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Germination test of *Cordia trichotoma* seeds: a forest species native to Brazil

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ABSTRACT: *Cordia trichotoma* (Vell.) Arrab. *ex* Steud. is a forest species native to Brazil, naturally propagated by seeds, whose quality assessment may be underestimated by the use of inappropriate methods for conducting the germination test. Given the potential use of this plant and the importance of conserving native species, the present work aimed to study parameters for conducting the germination test in louro-pardo seeds, during three consecutive years of evaluations. For this purpose, temperatures (20, 25 and 30 °C) and substrates (blotter paper, filter paper, sand and vermiculite) were tested in seeds collected in different crop seasons. The tests were carried out under a completely randomized experimental design, with four replications, in a factorial scheme for the germination test (temperatures x substrates), with the data obtained being subjected to analysis of variance and means compared by Tukey's test ($p \leq 0.05$). Germination percentage and speed index were determined, and seed health analysis was performed. It is concluded that the germination test for louro-pardo seeds should be carried out between vermiculite, at 30 °C, without light supply, with the first count carried out at 26 days and the last count at 48 days after setting up the test.

Index terms: *Cordia trichotoma*, substrate, temperature, physiological quality.

RESUMO: *Cordia trichotoma* (Vell.) Arrab. *ex* Steud. é uma espécie florestal nativa do Brasil, propagada naturalmente por sementes, cuja avaliação da qualidade pode estar sendo subestimada pela utilização de métodos não adequados para condução do teste de germinação. Diante do potencial de utilização desta planta e da importância de se conservar espécies nativas, o presente trabalho teve por objetivo definir as condições mais adequadas para condução do teste de germinação em sementes de louro-pardo, durante três anos consecutivos de avaliações. Para tanto, foram testados: temperaturas (20, 25 e 30 °C) e substratos (papel mata-borrão, papel filtro, areia e vermiculita) em sementes coletadas em diferentes safras. Os testes foram excecutados sob delineamento experimental inteiramente casualizado, com quatro repetições, em esquema fatorial (temperaturas x substratos), sendo os dados obtidos submetidos à análise de variância e as médias comparadas pelo teste de Tukey ($p \leq 0,05$). Foram determinados a porcentagem e o índice de velocidade de germinação, além da análise de sanidade das sementes. Conclui-se que o teste de germinação para sementes de louro-pardo deve ser conduzido entre vermiculita, a 30 °C, sem fornecimento de luz, sendo a primeira contagem realizada aos 26 dias e a última aos 48 dias após a instalação do teste.

Termos para indexação: Louro-pardo, substrato, temperatura, qualidade fisiológica.

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INTRODUCTION

Louro-pardo (*Cordia trichotoma* (Vell.) Arráb. *ex* Steud.) is a forest species of the Boraginaceae family, native to Brazil, with wide geographical distribution, having natural occurrence from Ceará to Rio Grande do Sul (Carvalho, 2002). With relatively fast growth, its wood acquires commercial value due to its good physical and mechanical properties (Mantovani et al., 2001), reaching commercial stem height similar to those of other native species, with good yield (Roman et al., 2009), considered noble and suitable for laminates and exquisite furniture (Grings and Brack, 2011). In addition, the trees have ornamental and landscape value and can be used in urban afforestation and in the recovery of degraded areas (Grings and Brack, 2011).

The propagation of louro-pardo occurs naturally by seeds (Mantovani et al., 2001), and the dispersal unit is the perianth, composed of fruit and seed, since the seed is attached to the fruit wall by the base of stigma (Carvalho, 2002). Native forest species usually have a low germination percentage, with few species reaching 80% (Wielewicki et al., 2006); however, the quality assessment may be underestimated using inappropriate methods to conduct the germination test, since the species under study does not have dormancy (Vaz et al., 2015).

For forest species, the Ministry of Agriculture, Livestock and Food Supply published the Instructions for Analysis of Seeds of Forest Species (Brasil, 2013), which contains recommendations for conducting the germination test for 319 native and exotic species, of which only 50 had their methodology validated, and louro-pardo is not included.

