seeds with visible damage in the radiographs tend to have reduced germination capacity. Such an analysis is favorable for the sexual propagation of palm trees and other crops on a commercial scale, as identified for *Tecoma stans* by Sokolowski and Cicero (2008). Therefore, the radiographic analysis allows a quick diagnosis of seed quality in a non-destructive manner facilitating sexual propagation of palm trees (CARVALHO; OLIVEIRA, 2006), (STURIÃO; LANDGRAF; ROSA, 2012) without influencing the physiological quality of the seeds submitted to low radiation (BINO; AARTSE; van der BURG, 1993; CARVALHO, 2009), as was used in the present study.

Several authors have studied the morphology of seeds in different crops such as *Eugenia pleurantha* (MASETTO et al., 2007), *Zea mays* (CÍCERO et al., 1998; CÍCERO; MONDO, 2005), *Lithraea molleoides* (MACHADO; CICERO, 2003), and *Tabebuia* spp. (OLIVEIRA et al., 2004). Hence, physiological tests are necessary to complement the findings obtained using the x-ray test according to the specimen used (MACHADO; CICERO, 2003).

The tetrazolium test used for non-germinated (NG) seeds revealed that those seeds that were not in a good physiological condition were unable to germinate.

Radiographic analysis with a radiation intensity of 37 kVp and a 0.25-s exposure to x-ray radiation was sufficient for assessing the internal morphology of seafortia palm seeds as follows: 78% perfect seeds, 15% of seeds with little damage, 4% of seeds with substantial damage, and 3% of seeds in which embryos were not visualized.

Seafortia palm seeds classified as perfect had a higher percentage of germination, whereas those that had some level of damage tended to have a low physiological quality that was inimical to germination.

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