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Morpho-physiological characterization of germination in *Senna siamea*[1]

Caracterização morfo-fisiológica da germinação de *Senna siamea*

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ABSTRACT - Establishing the appropriate procedures for conducting germination tests in forest species is important for marketing the seeds, especially species that are not yet included in the relevant Normative Instructions. The aim of this study was to determine the ideal conditions of temperature and substrate to carry out the germination test on seeds of *Senna siamea*, as well as: (i) to characterise the morpho-physiology of seedling establishment, (ii) the period for seedling evaluation, and (iii) validate the efficiency of the methodology in 10 batches of seeds. The experimental design was completely randomised in a 2 x 2 factorial scheme (temperatures x substrates), with 12 replications of 50 seeds in each treatment. To evaluate the different batches, the data were submitted to analysis of variance, and the mean values compared by the Scott-Knott test at 5% probability. The percentage and speed of germination, first count, seedling length, accumulated germination, and seedling morphology were evaluated. The germination potential of *S. siamea* seeds is greater on paper-roll substrate at temperatures of 25 °C and 30 °C (86% and 87%). The first germination count and final seedling evaluation should be carried out 15 and 21 days after sowing, respectively. The species has epigeal germination and the seedlings are phanerocotyledonous. It was found that the method for carrying out and evaluating the germination test recommended in this study is suitable for evaluating batches of *S. siamea* seeds.

Key words: Fabaceae. Seedling morphology. Physiological quality. Forest seeds. Germination test.

RESUMO - Estabelecer os procedimentos adequados para condução do teste de germinação em espécies florestais é importante para viabilizar a comercialização de lotes de sementes, sobretudo para espécies que ainda não constam nas Instruções Normativas. Objetivou-se com este estudo determinar as condições ideais de temperatura e substrato para a realização do teste de germinação de sementes de *Senna siamea*, bem como: (i) caracterizar a morfo-fisiologia do estabelecimento da plântula, (ii) o período para a avaliação das plântulas, e (iii) validar a eficiência da metodologia em 10 lotes de sementes. O delineamento utilizado foi inteiramente ao acaso em arranjo fatorial 2 x 2 (temperaturas x substratos) com 12 repetições de 50 sementes para cada tratamento. Para a avaliação dos diferentes lotes, os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Scott-Knott ao nível de 5% de probabilidade. Avaliou-se o percentual e a velocidade de germinação, primeira contagem, comprimento de plântulas, germinação acumulada, e morfologia de plântulas. Sementes de *S. siamea* possuem maior potencial germinativo em substrato rolo de papel sob temperaturas de 25 e 30 °C (86% e 87%). A primeira contagem de germinação e avaliação final de plântulas deve ser feita aos 15 e 21 dias após a semeadura, respectivamente. A espécie possui germinação do tipo epígea e suas plântulas são fanerocotiledonares. Observou-se que as condições de execução e avaliação do teste de germinação recomendadas neste estudo são adequadas na avaliação de lotes de sementes de *S. siamea*.

Palavras-chave: Fabaceae. Morfologia de plântulas. Qualidade fisiológica. Sementes florestais. Teste de germinação.

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INTRODUCTION

The Rules for Seed Analysis (BRASIL, 2009) and the Normative Instructions for the Seeds of Forest Species (BRASIL, 2013) do not yet include methods for evaluating seed viability in all forest species. The lack of standardisation in analysing some species makes it difficult to evaluate them in the laboratory and at other stages of the seed production process, especially where there are no established norms regarding test conditions. For this reason, it is necessary to evaluate methodologies for forest species of relevant economic and/or ecological interest.

Senna siamea (Lam.) H.S. Irwin & Barneby (Fabaceae) is one of the species for which there are no technical recommendations for carrying out or evaluating the germination test. Studies are therefore needed to establish the ideal conditions of temperature and substrate for germination, as well as the period for evaluating the seedlings. The species, popularly known as Siamese cassia, originated in Thailand, and occurs naturally in arid and semi-arid environments, and has the commercial potential for exploiting substances and nutrients for medicinal purposes and food (HASSAN *et al*., 2015). Determining the physiological quality of seed batches serves as a basis for the possible recommendation of biological material for the purposes of production and marketing, so that the selection of batches with high physiological potential reflects directly on the efficiency of production in the field, as well as on the end product (MOURA *et al*., 2016; YOKOMIZO *et al*., 2016).

