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SUBSTRATES AND TEMPERATURES FOR THE GERMINATION OF SEEDS OF *Senegalia tenuifolia* (L.) BRITTON & ROSE[1]

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ABSTRACT – The Rules for Seed Analysis and the Instructions for Seed Analysis of Forest Species have no recommendations for conducting the *S. tenuifolia* germination test. Therefore, the aim of the present study was to evaluate different temperatures and substrates to perform the germination test of *S. tenuifolia* seeds. The experimental design was completely randomized in a factorial 4 × 3 design and consisted of four substrates (paper roll; on paper; on sand and on vermiculite) and three temperatures (25, 30 and 35 °C), with four replicates of 25 seeds. The percentage of normal seedlings, the germination speed index, the shoot length, root length and dry mass of seedlings were evaluated. The Tukey test was used at 5% probability. There was a significant interaction between the temperatures and substrates tested for all variables, indicating that there is at least one ideal combination of the two factors that can increase the germination of seeds. The germination and vigor of *S. tenuifolia* seeds are influenced by the temperature and by the substrate used in the germination test. The combination of the paper roll substrates with a temperature of 25 and 30 °C was suitable for the germination of *S. tenuifolia* seeds.

Keywords: Caatinga. Fabaceae. Calumbi.

SUBSTRATOS E TEMPERATURAS PARA GERMINAÇÃO DE SEMENTES DE *Senegalia tenuifolia* (L.) BRITTON & ROSE

RESUMO – As Regras de Análise de Sementes e as Instruções para Análise de Sementes de Espécies Florestais não possuem recomendações para condução do teste de germinação da *S. tenuifolia*. Neste sentido, o presente trabalho foi desenvolvido com o objetivo de avaliar diferentes temperaturas e substratos para a condução do teste de germinação de sementes de *S. tenuifolia*. O delineamento experimental utilizado foi inteiramente casualizado, em esquema fatorial 4x3, sendo quatro substratos (rolo de papel, sobre papel, areia e vermiculita) e três temperaturas (25, 30 e 35 ºC), com quatro repetições de 25 sementes. Foram avaliadas a porcentagem de plântulas normais; índice de velocidade de germinação; comprimento da parte aérea; comprimento de raízes e massa de matéria seca de plântulas. Utilizou-se o teste de Tukey a 5% de probabilidade. Houve interação significativa entre as temperaturas e substratos testados para todas as variáveis, indicando que existe pelo menos uma combinação ideal entre os dois fatores, potencializando o processo germinativo das sementes. A germinação e o vigor de sementes de *S. tenuifolia* são influenciados tanto pela temperatura como pelo substrato utilizado no teste de germinação. A combinação dos substratos rolo de papel com as temperaturas de 25 e 30 ºC mostraram-se adequados para a condução do teste de germinação em sementes de *S. tenuifolia.*

Palavras-chave: Caatinga. Fabaceae. Calumbi.

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INTRODUCTION

Senegalia tenuifolia (L.) is widely distributed throughout South America and northeastern Brazil, especially in areas of arboreal Caatinga, 400–900 m above sea level. Throughout its range, *S. tenuifolia* is very variable in size (QUEIROZ, 2009) and is popularly known as “calumbi”, “calumbi-vermelho” (red calumbi) or “unha-de-gato” (cat’s claw).

Knowledge concerning seed germination and the factors that influence germination is useful to allow the species to be propagated more efficiently.

To conduct a germination test, the substrate and temperature are two of the most important factors (CARVALHO; NAKAGAWA, 2012). The substrate maintains appropriate conditions for germination and seedling development and its structure, aeration, water-holding capacity and degree of infestation of pathogens that might favor or inhibit germination is important (FIGLIOLIA et al., 1993).

The choice of substrate type depends on the seed requirements with respect to the amount of water, light sensitivity, and the ease with which the substrate allows the evaluation of counts and the assessment of seedlings, as well as on the availability and familiarity of the analyst with the material (BRASIL, 2009). In laboratory tests, the most frequently used substrates are paper rolls (towel, filter and blotter) and sand, although vermiculite is also often used in germination tests with forest species.

