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Morpho-physiological characterization and germinative response of *Delonix regia* (Bojer) raf seeds. subjected to different pregerminative treatments

Caracterización morfo-fisiológica y respuesta germinativa de semillas de *Delonix regia* (Bojer) raf. sometidas a diferentes tratamientos pregerminativos

Caracterização morfofisiológica e resposta germinativa das sementes de *Delonix regia* (Bojer) raf. sujeitas a diferentes tratamentos pré-germinativos

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ABSTRACT

Delonix regia is a species with a worldwide importance due to its multiple properties and applications. It has low the germination due seed characteristics like testa permeability and presence of phenolic inhibitors, which inhibits the necessary flow of water and oxygen to enhance the process. The objective of this study was to characterize the morphology and germination response of *D. regia* seeds subjected to different pre-



germination treatments. Methodologically, a completely randomized experimental design with four replicates of 25 seeds each was applied. The treatments were: physical, mechanical and combined scarification. In this context, it was possible to establish the quality of seeds by indicating their biometric traits and colorimetric characterization, which are advantageous tools to be used as classification systems for the species, since the size and color of the most suitable seeds for obtaining seedlings have been determined. In the assessment of the morphophysiological quality of the seeds, the interpretation of viability tests based on patterns and damage detection, by destructive and non-destructive methods, was achieved. Of the pregerminative treatments considered in this research, the physical scarification method by immersing the seeds for 15 minutes in water at 100 °C, with cooling in water at room temperature, had a direct influence on most of the parameters analyzed to promote seedling emergence.

Keywords: Dormancy; Emergence; Germination; Seeds; Viability.

RESUMEN

Delonix regia es una especie que adquirió importancia mundial debido a sus múltiples propiedades y aplicaciones. Esta presenta baja germinación por características propias de la semilla como: permeabilidad de la testa y presencia de inhibidores fenólicos que impide el flujo necesario de agua y oxígeno para desencadenarla. El objetivo del trabajo consistió en caracterizar la morfología y la respuesta germinativa de semillas de *D. regia* sometidas a diferentes tratamientos pregerminativos. Metodológicamente se aplicó un diseño experimental completamente al azar con cuatro repeticiones de 25 semillas cada uno. Los tratamientos fueron: escarificado físico, mecánico y combinado. En este contexto, se logró establecer la calidad de semillas indicando sus rasgos biométricos y caracterización colorimétrica, que son herramientas ventajosas para utilizarlas como sistemas de clasificación para la especie, ya que se ha determinado el tamaño y el color de las semillas más idóneas para obtener plántulas. En la evaluación de la calidad morfofisiológica de las simientes, se logró la interpretación de pruebas de viabilidad basados en patrones y detección de daños, por métodos destructivos y no destructivos. De los tratamientos pregerminativos considerados en esta investigación, el método de escarificado físico mediante la inmersión de las semillas por 15 minutos en agua a 100 °C, con enfriamiento en agua a temperatura ambiente, ha influido de manera directa en la mayoría de los parámetros analizados para promover la emergencia de las plántulas.

Palabras clave: Dormancia; Emergencia; Germinación; Semillas; Viabilidad.

RESUMO

Delonix regia é uma espécie que adquiriu importância mundial devido a suas múltiplas propriedades e aplicações; mas apresenta deficiências na germinação de suas sementes; devido à variação no processo por suas características intrínsecas (problemas de permeabilidade do testa e presença de inibidores fenólicos), o que impede o fluxo necessário de água e oxigênio para desencadeá-lo. O objetivo deste estudo era caracterizar a morfologia e a resposta germinativa das sementes *D. regia* submetidas a diferentes tratamentos pré-germinativos. Metodologicamente, foi aplicado um projeto experimental completamente aleatório com quatro réplicas de 25 sementes cada. Os tratamentos foram: físicos, mecânicos e combinados de escarificação. Neste contexto,



foi possível estabelecer a qualidade das sementes indicando seus traços biométricos e caracterização colorimétrica, que são ferramentas vantajosas para uso como sistemas de classificação das espécies, uma vez que o tamanho e a cor das sementes mais adequadas para a obtenção das mudas foram determinados. Na avaliação da qualidade morfofisiológica das sementes, foi feita a interpretação dos testes de viabilidade baseados em padrões e detecção de danos por métodos destrutivos e não-destrutivos. Dos tratamentos pré-germinativos considerados nesta pesquisa, o método de escarificação física por imersão das sementes durante 15 minutos em água a 100 °C, com resfriamento em água à temperatura ambiente, teve uma influência direta na maioria dos parâmetros analisados para promover o surgimento de mudas.