In turn, germination is the principal test used to determine the percentage of germinable seeds in a sample under controlled laboratory conditions (BRASIL, 2009). During this process, viable embryos exit the resting state and resume metabolic activities until the seedling becomes established (CARVALHO; NAKAGAWA, 2012). The conditions for carrying out the germination test, such as water content, temperature, light, substrate and oxygen availability, directly affect the germination process (BENEDITO *et al*., 2019; CARVALHO *et al*., 2020; DINIZ; CHAMMA; NOVEMBRE, 2020; FELIX *et al*., 2017; MENDONÇA *et al*., 2014).

Among the regulatory factors in the process of resuming seed metabolism, temperature can affect the speed of water absorption and the biochemical reactions necessary for the resumption of embryo growth, as well as the speed and uniformity of germination and seedling establishment (MARCOS-FILHO, 2015). The optimal temperature range for germination of tropical forest seeds is between 25 °C and 35 °C (BENEDITO *et al*., 2019; CARVALHO *et al*., 2020; FELIX *et al*., 2020; OLIVEIRA *et al*., 2016).

The substrate, in turn, influences seed germination in line with its physical characteristics, affecting water potential and thermal conduction capacity (MARCOS-FILHO, 2015). The choice of substrate used in the germination test should take into account the requirements of the seed in relation to its size, amount of water, and sensitivity to light, in addition to offering ease for counting and evaluating the seedlings (BRASIL, 2009). The substrate must be easily accessible and readily available for transport, free from pathogens and weed propagules, as well as having suitable pH, texture and structure (BENEDITO *et al*., 2019; CARVALHO *et al*., 2020; FELIX *et al*., 2020; FELIX *et al*., 2017).

In order to achieve good performance in comparing the seed batches under evaluation, each of the above-mentioned aspects must be taken into account. Inadequate performance resulting from the incorrect installation of tests used to compare the viability of seed batches can lead to a loss of field productivity, as well as to batches of less than 80% viability being marketed (PIÑA-RODRIGUES; FIGLIOSA; SILVA, 2015).

The aim of this study, therefore, was to determine the ideal conditions of temperature and substrate for carrying out the germination test on seeds of *S. siamea*, as well as to: (i) characterise the morpho-physiology of seedling establishment, (ii) determine the best time for evaluating the seedlings, and (iii) validate the efficiency of the methodology in different seed batches.

MATERIAL AND METHODS

Location of the experiment and plant material

The batches of *S. siamea* seeds were obtained from fruit collected from 10 naturally occurring trees on the central campus of the Federal University of Rio Grande do Norte (UFRN) (5°50′20.3″ S and 35°12′3.49″ W), in Natal in the state of Rio Grande do Norte, Brazil. The trees were spaced at two and a half times their height. The seeds were extracted manually by shaking to detach them from the fruit. Visibly healthy and well-formed seeds were then selected. Each of the 10 batches included the seeds from each tree.

The moisture content of the seeds was previously determined in two samples of 4.5 g of seeds using the oven method at 105 ± 3 ºC for 24 h (BRASIL, 2009). Before the tests, the seeds were disinfected with 5% sodium hypochlorite (NaClO) for one minute, overcoming physical dormancy (impermeability to the entry of water) by the use of 80 sandpaper on the side opposite the micropyle (scarification).

The collected batches were sampled to make up one single batch for evaluating the ideal conditions of temperature and substrate.

Table 1 - Data adjustment and conversion functions proposed by Hara (1999), used in evaluating the accumulated germination curve in *Senna siamea*

Population parameters	Richards function $\gamma_{\pi} = \frac{\alpha}{\{1 + b \cdot d \cdot \exp(-c \cdot t)\}1/d}$		Logístic function $\gamma_{\pi} = \frac{\alpha}{1 + b \cdot \exp(-c \cdot t)}$
Viability (Vi)	$= \alpha$	$d = 1 \rightarrow$	α
Median (Me)	$= \cdot \frac{1}{c} \cdot \in \frac{b \cdot d}{2^d - 1}$		$\frac{Inb}{c}$
Dispersion (Qu)	$\frac{1}{2c} \cdot \in \frac{4^d - 1}{(4/3)d_{-1}}$		$\frac{3}{c}$
Asymmetry (Sk)	$2 \cdot$		0

Variables under analysis

The germination test was carried out in germination chambers set to a constant temperature of 25 °C and 30 °C under a photoperiod of 8 hours, using daylight fluorescent lamps (4 x 20W) and two substrates (paper towels and sand). Paper towels: the seeds were distributed over rolls made from three sheets of paper towels moistened with distilled water equivalent to 2.5 times the weight of the dry paper. Sand: the seeds were placed on sand (medium texture) in transparent acrylic boxes (11 x 11 x 3 cm), and moistened with distilled water to 60% of field capacity (BRASIL, 2009).