Another important factor that influences germination is temperature, which is directly related to the metabolic processes of the seed and affects the water absorption rate and biochemical reactions that influence the speed and uniformity of germination (MARTINS et al., 2008; PASSOS et al., 2008). Notably, most tropical species germinate at temperatures between 20 and 30°C (BORGES; RENA, 1993).

Studies involving the interaction between temperature and substrate produce different results among species of the Fabaceae, such as those found by Lima et al. (2006), for seeds of *Caesalpinia ferrea* Mart. ex Tul.; Guedes et al. (2010), with *Amburana cearensis* (Allemao) A.C. Smith.; Pacheco et al. (2010) with *Dimorphandra mollis* Benth.; Lima et al. (2011) for seeds of *Caesalpinia pyramidalis* Tul.; Azerêdo et al. (2011) with *Piptadenia moniliformis* Benth. and Nogueira et al. (2013) for *Mimosa caesalpiniifolia* Benth.

The Rules for Seed Analysis (BRASIL, 2009) and the Instructions for Seed Analysis of Forest Species (BRAZIL, 2013) do not contain recommendations for the germination of *S. tenuifolia*. Therefore, this study aimed to evaluate different temperatures and substrates for conducting seed germination tests with *S. tenuifolia*.

MATERIAL AND METHODS

Seeds of *S. tenuifolia* were obtained from dry fruits of different plants of the native vegetation from Serra de Santana, Florânia, RN (6°7'24"S, 36° 49'11"W and an altitude of 282 m). After the harvest, the seeds were extracted manually, cleaned, packaged in paper packaging and kept in a cold and dry chamber (10°C and 50% RH environment) until the experiment.

The experimental design was completely randomized, in a factorial 4 × 3 (substrates and temperatures) design, with four replicates of 25 seeds each.

At the start of the experiment, the seeds were topped and tailed with scissors by cutting at the opposite end to the hilum. Then, the seeds were sown on the substrates: washed sand, vermiculite, blotting paper (packed in transparent plastic boxes of the Gerbox type) or germitest paper towels (wrapped in transparent plastic bags to prevent moisture loss).

The paper towels and the blotting paper were moistened with distilled water with an amount equivalent to 2.5 times their dry weight; vermiculite and sand were moistened to 60% of their water-holding capacity.

The germination tests for each substrate were conducted in a Biochemical Oxygen Demand (BOD) germinator regulated at a constant temperature of 25, 30 or 35 °C, with a photoperiod of 8 h light and 16 h dark.

Counts were performed daily until the seventh day after sowing, with seeds considered to have germinated when they issued the primary root or apparently had a healthy seedling shoot (BRASIL, 2009). The results were expressed as a percentage.

The variables analyzed were: a) the percentage germination – expressed as a percentage of normal seedlings; b) germination speed index – performed simultaneously with the germination test; seedlings were assessed daily from the day that the first seedlings emerged, and the evaluations were performed until the seventh day after sowing and the index was calculated according to the equation proposed by Maguire (1962); c) length of the shoot and root – on the seventh day after sowing, all normal seedlings of the experimental unit had a root (measured from the base of the neck to the root end) and a shoot (measured from the base of the neck to the seedling apex); d) total dry mass of seedlings - all normal seedlings of the experimental unit were dried in a forced-air circulation oven at 65 °C to constant weight (48 h), and were subsequently weighed on a precision analytical balance (0.01 g).

The results were submitted to an analysis of variance using the F test, at 5% probability; in the case of significance, the Tukey test was applied at 5% probability with the aid of the statistical program SISVAR (FERREIRA, 2011).

RESULTS AND DISCUSSION

There was a significant interaction between the temperature and substrate tested for all variables (Table 1), indicating that at least one ideal combination of the two factors existed, to optimize the germination of seeds.