Palavras-chave: Dormência; Emergência; Germinação; Sementes; Viabilidade.

INTRODUCTION

Delonix regia is a forest species from the native dry deciduous forest of Madagascar, where is declared threat of extinction (Du Puy *et al.*, 1995). This species has several uses around the world, among them are: ornamental and as a shade tree in streets and public spaces, medicinal for the presence of triterpene lupeol and sterol beta-sitosterol in the bark that can be use to treat rheumatism. The flowers contain flavonoids camferol, 3-O-beta-genobioside, 3-O-beta-cyanidin glucoside and quercetin, also present in the seed and are used for respiratory problems such as cough and bronchial asthma. Besides, it is a rich source of potentially useful natural antioxidants such as polyphenols and flavonoids (Tapia *et al.*, 2014).

In recent years, it was determined that the characteristics of the oil contained in its seeds show that it can potentially be used as a feedstock for the production of a renewable fuel such as biodiesel (Adejumo *et al.*, 2019).

Therefore, *D. regia* is a species that has acquired worldwide importance due to its properties. Thus, it is relevant to have an efficient protocol for obtaining plants, since its seeds need pregerminative treatments. Smith *et al.*, (2010) mentioned that this species presents dormancy by seed coat with viable embryo; this type is induced by one or more water impermeable layers of palisade cells in the seed or fruit coat.

On the other hand, the validation of biometric characteristics of fruits and seeds provides information on the variability of these characteristics among individuals in an established area. Also the classification of seeds by size to determine the physiological quality has been widely used in the reproduction of different plant species (Souto *et al.*, 2008).

The present study aims to establish a protocol to determine the quality of *D. regia* seeds through their morphological traits and germination response by evaluating different pregermination treatments to promote seedling emergence.



Radiography System®, Illinois, USA) and subjected to 18 kV exposures for 10 s. Subsequently, the plates were developed in a digital X-ray printer (Hope X-Ray processor; Micro-Max model 319®).

Pre-germinative treatments to promote emergence

(physical, mechanical and combined scarification).

The pre-germinative treatments applied to *D. regia* seeds are detailed below:

Physical scarification:

1. Immersion of the seeds in water at 100 °C allowing the water to cool naturally for 24 h.
2. Immersion of the seeds for 15 minutes in water at 100 °C, with cooling in water at room temperature.
3. Immersion of the seeds for 30 minutes in water at 100 °C, with cooling in water at room temperature.
4. Immersion of the seeds in water at room temperature for 24 h.

Mechanical scarifying:

1. Dry blend at low speed for 15 seconds.
2. Dry blend at low speed for 30 seconds.
3. With sandpaper No. 80 until the cotyledons are exposed.

Combined or mixed scarifying:

1. Immersion of the seeds in water at 100 °C for 24 hours allowing the water to cool naturally followed by cutting the seed coat with a scalpel.

Sowing:

The substrate used was sterilized sand contained in rectangular plastic trays measuring 43 x 37 x 17 cm in length, width and depth, respectively. The sowing depth was approximately 0.5 cm. The trays were kept in an incubation chamber under controlled conditions of light (PAR radiation, 160 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; photoperiod, 14 hours) and temperature ($27\pm 2^\circ\text{C}$).

Parameters evaluated:

The percentage of total emergence (% E), time to initiate emergence (TI), time to reach 50 % of total emergence (TE 50) and time between the occurrence of 10 and 90 % of total emergence (TE 10-90) were determined, all based on average values.

The experimental design was completely randomized with four replications of 25 seeds each. Emergence or germination was considered as the emergence and development of the seedling to a stage in which the appearance of its essential structures indicates whether or not it is capable of developing into a satisfactory plant under favorable



conditions in the field. Counts were made every two days after the initiation of the emergence process and were maintained until the values remained constant for 10 days.

Data were transformed ($y = \log_e x$) and statistically analyzed with Infostat software. Analysis of variance was performed by comparing the means of morphometric measurements of seeds from different pods ($n=50$) through Duncan's test ($p \leq 0.05$). For the color variable, the frequency distribution was analyzed.

RESULTS AND DISCUSSION

Biometric traits in seeds

In the morphometric characterization of the seeds analyzed, an average of 25.5 seeds per pod were counted. The batch of seeds subjected to biometric characterization showed 85.51 % of specimens with healthy or undamaged health status. While the variables width and length/width ratio showed no differences between pods, the length variable was statistically different (Table 1). Based on the high heterogeneity in the germination response and the effectiveness of mechanical scarification published by Quiroz Marchant *et al.*, (2009), it was decided to practice treatment seven, as a control, on a batch of seeds classified by size (taking as a reference the length in mm) in: large - 20 mm; medium - 15-20 mm and small - ≤ 15 mm (Table 1).