The following variables were analysed: (a) germination – percentage of normal seedlings obtained up to the 21st day after sowing; (b) germination speed - determined by means of the germination speed index (GSI), obtained from daily counts of the number of seeds that emitted a primary root (> 2 mm), and calculated as per the formula proposed by Maguire (1962); (c) first germination count – accumulated percentage of normal seedlings obtained up to the 15th day after the start of the germination test; (d) relative frequency of germination – obtained by counting the number of seeds germinated each day until the germination process stabilised; (e) cumulative germination – cumulative germination curve generated as proposed by Richards (1959), which characterises the change in biological behaviour following seed germination. The values obtained after calculating the Richards function were converted by the logistic function and adjusted for the population parameters (HARA, 1999) (Table 1).

The germination and morphology of the seedlings were characterised at the same time as the germination test. Seedlings that showed changes in tissue structure during the germination test were transferred to a sheet of Ethyl Vinyl Acetate (EVA). Based on the methodology proposed by Silva and Matos (1991), the seedlings were classified into three stages of development and morphological differentiation: I – swelling of the seed until root protrusion; II - emission of cotyledons, without the formation of eophylls; and III - normal seedling, following complete eophyll formation.

After determining germination and the most appropriate evaluation period, the germination-test methodology was assessed in 10 seed batches, based on germination, first count, speed of germination, length of the root and hypocotyl, and total length of the seedlings. The latter was carried out by selecting 10 normal seedlings in each batch from the germination test, using a ruler graduated in millimetres, with the results expressed in centimetres per seedling (cm.seedling^{-1}).

Experimental design and statistical analysis

The experimental design was completely randomised in a 2 x 2 factorial scheme (temperatures x substrates), with 12 replications of 50 seeds in each treatment. The data relating to germination were submitted to analysis of variance and the mean values compared by Tukey's test at 5% probability. To evaluate the different batches, the data were submitted to analysis of variance and the mean values compared by the Scott-Knott test at 5% probability. Multivariate principal component analysis was carried out and represented on biplots to verify the relationship between the different batches and the variables under evaluation. The R statistical software v. 3.5.0 was used (R CORE TEAM, 2018).

RESULTS AND DISCUSSION

The seeds of *S. siamea* expressed a moisture content of 13.1% to 14.1%. This variation is within the limits for comparing the physiological quality of seed batches (MARCOS-FILHO, 2015). The moisture content between the batches should be similar so that any differences in metabolic activity and in the intensity of seed deterioration do not alter the tests.

Thus, the water content of the seeds is linked to several aspects of their physiological quality, and it is essential that the water content be determined using official tests for comparing seed batches (SARMENTO, 2015). In this study, the moisture content of the *S. siamea* seeds showed low percentages. In orthodox seeds, a high water content can negatively affect physiological quality during storage, processing operations and laboratory evaluations (CARVALHO; NAKAGAWA, 2012).

The paper-roll substrate provided more-suitable conditions for germination and the expression of vigour in the seeds of *S. siamea*, with no difference in germination performance at the two temperatures under test (Table 2). The seeds on the sand substrate showed higher germination percentages at 25 °C, albeit lower than those found for the paper rolls.

Germination performance in forest species is one of the most important aspects of seed technology and seedling production. This process can be determined by a set of factors that vary depending on the species, as evaluated in seeds of *Campomanesia xanthocarpa* O. Berg and *Eugenia involucrata* DC. (Myrtaceae), which showed good germination performance on paper rolls (CARVALHO *et al*., 2020).

The reduction in seed germination on sand may have been due to the high drainage capacity of the substrate. This substrate may have promoted a lower water retention capacity, especially in the surface layer where the seed was located. As a result, it caused dehydration of this region of the substrate, reducing the water available for resuming embryo growth and for seedling development (PIÑA-RODRIGUES; FIGLIOSA; SILVA, 2015).

The water retention capacity of the substrate, together with the morphological characteristics of the seeds, can affect the percentage of germinated seeds. In this respect, the paper-roll substrate affords better distribution and maintenance of the moisture during the germination process. This was verified in this study, and helped maintain metabolic processes during germination and seedling development.

In seeds of *Simira gardneriana* M.R. Barbosa & Peixoto (Rubiaceae), water distribution on a paper-roll substrate during the germination process afforded better performance during the initial phase (root protrusion) and during seedling establishment, resulting in individuals of greater length (OLIVEIRA *et al*., 2016).