Table 1. Summary of analysis of variance for the variables germination (G), germination speed index (GSI), length of the shoot (LS), length of the root (LR) e total dry mass of seedlings (TDMS) in *S. tenuifolia* seeds, in relation to substrates (S) and temperatures (T).

Source of Variation	F values				
	G (%)	GSI	LS (cm)	LR (cm)	TDMS (mg)
S	27.39 **	11.41**	18.98**	200.22**	15.19**
T	6.4755 **	104.85**	57.82**	29.46**	17.38**
SxT	3.5276 **	5.01**	2.94*	8.81**	6.76**
Média	82.92	14.58	5.44	6.36	50.78
CV (%)	10.11	9.92	9.85	7.79	5.20

**, * Significant at 1% and 5% probability by F test, respectively.

The highest germination percentages were obtained at 25 and 30 °C, regardless of the tested substrate and low germination percentages were obtained at 35 °C in combination with the paper and vermiculite substrates (Table 2).

Table 2. Germination, germination speed index, length of the shoot, length of the root and Total dry mass of seedlings de *S. tenuifolia*, subjected to different temperatures and substrates.

Temperature (ºC)	Substrates			
	Germitest paper towels	Blotting paper	Vermiculite	Washed sand
Germination (%)				
25	86 aA*	90 aA	86 aA	94 aA
30	92 aA	85 aA	87 aA	94 aA
35	87 aA	54 bC	64 bBC	76 bAB
Germination speed index				
25	18.0 cA	11.9 bB	11.5 aB	11.9 abB
30	21.0 bA	12.5 abB	12.1 aB	13.2 aB
35	23.9 aA	14.7 aB	13.8 aB	10.6 bC
Length of the shoot (cm)				
25	5.4bA	3.1 bB	5.1 bA	5.7 bA
30	6.1abA	4.5 aB	6.8 aA	6.5 abA
35	6.4 aA	3.6 bC	5.3 bB	6.8 aA
Length of the root (cm)				
25	5.9 bB	7.0 aA	7.0 bA	5.2 bB
30	9.6 aA	7.2 aB	9.0 aA	6.9 aB
35	5.0 bA	4.5 bAB	5.2 cA	3.9 cB
Total dry mass of seedlings (mg)				
25	54.94 aA	54.47 aA	53.60 abA	51.59 aA
30	45.91 bBC	53.44 aA	50.68 bAB	44.69 bC
35	42.69 bC	52.45 aAB	56.54 aA	48.36 abB

*Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by the Tukey test at 5% probability.

The significant interaction between temperature and substrate can be explained by the water-holding capacity and the amount of light that the substrate allows to reach the seed, which causes different responses to the same temperature (FIGLIOLIA et al., 1993), as observed for the seeds of *S. tenuifolia*.

The higher germination percentage for the combination between temperatures of 25 and 30 ºC and the sand substrate (although there was no statistical difference among the other substrates) was possibly due to the water-holding capacity of the sand and the area of contact of the seed with this substrate; this provided a greater conservation in the amount and availability of water for the seed. In combination with the substrate, the lowest temperature (25 ºC) might have also caused a lower moisture loss.

In the germination test, the area of contact between the moistened substrate and the seed is important and can be critical for both total germination and the germination rate (ALVES et al., 2014).

A greater germination percentage of seeds was obtained for seeds of *Caesalpinia echinata* Lam., when the test was performed 25 ºC using paper roll as a substrate (Melo and Barbedo, 2007). For seeds of *Mimosa caesalpiniifolia* Benth., the greatest germination was obtained when germination was conducted on paper roll at a constant temperature of 30 or 25 ºC (Nogueira et al. 2013). Comparing constant (25 ºC) and alternating (20-30 ºC) temperatures, Scalon et al. (2007) obtained higher values for the germination percentage and speed index with a constant temperature for seeds of *Dimorphandra mollis* Benth. Lima et al. (2006) recommends a temperature of 30 ºC and sand as substrate for the rapid germination of seeds of *Caesalpinia ferrea* Mart., a finding that was confirmed by Souza et al. (2007) for *Adenanthera pavonina* L.