Table 1. - Morphometric characterization and germination percentage of *D. regia* seeds sorted by size and subjected to mechanical scarification (treatment seven-control)

N° of seeds/ pod	Health status (%)		Average length (L) mm	Width (A) mm	L/A
	With damage	No damage			
	14,48±9,82	85,51±9,82	18,67±1,01*	5,93±0,63	3,16±0,26
25,5±4,87	Categories (%)				
			large	medium	girls
			10,60±1,14	79±0,83	10,40±0,89
% germination			16±1,01	38±0,74	14±0,8

Values express the average of four replicates of 25 seeds \pm SD ($n= 100$).
*significant statistical differences ($p \leq 0.004$) for the variable Length.

Analyzing the germination percentages of the seeds according to their size classification, it can be observed that medium seeds (15-20 mm) showed a greater response to the pregermination treatment recommended for the species (Table 1).

Although no antecedents have been found in this regard for the species under study, there are precedents for other forest species where seed morphometry is a useful tool for classification (Fontana *et al.*, 2015). In this case, knowledge of the biometry or size of the seeds most suitable for obtaining plants (medium seeds of 15-20 mm), is a useful data either for the selection of sieves, machines for testa elimination, for planting and marketing. Either of the alternatives used in this study to determine biometric traits in seeds (caliper measurement or image analysis) are useful, the difference lies in the time required for each. Image analysis allows collecting data mechanically and quantifying



some of its variables in an agile way, but requires specific equipment; on the other hand, the use of calipers that have a digital screen where the exact measurement is reflected implies a more laborious task, therefore the technique is intended for high precision work.

Image analysis has been used by numerous researchers, as a tool for the morphological description of seeds focusing on biometric aspects such as length, width, area and shape (Espitia-Camacho *et al.*, 2018).

Indeed, the morphometric characterization of seeds offers an interesting tool for the determination of quality and/or homogenization of lots. Even in the agronomic context, the biometric characterization of seeds, particularly considering their size, constitutes an evaluation of their physiological suitability since, in the same lot, small seeds may have lower germination rate and vigor compared to those with medium or large size (Biruel *et al.*, 2010).

Colorimetric characterization of seeds

Table 2. - Colorimetric characterization of seeds with the Munsell color chart for soils to know their variability

Color	% de semillas
 10 YR 3/6 marrón amarillento oscuro	21,86 ± 0,24
 10YR 4/6 marrón amarillento oscuro	21,13 ± 0,27
 10YR 5/6 marrón amarillento	14,72 ± 0,18
 10YR 5/8 marrón amarillento	4,41 ± 0,66
 10YR 6/6 amarillo amarronado	26,96 ± 0,37
 7,5YR 3/4 marrón oscuro	8,46 ± 0,11
 7,5YR 4/4 marrón	0,50 ± 0,07
 7,5YR 4/6 marrón fuerte	0
 7,5YR 5/4 marrón	0
 7,5YR 5/6 marrón fuerte	0
 7,5YR 5/8 marrón fuerte	1 ± 0,1
 2,5Y 5/4 marrón oliva claro	1 ± 0,1
 2,5Y 5/6 marrón oliva claro	0
 2,5Y 6/8 amarillo oliva	0



Regarding the analysis of color and uniformity of the seed coat, 26.96 % of the seeds categorized for brownish yellow tone (10YR value 6, intensity or saturation 6); 21.86 % for dark yellowish brown (10YR value 3, intensity or saturation 6) both varying only by value not in hue or saturation; 21.13 % for dark yellowish brown (10YR value 4, intensity or saturation 6) and 14.72 for yellowish brown (10YR value 5, intensity or saturation 6) (Table 2).

There is a precedent of a colorimetric classification of *D. regia* seeds where they are described as light brown and mostly homogeneous (Fontana *et al.*, 2015), using the Royal Horticultural Society Color Chart.

In the present study, although another source of colorimetric characterization such as the Munsell color chart was used, most of the seeds have been categorized in a brownish-yellow and dark yellowish-brown tone, which makes it possible to choose homogeneous samples.

Colorimetric characterization for forest seed classification, has been recorded for different *Prosopis* species (Fontana *et al.*, 2015) especially the Munsell color chart; while the use of Royal Horticultural Society Color Chart is more used to classify different organs (flowers, flower stalks, epidermis, fruit pulp, etc.) (Bologna 2018).