The speed of germination in *S. siamea* was greater at 30 °C on paper-roll substrate, and slower on sand at the same temperature (Table 2); at 25 °C, the paper rolls afforded greater speed of germination compared to the sand substrate. This is due to more water being available during the initial germination process, with reactivation of the metabolic processes and activation of reserve mobilising enzymes, which lower the nutrient reserves for root protrusion and seedling establishment (CARVALHO; NAKAGAWA, 2012).

The speed of germination or emission of the primary root depends on a series of soil and climate conditions that play a part in regulating the metabolism, in water absorption and in the biochemical reactions (BEWLEY *et al*., 2013; GODOI; TAKAKI, 2005). Germination only occurs within certain temperature limits, there being an optimal temperature for the process to occur with maximum efficiency and achieve maximum germination in the shortest possible time (CARVALHO; NAKAGAWA, 2012). Thus, temperature directly influences the speed of seed germination.

Table 2 - Viability and speed of germination in seeds of *Senna siamea* submitted to the germination test on different substrates and temperatures (25 °C and 30 °C)

---------- Germination (%) ----------			
		Temperature (°C)	
		25	30
Substrate	Paper roll	86 aA	87 aA
	Sand	48 aB	2 bB
Coefficient of variation		17.7%	
---------- Speed of germination (index) ----------			
		Temperature (°C)	
		25	30
Substrate	Paper roll	13.97 aA	14.93 aA
	Sand	12.65 aB	10.21 bB
Coefficient of variation		8.4%	

Mean values followed by the same lowercase letter in a row and uppercase letter in a column do not differ by Tukey's test ($p \leq 5\%$)

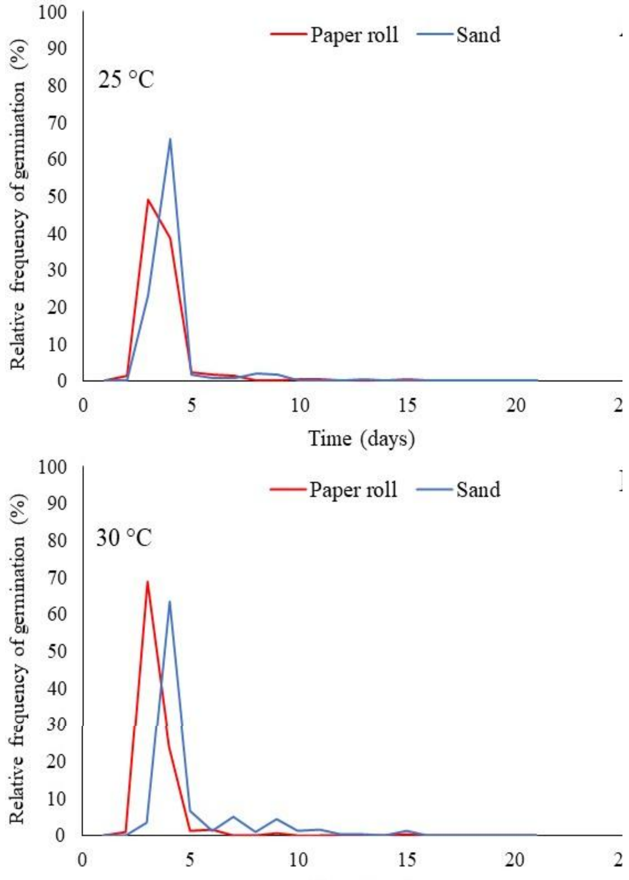
Root protrusion was established from 48 h after sowing on paper rolls, and after 72 h on sand. The maximum daily germination in seeds of *S. siamea* occurred on the third day on paper rolls (69%) and on the fourth day on sand (67%), both at 30 °C, with primary root emission stabilising 16 days after sowing (Figure 1).

The uniformity of normal seedlings, and the ability to establish themselves under uncontrolled conditions is due to their good performance in the speed of root protrusion. This can ensure the resilience of several plant species in seasonal areas and tropical forests that present different ranges of climate and temperature throughout the year.

When evaluating germination performance in seeds of *Leucaena leucocephala* (Lam.) de Wit. (Fabaceae), *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae), and *Caesalpinia ferrea* mart. ex tul. (Fabaceae), an average of 1 ± 3 days was seen for the start of root emission on paper roll at 25 °C and 30 °C (FONSECA; JACOBI, 2011).

Germination performance in seeds of *S. siamea* varied according to the temperature and substrate (Figure 2).