The highest germination speed indices were obtained using paper roll as a substrate, regardless of the temperature. Carvalho and Nakagawa (2012) found that the optimum temperature for the maximum germination percentage was different from that for the germination speed index, which was higher, i.e. a temperature of 25 °C or 30 °C was the best for the percentage of germination, whereas for the speed of germination, a temperature of 35 °C led to the highest index.

The superiority of the paper roll was found in germination tests conducted by Nogueira et al. (2013) for seeds of *Mimosa caesalpiniifolia* Benth., demonstrating that the germination speed is important and can indicate the superiority of a substrate, since the tests can be conducted in a shorter time.

For seeds of *Acacia mangium* Willd., Lima and Garcia (1996) observed that a paper roll substrate at 35 °C provided a high germination speed index, corroborating the results found in this experiment using *S. tenuifolia*. Guedes et al. (2011), evaluated the effect of different temperatures and substrates on the germination of *Myracrodruon urundeuva* Allemao, which showed higher germination speed indices at a temperature of 30 °C and paper roll as a substrate. These results differ from those of Pacheco et al. (2014), in which the germination speed index of *Combretum leprosum* Mart. was higher at 25 to 30 °C, and Guedes and Alves (2011) showed higher values with *Chorisia glaziovii* O. Kuntze. at 25 °C. These results show that the germination speed index varies in native species when different substrates and temperatures are used.

Vermiculite and paper roll substrates at a constant temperature of 30 °C resulted in the greatest radicle growth, possibly by providing better aeration and promoting root development. The longest shoots were obtained at higher temperatures (30 and 35 °C), on sand, paper roll and vermiculite substrates.

Similarly, Gonçalves et al. (2007) observed that the greatest length of the primary roots and shoots of seedlings of *Crataeva tapia* L.were obtained on the paper roll substrate. The high water-holding capacity and the suitable aeration conditions of vermiculite, coupled with its physical properties, such as thickness and texture, might have contributed to increased the radicle system of these seedlings compared to that on other substrates (AZERÊDO et al., 2011). Pacheco et al. (2006) demonstrated that the longest shoots of *Myracrodruon urundeuva* Allemão occurred on the substrates only at constant temperatures (30 and 35 ºC). However, Lima et al. (2011) found that there was no significant difference in the length of seedlings of *Caesalpinia pyramidalis* Tul. between different temperatures (25, 30, 20-30 and 20-35 ºC), regardless of the substrate.

Although the test conducted in the vermiculite substrate led to high mean values for the lengths of shoots and roots, it also led to a high degree of fungal contamination, regardless of the temperature. Fungal contamination was also high on all substrates at a temperature of 35 °C, which was also observed by Nogueira et al. (2013), who tested the effect of substrates and temperatures on the germination of *Mimosa caesalpiniifolia* Benth seeds.

The vermiculite and paper roll substrates led to higher total dry mass accumulation, regardless of the temperature. At a temperature of 25 °C, the dry mass accumulation was not affected by the substrate.

Guedes et al. (2010) found that a temperature of 35 °C favored the dry mass accumulation *of Amburana cearensis* (Allemao) A.C. Smith, on all substrates; however, for *C. pyramidalis* seedlings, Lima et al. (2011) found that alternating temperatures favored both the growth and the transfer of dry mass from the cotyledons to the embryonic axis.

According to Stockman et al. (2007), the temperature and the substrate are basic environmental factors of the germination test. Because seeds show variable physiological responses to different temperatures and substrates, the influence of these components on the germination process can improve the area of analysis of forest seeds.

CONCLUSIONS

The germination and vigor of *S. tenuifolia* seeds are influenced by the temperature and by the substrate used in the germination test.

The combination of the paper roll substrate with a temperature of 25 or 30 °C was suitable for the germination of *S. tenuifolia* seeds.

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