Viability testing: viability testing by destructive and nondestructive methods

With the information obtained from the developed pattern, it was possible to determine the percentage of viability of the seeds studied for both techniques. This made it possible to reduce the time required to perform the tests, obtaining the results more quickly in comparison with germination tests, which in most cases require a long period of time to obtain similar results.

Referring to the pattern adapted for *D. regia* seeds by Porger and Luna (2018), viability percentages were determined for both destructive methods. With the tetrazolium test, 73.49 % of viable seeds were identified (Figure 1 and Table 3).

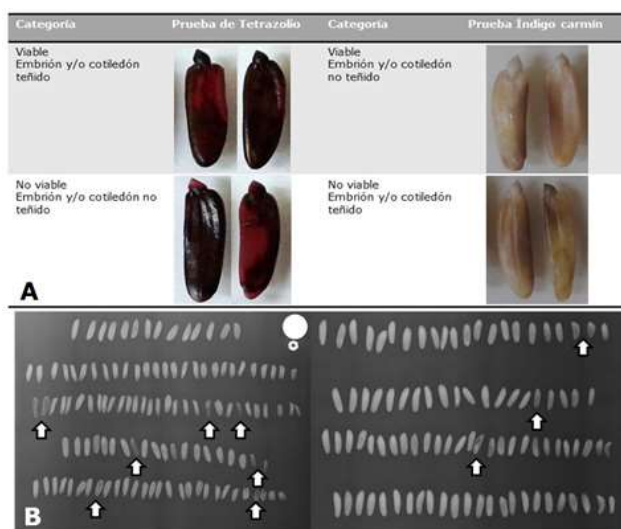


Figure 1. Destructive (A) and nondestructive (B) methods for viability determination. References: arrows indicate non-viable seeds on the radiographic plate.



Table 3. - Percent of viability of *D. regia* seeds by destructive methods: indigo carmine test (IC) and topographic test by tetrazolium (TZ) according to reference standard adapted from Porger and Luna (2018); and non-destructive: radiographic plates (RX). Values express the average of 4 replicates of 25 seeds \pm SD (n= 100)

Methods		Viable (%)	Not viable (%)
Non-destructive	RX	86,16 \pm 5,93	13,81 \pm 2,15
Destructive	TZ	73,49 \pm 1,60	26,50 \pm 1,37
	IC	51,21 \pm 0,83	48,78 \pm 1,73

The Tetrazolium test and the viability test with indigo carmine, have been successfully used for another Fabaceae, *Enterolobium contortisiliquum*, as reported by Porger and Luna (2018). They also concluded that the first is a rapid staining test that requires little time and few resources and allows obtaining viability values as reliable as direct germination tests; while the second, despite being a fairly widely used system, due to the ease of considering damage by staining dead zones, is not yet accepted by the International Seed Testing Association-ISTA (Álvarez *et al.*, 2020).

These tests have a strong load of subjectivity in the interpretation of the results, based mainly on the experience of the analyzer. This can be due to the lack of tonality or errors in the manipulation, being difficult to interpret the importance of zones that can be basic for the development of the embryo and later germination of the seeds. This ambiguity is less frequent in the case of indigo since the staining of dead zones clearly defines how the seed is found (Prieto *et al.*, 2011).

The studies for the detection of damage in this sample of seeds showed a high percentage of undamaged (viable) seeds with the X-ray technique: 86.16 % (Figure 1; Table 3) and with the Indigo carmine technique (51.21 %), which clearly defines the damage in the seeds (Table 3).

With the use of the X-ray technique, it was possible to verify the existence of a high percentage of undamaged seeds in the lot studied, which were considered viable. The technique allows a quick interpretation of some attributes of seed quality such as vigor, as well as making it possible to diagnose the existence of physical damage of different nature and degree of integrity of the different embryonic structures.

Through the observation of radiographic images it is possible to identify malformations of the embryo or nutritive tissues that may affect the viability of the seeds; insect attack can be observed, allowing to know the degree of hidden infestation and to identify insects that attack different forest species. Also, as the seeds of forest species are marketed by weight, and due to their high cost, it is important to determine the percentage of full and/or healthy seeds so that the user knows the amount to buy. This method has been previously accepted by ISTA as well as by the Official Seed Analysts Association, which have manuals of the technique for different species, and propose it as a valid alternative to the cutting test to detect empty and/or insect-damaged seeds (Salinas *et al.*, 2016).



The evaluation of the final classification of seeds with the confusion matrix is presented in Table 4. The results obtained show that the coincidence in both techniques that detect dead tissue is 71.68 % in seeds with damage, which is considered satisfactory since the importance of this categorization lies in the acceptance of deteriorated seeds in a conclusive and fast way, counting on a non-destructive technique.