Figure 1 - Daily germination in seeds of *Senna siamea* under different temperatures and substrates: (A) paper roll and sand at 25 °C; (B) paper roll and sand at 30 °C



Source: The authors (2022)

The percentages shown in Figure 2 demonstrate the similarity in viability (primary root emission) between the treatments, with a percentage base performance from 87% to 89%. When evaluating 50% of the period for seed germination after sowing, it was noted that the treatments on the paper-roll substrate (25 °C and 30 °C), and on sand at 25 °C, presented a similar time range, in contrast to that found on sand at 30 °C, which took seven days to achieve 50% seed germination in *S. siamea*.

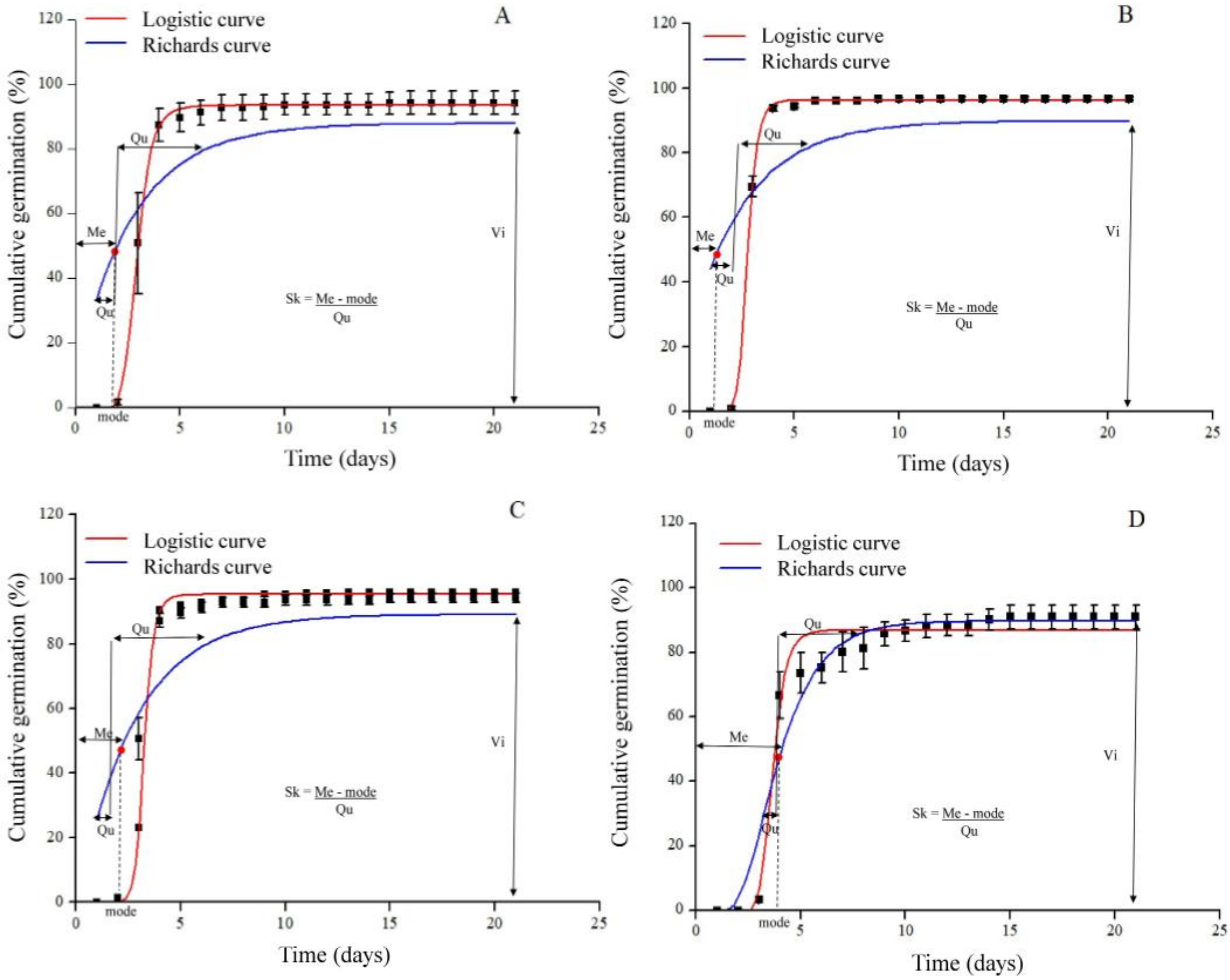
Although the performance of seeds under different conditions of temperature and substrate shows no variability in germination between any of the population parameters, in comparison with normal seedlings, it can be seen that biological development undergoes several modifications throughout the germination process. As such, verifying germination from the time of root emission will not establish a fully effective method for selecting suitable conditions or batches for sowing.