Among the components of the germination test, temperature and substrate stand out. Germination is driven by enzymatic systems that control seed metabolism and require an adequate temperature. The ideal temperature for the species is the one that promotes the highest germination of seeds in a shorter period (Marcos-Filho, 2016). The ideal substrate is defined based on its capacity to supply water to the seed, control the dispersion of pathogens and aeration, also considering the size of seeds and aspects of their morphology (Brasil, 2009a, Marcos-Filho, 2016). Studies conducted by Felippi et al. (2012) with louro-pardo, using the temperature of 25 °C and paper roll substrate, were not conclusive, and it is necessary to establish more specific criteria for conducting the germination test with seeds of this species.

Thus, there is a need for studies to define the most appropriate methodology for the germination test of louro-pardo seeds, aiming to standardize the evaluations in the laboratory and support new production techniques, thus boosting the use and commercialization of seeds of this native species. In view of the above, the present study aimed to assess parameters for conducting the germination test in louro-pardo seeds, during three consecutive years of evaluations.

MATERIAL AND METHODS

Obtaining seeds

The experiments were conducted for three consecutive years, using material collected from louro-pardo plants (Figure 1a) located in the municipality of Ribeira, São Paulo, Brazil (24°39'25" S and 49°00'32" W) (Figure 2).

In the months of July of each year studied, when the beginning of the natural dispersal of seeds in the region was observed, branches containing the dispersal units (Figure 1b), that is, the perianth (Figure 1c), composed of fruit and seed, were collected (Souza, 2008).

Initially, the perianths were extracted from the branches with subsequent removal of petals, leaving only what is considered the seed of louro-pardo (calyx + fruit + seed) (Figure 1d). Samples were homogenized by manual method (Brasil, 2009a) and divided into four subsamples, which represented statistical replications. During the experimental period, the seeds were stored in Kraft paper bags in a controlled environment (temperature 16 ± 2 °C and 50 - 60% relative humidity), to minimize deterioration.

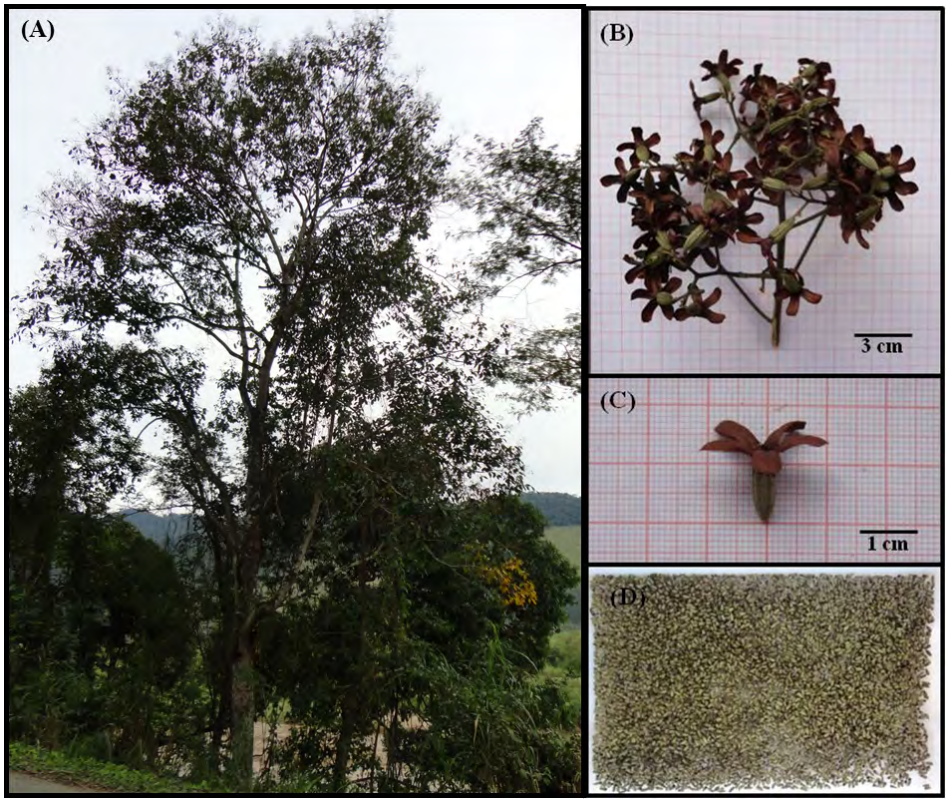

Figure 1. Details of the species *Cordia trichotoma*: (A) tree; (B) branches with dispersal units; (C) perianth (fruit + seed); (D) seeds (calyx + fruit + seed).