Table 4. - Assessment of the final classification of the viability/damage determination system by destructive/non-destructive method on *D. regia* seeds (Viable/Non-viable) by confusion matrix

IC Classification		RX Classification		Coincidence (%)
		Viable	Not viable	
Viable	51,21	86,16	34,95	68,26
Not viable	48,78	13,81	34,97	71,68

Pregerminative treatment trials for emergence promotion

Table 4 shows the results of the different pre-germination treatments for the promotion of seed emergence of *D. regia*. On the final percentage (% E), the physical scarification applied with treatment two is the one that presented the highest values of emergence reaching 95 %, while, among the mechanical scarification treatments, the best result was the liquefaction at low speed for 30 seconds (treatment six) that registered 62 % of emergence. The different scarification treatments tested also affected the speed of the emergence process (Table 5): the onset of emergence (TI) was favored by treatment seven starting the process at 8 days and, as for the time required to reach 50 % of total emergence (E50), treatment two was the most favorable (12.33 days), coinciding with the highest percentage of total emergence. The only one of all the pregerminative treatments applied that accelerated the lapse between the occurrence of 10 and 90 % of seedling emergence (E10-90) was treatment two with 12 days.

Table 5. - Promotion of *D. regia* seed emergence

Scarified	Treatment	(% E)	IT (days)	TE 50 % (days)	TE 10-90 (days)
physical	1	25±1 c	11±1 c	-	-
	2	95±2 f	12,67±1,53 c	12,33±1,15 b	12±1 a
	3	62±2 e	18,67±1,53 d	19,67±0,58 d	-
	4	-	-	-	-
mechanic	5	56±1 d	17,33±0,58 d	18±1 c	-
	6	63±1 e	11±1 c	-	-
	7	7±1 b	8±1 b	-	-
combined	8	6,67±15 b	12±1 c	-	-



Percentage of total emergence (% E), time to initiate emergence (TI), time to reach 50 % of total emergence (TE 50) and time between the occurrence of 10 % and 90 % of total emergence (TE 10-90).

The findings of this trial demonstrate that the method of physical scarification by immersing the seeds for 15 minutes in water at 100 °C, with cooling in water at room temperature, has a direct influence on the final percentage of emergence and time to reach 50 % of total emergence, in addition to accelerating the time between the occurrence of 10 % and 90 % of seedling emergence.

The seeds of *D. regia* present a state of dormancy due to the hardness of their teguments; to increase the germination percentage it is necessary to break it using scarification methods (Tapia *et al.*, 2014). This type of dormancy is frequent in Fabaceae species, Rolston (1978) reported that, of 260 evaluated species of this family, approximately 85 % presented seeds with totally or partially impermeable teguments, which confer dormancy or dormancy, also pointing out that it is due to the presence of a layer of tegumentary epidermal cells in the form of a palisade associated with a waxy cuticular layer.

The use of physical scarification, such as exposure to hot water, has been found to be effective in breaking dormancy in several species and although it requires special care, it is economical, easy and safe to apply. Heat shock on seeds may be more effective than mechanical scarification for some species, but the optimums of temperature and soaking time are species-dependent and failure to determine them may have adverse results (Piroli *et al.*, 2005).

Although Tapia *et al.*, (2014) have presented a scarification protocol for *D. regia* that includes 2H₂SO₄ 98 % with which 60% emergence was achieved, seed sanding increased germination to 75 %. In the present work, these parameters have been improved without resorting to chemical scarification, although it has been effective in improving germination of countless species (Piroli *et al.*, 2005). Mechanical scarification has been used by rubbing the seed with sandpaper or cutting the testa with a razor in different legume species (Quiroz Marchant *et al.*, 2009). Ataíde *et al.*, (2013) obtained 71 % cumulative germination with combined scarification, speeding up emergence from the 6th to the 16th day; in this trial, the percentage of emergence was optimized in the same average time, but with a different pregermination treatment.

CONCLUSIONS

The morphology of *D. regia* seeds was characterized by biometric traits and colorimetric classification. Regarding the germination response of seeds subjected to different pre-germinative treatments, the most effective method was physical scarification by immersing the seeds for 15 minutes in water at 100 °C, with cooling in water at room temperature (two).



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The authors declare not to have any interest conflicts.

Authors' contribution:

Claudia Verónica Luna: Design, data collection, data analysis, conceptualization, writing, original draft, resource acquisition, logistics, project manager, supervision, writing, review and editing.

María Laura Fontana: Data analysis, review and editing.



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