Adjusting the experimental parameter data using the Richard equation, and then converting by the logistic function, assumes tissue behaviour (germination kinetics) from modelling the curves, and demonstrates the impact of the conditions to which the seeds are exposed (SHAPIRA *et al*., 2017). In studies with seeds of *Erythrina velutina* Willd (Fabaceae), similar results were found using paper rolls, with viability rates of 70 ± 3, a median of 6.44 ± 0.62, dispersion of 2.81 ± 0.18, and asymmetry of 0.22 ± 0.02, and reaching 50% germination between 8 and 10 days after sowing (ALVES JUNIOR *et al*., 2016).

Germination in seeds of *S. siamea* was characterised as epigeal-phanerocotyledonous. The cotyledons were lifted above the surface of the substrate by elongation of the hypocotyl, and were freed from the seed coat (Figure 3f). The process of classifying the type of seedling has several typologies, such as cryptocotyledonous (cotyledons surrounded by the seed coat) and phanerocotyledonous (cotyledons free of the seed coat), that take the position of the cotyledons into account. This may be below ground (hypogean), above the surface (epigean) or at ground level (semi-hypogean) (SOUZA, 2009).

During stage I of germination (24 and 36 h), the seeds were already swollen, with visible detachment of the testa. After three to four days, the brownish-coloured root broke through the seed coat in the basal region of the seed, close to the hilum, (Figure 3b). Stage II was determined by the appearance of the cotyledons 10 days after sowing (Figure 3c). The cotyledons were opposed and equal, light-green and foliate, with a cylindrical curved hypocotyl of a light-green colour. During stage III, it was possible to see the formed eophylles from 21 days onwards (Figure 3e). At this stage, the basic structures that characterise a normal seedling were

Figure 2 - Cumulative germination in seeds of *Senna siamea*: A) paper roll at 25 °C; B) sand at 25 °C; C) paper roll at 30 °C; D) sand at 30 °C. Viability (Vi); median (Me); dispersion (Qu) and asymmetry (Sk)



Source: The authors (2022)

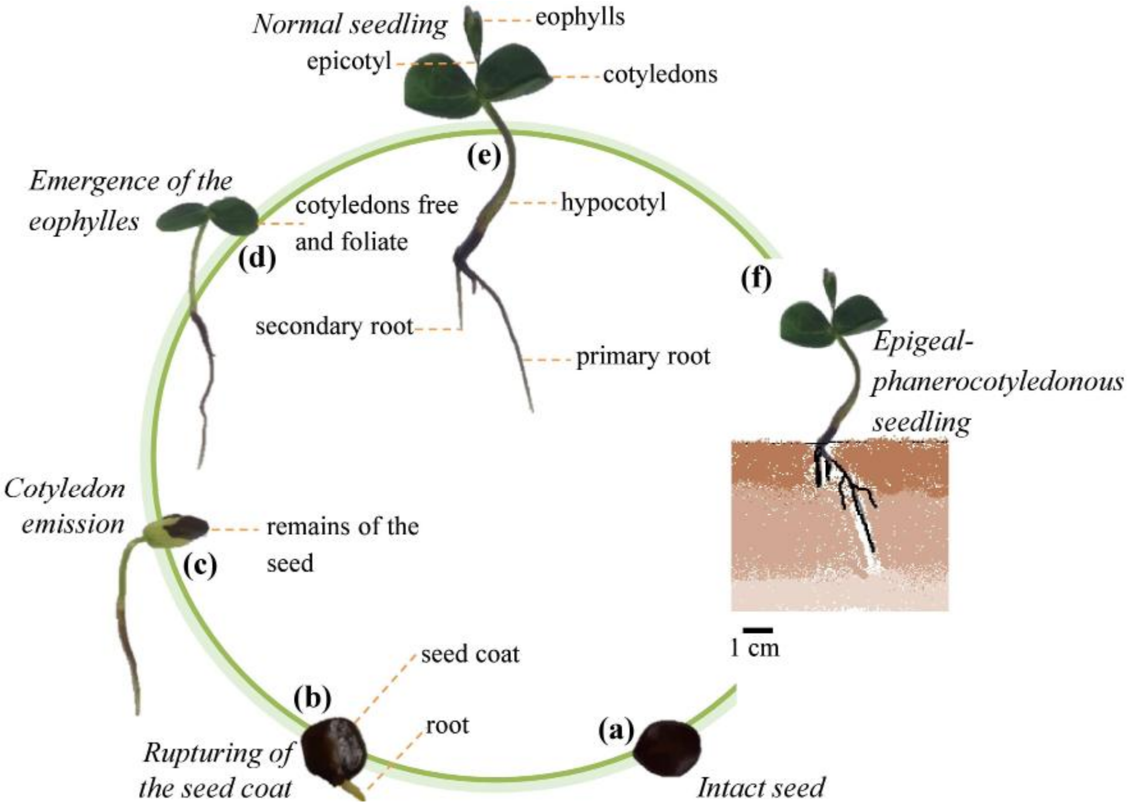
visible: well-developed primary and secondary roots, and an elongated hypocotyl curving away from the roots, dark green in colour.