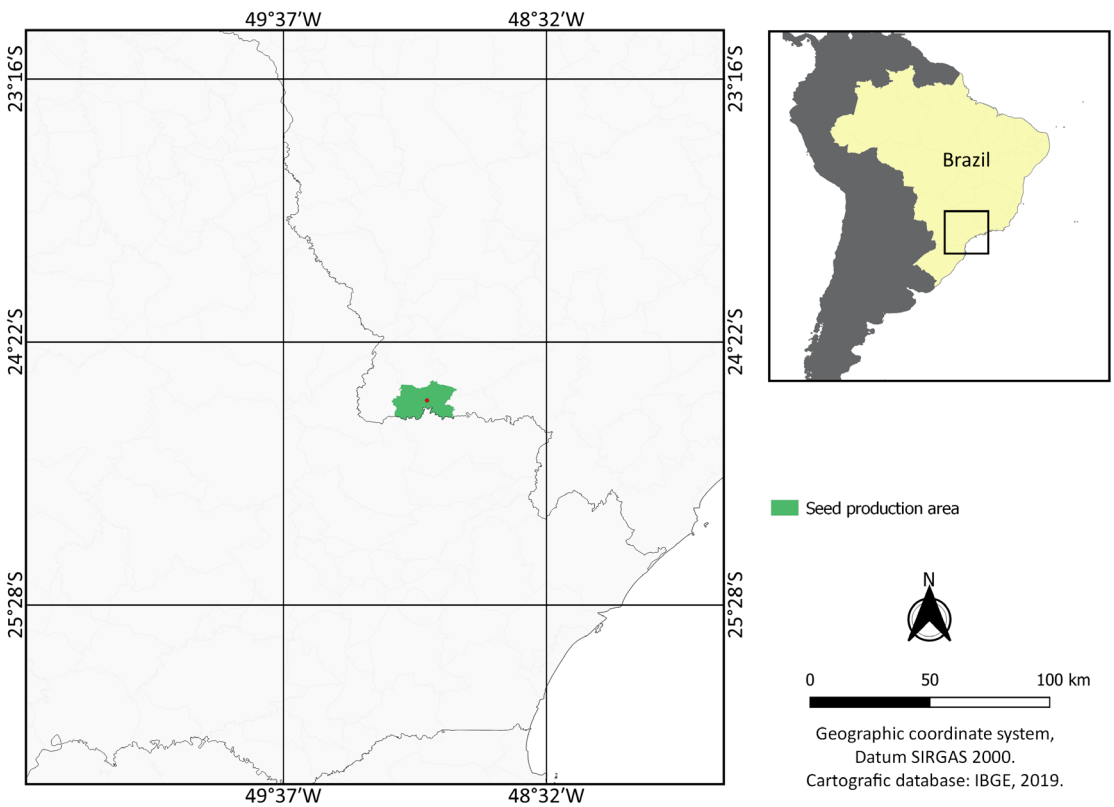

Figure 2. Production site of the *Cordia trichotoma* seeds used in the experiment.

Determination of seed moisture content

Seed moisture content was determined before germination studies, using the oven method at 103 ± 2 °C for 17 hours (Brasil, 2009a), with two replications of 5 g. Results were expressed as a percentage.

Germination test study

This study was conducted in three stages, represented by three consecutive years of seed collection, and the tested methodology was defined according to the results obtained in each stage and to the number of seeds available. Before conducting the experiment, the seeds were disinfested by immersion in 1% hypochlorite for three minutes, washed in distilled water and dried on paper towel for 30 minutes, under laboratory ambient conditions (temperature 20 ± 3 °C and 50 - 70% relative humidity).

First year of seed collection

Germination test was conducted on blotter paper, between medium-size sand and in paper roll. The tests using blotter paper and sand had four replications, each with two subsamples of 25 seeds, sown inside transparent plastic boxes (11.0 x 11.0 x 3.5 cm). The paper roll test was conducted using four replications of 50 seeds, sown in a roll of paper towel (Germilab). For sowing on paper, moistening was performed with water in a volume equivalent to 2.5 times the mass of the dry substrate, whereas in sand, sowing was carried out on the substrate previously sterilized in autoclave (at 1 atm and 120 °C for 1 hour), with moisture at field capacity.

The seeds were placed in Mangelsdorf germinator, and three germination temperatures (20, 25 and 30 °C) were tested, without light supply for all substrates tested.

Second and third years of seed collection

The seeds were sown in a paper roll, between sand and between vermiculite, as described above, using four replications of 50 seeds for paper roll and four replications (each with 2 subsamples of 25 seeds) for plastic boxes. The temperatures of 25 and 30 °C were tested in germinator without light supply. Vermiculite showed medium particle size and was subjected to the same sterilization and moistening treatment as the sand substrate previously described for the first year of collection.

Counts were performed periodically for all treatments tested, characterizing normal and abnormal seedlings, from the observation of the first normal seedling, until the counts became constant, when the germination percentage was calculated and the dates for the first and last counts of the test were defined. Germination speed index – GSI was also determined (Maguire, 1962).

The test for the third year of collection was conducted similarly to that of the second year, aiming to confirm the data obtained in the previous year and obtain greater consistency of the results.

Seed health analysis

Seed health was evaluated by the blotter test method with incubation under white fluorescent light lamps, with 10 replications of 20 seeds distributed on moistened blotter paper. Evaluation was performed in seeds with and without the floral envelope, which were kept in a chamber with photoperiod of 12 hours for 7 days, at 20 °C (Brasil, 2009b) and at 30 °C.

After incubation, the occurrence of fungi was observed with the aid of a stereoscopic microscope, analyzing the seeds individually and identifying fructifications typical of fungal occurrence (Brasil, 2009b). When some fungal structure was found, it was transferred to slides with lactophenol dye with methylene blue and analyzed using an optical microscope to identify the species. The results were expressed as an average percentage of fungal incidence.

Data analysis

The experimental design was completely randomized, with four replications, in a factorial scheme for the

germination test (temperatures x substrates). The data obtained were subjected to analysis of variance and the means were compared by Tukey test ($p \leq 0.05$). The percentage of incidence of pathogens was not statistically analyzed.

RESULTS AND DISCUSSION

Germination percentages and germination speed index (GSI) of louro-pardo seeds obtained in the first year of the germination test study are presented in Table 1. Statistical differences were found between treatments, and the temperature of 20 °C, without light supply, in general led to lower germination percentage and speed in all substrates tested.

Considering the other temperatures tested (25 °C and 30 °C), the substrate between sand promoted better results of germination (G) than paper substrates (Table 1). In terms of germination speed (GSI), the paper roll substrate stood out, followed by between sand (at 30 °C). The superior performance of louro-pardo seeds at higher temperatures can be expected, because it is a tropical forest species, whose group usually has the interval from 25 to 30 °C as optimum temperature for germination (Brancalion et al., 2010).

It is worth pointing out that the germination test conducted on paper substrate, despite having promoted germination results close to those obtained with the other substrates, was more complicated for routine analysis in seed laboratories, since the substrate dried quickly, so it was necessary to remoisten it every day; in addition, considerable fungal contamination was observed.

In the second year of evaluation, the substrates paper roll and between sand were maintained and the vermiculite substrate was added (Table 2), choosing to work without the light supply due to the results obtained in the first year of seed collection, and also because, in routine laboratory analysis, the germination test conducted without the need for artificial light allows the use of less sophisticated equipment.