The morphological characterization of germination in seeds of *S. Siamea* is similar to that found by Nogueira, Medeiros Filho and Gallão (2010) in *Dalbergia cearensis* Ducke (Fabaceae), by Braga, Oliveira and Souza (2013) in *Schizolobium amazonicum* Ducke (Fabaceae), and by Piveta *et al*. (2018) in *Senna macranthera* (DC. ex Collad.) H.S. Irwin & Barneby (Fabaceae) and *Senna multijuga* (Rich.) H.S. Irwin & Barneby (Fabaceae), who also characterised the germination of the above species as epigeal-phanerocotyledonous. A positive sign for the systematic characterization of species of family Fabaceae.

The morphological aspects seen during the germination process, daily germination analysis and cumulative germination showed that evaluating the first germination count is best done 15 days after sowing. During this period, the root, hypocotyl and epicotyl are seen to develop, with 50% seed germination in *S. siamea*. As for the final germination count, although, in each of the treatments the process of root emission may have stopped 16 days after sowing, it should be noted that it was only possible to see the essential structures (eophylls, epicotyl, cotyledons, hypocotyl, and primary and secondary roots) 21 days after sowing, guaranteeing permanence of the seedlings in the field.

Having determined the ideal conditions of temperature and substrate for conducting and evaluating the germination test, it was seen that batches 3, 4, 6

Figure 3 - Morphological aspects of seed germination and seedling establishment in seeds of *Senna siamea*: (a) seed not hydrated, (b) beginning of root protrusion (3 to 4 days), (c) emission of the cotyledons (10 days), (d) emergence of the eophylls (21 days), (e) normal seedling, and (f) characterization of an epigeal-phanerocotyledonous seedling



Source: The authors (2022)

and 9 afforded greater percentage germination and a greater germination speed index (Table 3). For the first germination count, the seed batches were divided into three classes. Batches 2, 3, 4 and 6 had greater percentage germination in the first count. Therefore, based on the final evaluation and the first germination count recommended in this study, it was possible to differentiate seed batches in terms of seed viability and vigour.

The *S. siamea* seedlings did not differ significantly in terms of hypocotyl length (5.5 to 6.5 cm), and only batch 10 differed from the others in terms of primary root length (1.5 to 3.7 cm), and batches 1, 3, 5 and 10 in relation to total seedling length (7.0 to 9.7 cm). The results when evaluating seedling length showed the batches divided into only two classes, which contributed little to distinguishing the seed batches.

The data set from the tests carried out to compare the 10 batches and six characteristics was used for the biplot of the multivariate principal component analysis (PCA) (Figure 4). The two components (Dim 1 and Dim 2) achieved a variability of 80% of the total value of the data, allowing the effects of the treatments to be projected for two components.