It can be verified that the substrate between vermiculite stood out from the others for the evaluated species (Table 2), since it promoted results of higher germination percentage and speed, corroborating the data obtained for seeds of other native forest species, such as: *Sebastiania brasiliensis* - Branquilho-leiteiro (Bassaco et al., 2014), *Amburana cearensis* -

Table 1. Germination percentage and germination speed index of *Cordia trichotoma* seeds under different temperatures and substrates without light supply in the first year of collection.

Germination (without light supply) (%)			
Temperatures	Substrates		
	Paper roll	On paper	Between sand
20 °C	45 bB	56 aA	48 cAB
25 °C	56 aB	64 aAB	70 aA
30 °C	34 cB	37 bB	58 bA
CV (%)		10.19	
Germination speed index (without light supply)			
Temperatures	Substrates		
	Paper roll	On paper	Between sand
20 °C	0.805 cA	0.423 bB	0.453 bB
25 °C	2.058 aA	0.916 aB	1.188 aB
30 °C	1.244 bA	0.522 bB	1.259 aA
CV (%)		17.50	

Means followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other by Tukey test ($p \leq 0.05$). CV: coefficient of variation.

Cumaru (Guedes et al., 2010) and *Stryphnodendron adstringens* - Barbatimão (Martins et al., 2011).

The following fungi were identified in the louro-pardo seeds (Table 3) by the Filter Paper method: *Aspergillus* sp., found in higher percentage (42%) in seeds with floral envelope, at 20 °C, and *Colletrotrichum* sp. (28%) and *Rhizopus* sp. (17%), at 20 °C, in seeds without floral envelope. The presence of sterile mycelium, thus classified for not producing reproductive structures for identification, was also observed under all tested conditions.

Low percentages of *Fusarium* sp. were found (less than 10%), regardless of the treatment used (Table 3), different from that found in a study conducted by Berghetti et al. (2015), with seeds collected in Santa Maria-RS, where the presence of this fungus was observed (78.1%), with indications that it may have negative effects on the germination of the forest species for causing lodging.

In the last stage of the study (Table 4), the results obtained in the second year of seed collection were confirmed, indicating that the best combination for evaluating the viability (germination) and vigor (GSI) of louro-pardo seeds was the substrate between vermiculite and temperature of 30 °C.

Vermiculite was included in the Instructions for Analysis of Seeds of Forest Species (Brasil, 2013) as an option for conducting the standard germination test. It is a substrate commonly used in the production of forest seedlings and has several advantages, such as: uniformity in chemical and particle-size composition, porosity, water retention capacity

Table 2. Germination percentage and germination speed index of *Cordia trichotoma* seeds, under different temperatures and substrates without light supply, in the second year of collection.

Germination (without light supply) (%)			
Temperatures	Substrates		
	Paper roll	Between vermiculite	Between sand
25 °C	17 aB	32 aA	11 aB
30 °C	9 bB	34 aA	9 aB
CV (%)		17.39	
Germination speed index (without light supply)			
Temperatures	Substrates		
	Paper roll	Between vermiculite	Between sand
25 °C	0.328 aA	0.301 bA	0.074 aB
30 °C	0.250 aB	0.429 aA	0.132 aC
CV (%)		22.17	

Means followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other by Tukey test ($p \leq 0.05$). CV: coefficient of variation.

Table 3. Average percentage of pathogen incidence in *Cordia trichotoma* seeds, with and without floral envelope, by the Filter Paper method at 20 and 30 °C, in the second year of collection.

Pathogen	With floral envelope (%)		Without floral envelope (%)	
	20 °C	30 °C	20 °C	30 °C
Aspergillus sp.	42	7	23	8
Rhizopus sp.	3	17	17	9
Colletrotrichum sp.	5	1	28	11
Fusarium sp.	2	4	7	8
Sterile mycelium	100	100	100	100

and low density, besides being easily obtained (Martins et al., 2009).

The percentages of normal seedlings obtained each day of evaluation, using the vermiculite substrate and the temperature of 30 °C, are presented in Figure 3, for the last two years of seed collection.

The objectives of defining the periods indicated for the first and last counts are, respectively, to prevent seedlings already considered normal from interfering in the growth of other seedlings and to determine the maximum germination of the seed lot (Brasil, 2009a). To establish the day of the first count, the day with the highest germination peak (period with the highest percentage of normal seedlings) was evaluated.

It was verified that most of the seeds germinated up to 26 days after setting up the test and there was no formation of seedlings from the 48th day, that is, the first and last counts of the germination test should be performed at 26

Table 4. Germination and germination speed index of *Cordia trichotoma* seeds, under different temperatures and substrates, without light supply, in the third year of collection.

Germination (%)			
Temperatures	Substrates		
	Paper roll	Between vermiculite	Between sand
25 °C	3 aB	34 bA	20 aA
30 °C	5 aB	56 aA	13 aB
CV (%)		39.52	
Germination speed index			
Temperatures	Substrates		
	Paper roll	Between vermiculite	Between sand
25 °C	0.072 aB	0.329 bA	0.204 aAB
30 °C	0.054 aB	0.534 aA	0.131 aB
CV (%)		22.17	

Means followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other by Tukey test ($p \leq 0.05$). CV: coefficient of variation.

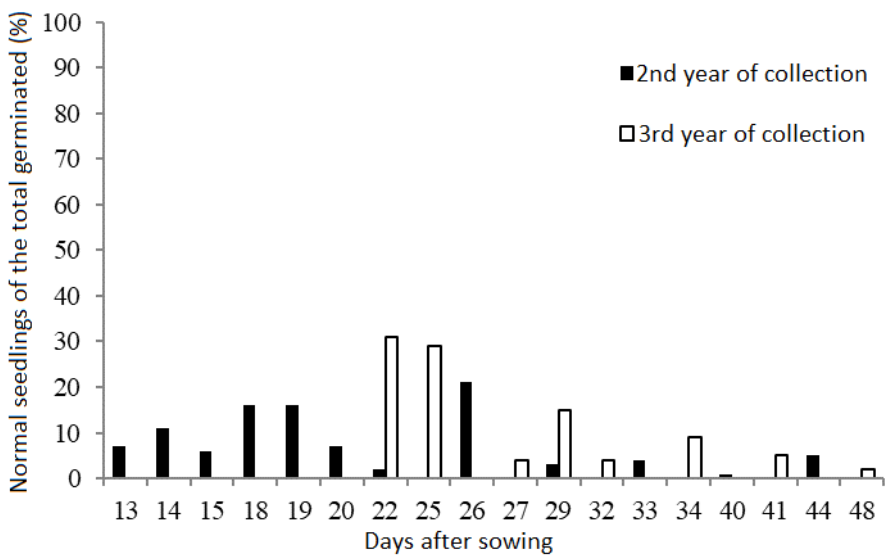

Figure 3. Percentage of normal seedlings obtained each day of evaluation of *Cordia trichotoma* at 30 °C, in the substrate between vermiculite, in the second and third years of collection.

Figure 4. Normal seedlings of *Cordia trichotoma*.

and 48 days after sowing, respectively. These results for the first and last count of the germination test are similar to those obtained by Felippi et al. (2012), who indicated 24 and 46 days after sowing, respectively. The small variation between the results of both studies can be explained by the germination criterion considered by the authors during the evaluation. Felippi et al. (2012) considered as germinated seeds those with the emergence of the primary root, with at least 2 mm, whereas the present study considered the seed technology criterion (formation of normal seedling).

In seedling development (Figure 4), normal seedlings were considered as those that showed well-developed, complete, proportional and healthy essential structures (Brasil, 2009a). This group included seedlings with cylindrical primary root more tapered at the base (indicating the root cap region), shoots with cylindrical hypocotyl and leaf cotyledons of light green color, in expansion (Berghetti et al., 2015). In abnormal seedlings, the following characteristics were observed: necrosis in the collar region and burning of the meristematic region of the primary root; primary root undeveloped and disproportionate to the aerial part; and necrotic cotyledons.

CONCLUSIONS

The germination test for louro-pardo seeds should be conducted between vermiculite at 30 °C, without continuous light supply, with the first count performed at 26 days and the last count at 48 days after setting up the test.

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