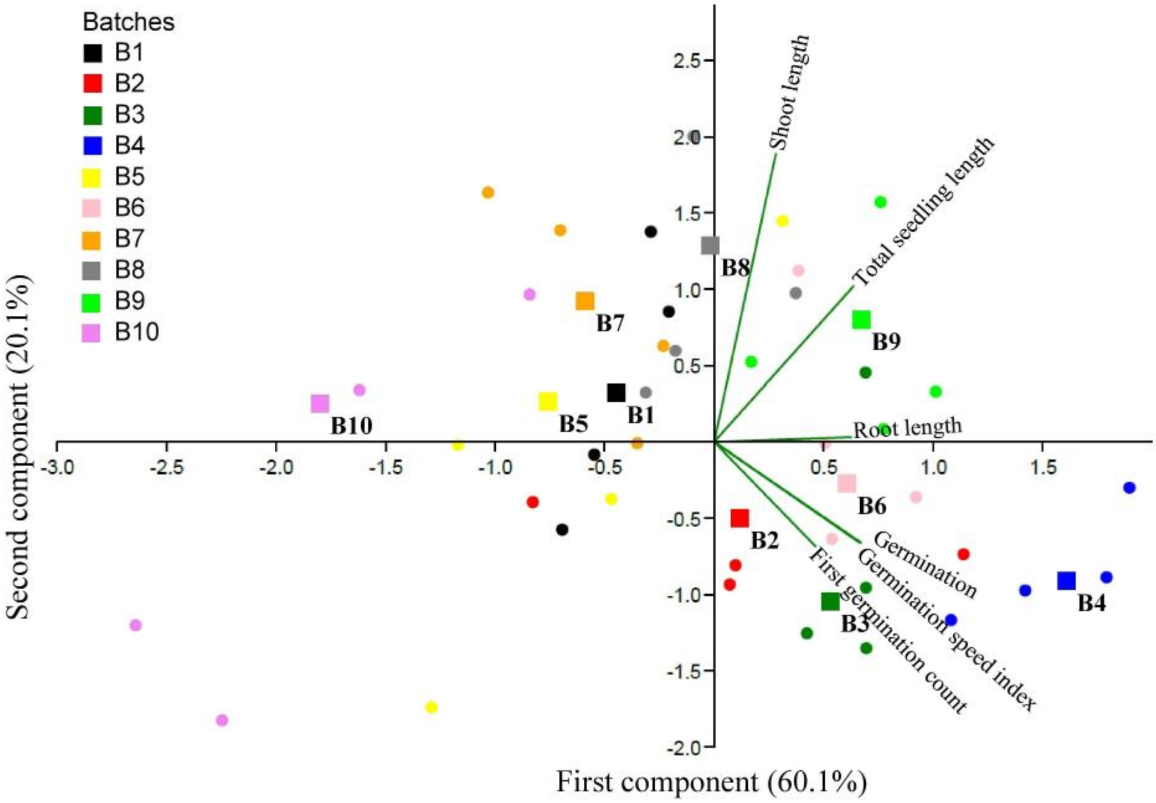
The germination performance of the seeds from batches 1, 5, 7 and 10 was inversely proportional to the other batches classified as of higher quality (4, 9 and 3). Genetic variability between the parent plants, and environmental factors may have contributed to the difference in quality between the seed batches. When evaluating the spatial distribution of the vectors, it can be seen that batches 2, 3, 4 and 6 resulted in more significant ordering of the vectors of each characteristic (germination speed index, first count and germination). Evaluating germination and the first germination count as described here is therefore important, and contributes as a recommendation of tests for assessing the physiological quality of *S. siamea* seeds. Finally, it is important to carry out future studies to establish ideal conditions for the germination test and seedling evaluation in forest seeds that are not included in the national and international instructions for seed analysis.

Table 3 - Germination (G), germination speed index (GSI), first germination count (FGC), root length (RAL), hypocotyl length (HYL) and total seedling length (TSL) in ten batches of *Senna siamea* seeds

Batches	G	GSI	FGC	RAL	HYL	TSL
	%	index	%	cm. seedling^{-1}		
1	60 d	9.88 d	60 b	2.7 a	6.5^{ns}	9.2 a
2	69 c	11.77 c	65 a	3.0 a	6.0^{ns}	9.0 a
3	80 b	13.19 b	77 a	2.7 a	6.2^{ns}	8.9 a
4	88 a	14.63 a	83 a	3.7 a	6.0^{ns}	9.7 a
5	59 d	10.02 d	54 b	2.5 a	6.0^{ns}	8.5 b
6	76 b	12.72 b	73 a	2.7 a	6.2^{ns}	8.9 a
7	56 d	8.60 d	48 c	3.0 a	6.5^{ns}	9.5 a
8	58 d	9.92 d	60 b	3.0 a	6.2^{ns}	9.2 a
9	78 b	13.5 b	43 c	3.2 a	6.2^{ns}	9.4 a
10	49 e	7.64 e	49 c	1.5 b	5.5^{ns}	7.0 b
CV (%)	8.05	7.75	13.85	9.20	8.40	7.07

Mean values followed by the same lowercase letter in a column do not differ by the Scott-Knott test ($p < 0.05$); *, ns = significant and non-significant by F-test ($p < 0.05$); CV = Coefficient of variation

Figure 4 - Biplot obtained by the linear combination of the variables evaluated in comparing the batches of *Senna siamea* seeds



Source: The authors (2022)

CONCLUSIONS

The germination test in seeds of *S. siamea* should be carried out on paper-roll substrate at a temperature of 25 °C or 30 °C. The first germination count and final seedling evaluation should be performed 15 and 21 days after sowing, respectively. The species has epigeal germination and the seedlings are phanerocotyledonous. The conditions for carrying out and evaluating the germination test recommended in this study can be considered suitable for evaluating batches of *S. siamea* seeds.

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