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Temperature and substrate effects on the germination of *Caesalpinia ferrea* Mart. Ex Tul.

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***Caesalpinia ferrea* Mart. Ex Tul. is a tropical arboreal species used in naval and civil construction, and in the recovery of degraded areas. The substrate used and temperature can interfere directly in the germination of this species. This work aimed to evaluate the influence of different substrates and temperature regimes on the seed germination potential and initial growth of *C. ferrea* seedlings. The experiment was conducted at the Plant Propagation Laboratory of the Federal University of Alagoas, Brazil. A completely randomized design was used in a 5 × 4 factorial scheme with five substrates (paper roll, paper, sand, and vermiculite) and four temperatures (20, 25, 30 and 35°C). The evaluated characteristics were first count of germination, germination, germination speed index, dry mass, and seedling length. Results showed that *C. ferrea* seeds could germinate under different temperatures and substrate conditions. The sand substrate and the temperature of 30°C are excellent for evaluating the physiological quality of the seeds.**

Key words: Physiological potential, substrate, temperature.

INTRODUCTION

Caesalpinia ferrea Mart. Ex Tul. is a leguminous native of the Atlantic forest of arboreal growth and known commonly as pau ferro. It is distributed in the Brazilian northeast in the states of Pernambuco and Ceará, but it is predominant in Pernambuco, specifically in the Region of São Francisco and in the municipalities of Floresta and Buíque (Lorenzi, 2008).

The germination process involves a series of metabolic activities, in which a sequence of chemical reactions occurs with their own temperature requirements because they depend on specific enzymatic activities (Marcos Filho, 2015). Germination is affected by a series of intrinsic and extrinsic conditions, such as humidity, temperature, substrate, light, and oxygen. All these

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conditions are essential for the process to be carried out normally, and the absence of one of them prevents seed germination (Carvalho and Nakagawa, 2012).

Among the conditions that affect germination, temperature is one of the factors with a significant role. Temperature variations influence the speed, percentage, and uniformity of germination (Marcos Filho, 2015). The optimum temperature for each species is the one where the highest percentage of germination occurs in the shortest time. For most species, the optimum temperature is between 20 and 30°C (Marcos Filho, 2015). In addition to an optimum temperature range to germinate, some species require temperature alternation for the process to be satisfactory (Santos and Aguiar, 2000).

The substrate is another important factor in germination, and its function is the maintenance of moisture for the seeds, providing the resumption of embryo growth and conditions suitable for germination and subsequent fixation and development of seedlings (Figliolia et al., 1993). The substrate must maintain balanced humidity so that the amount of water does not affect aeration and prevent the availability of oxygen (Popinigis, 1985).

Knowledge of the factors that influence seed germination is extremely important, especially for forest species. Despite the great diversity of native species in Brazil, few are included in the Rules for Seed Analysis. Thus, several studies have been carried out to facilitate this insertion, such as those by Wagner et al. (2007) with three species of jaboticabeira, *Myrciaria jaboticaba* (Vell.) Berg, *Myrtus cauliflora* (Mart.) Berg, and *Myrtus peruviana* var. *trunciflora*; they recommended the temperature of 24°C for germination. Guedes et al. (2009) used the substrate paper roll at 30°C for tests on germination and vigor in seeds of *Cereus jamacaru*. For seeds of *Amburana cearensis* (Allemão) A.C. Smith, sand and vermiculite substrates were found to be the most appropriate for conducting germination and vigor tests at 35°C (Guedes et al., 2010).

In spite of the great importance of *C. ferrea*, the rules for seed analysis do not include information about optimum conditions for the evaluation of germination and vigor of the seeds of this species. These conditions are mainly temperature and substrate because they are responsible for triggering the germination process. Moreover, their requirements vary between seeds of the same species and different species. The lack of such information makes seed marketing difficult.

This work aimed to evaluate the influence of temperature and substrates on the physiological potential of *C. ferrea* seeds to determine the procedures for the germination and vigor tests of these seeds.

MATERIALS AND METHODS

The seeds of *C. ferrea* Mart. Ex Tul. were collected from eight

matrix trees in the rural area of the municipality of Garanhuns, Pernambuco, Brazil. The experiment was conducted at the Plant Propagation Laboratory of the Federal University of Alagoas. Given that they had impermeable integument, the seeds were scarified manually with sandpaper no. 80 on the opposite side to the micropyle (Melo et al., 2010). Before sowing, the seeds were disinfected with 2% sodium hypochlorite solution (12.5 mL of sodium hypochlorite and 487.5 mL of distilled water) for 5 min, and washed in running water for 4 min, followed by washing with distilled water for 1 min.

The seeds were distributed in five substrates: paper towels in the form of a roller, between blotting paper, on blotting paper, between sand, and between vermiculite. The last four substrates were placed in gearboxes (11 × 11 × 3 cm), and the latter two were moistened with distilled water at 60% of their water retention capacity. The substrates were subjected to dry heat sterilization in a sterilization oven, and drying at 105°C for 2 h.

First germination count

The counts were performed together with the germination test. The normal seedlings of the first germination test count were computed on the fourth day after the test installation. The results were obtained by the arithmetic mean of the four subsamples and expressed as a percentage.

Germination speed index (IVG)

IVG was determined with the germination test, in which the normal seedlings were evaluated daily at the same time from the first germination count. This procedure followed until the end of the test, and the index was calculated using the proposed formula by Maguire (1962).

Length of root and aerial part of the seedlings

At the end of the germination test, the hypocotyl and primary root of the normal seedlings of each subsample were measured using a graded ruler. The results are expressed in centimeters per seedling.

Dry mass of the root and aerial part of the seedlings

After the germination test, the normal seedlings of each replicate were separated in the aerial part and root, packed in paper bags, and placed in a forced ventilation oven at 80°C for 24 h. Subsequently, the samples were placed in desiccators with activated silica gel and weighed in an analytical balance with an accuracy of 0.0001 g. The results are expressed in grams per seedling (Nakagawa, 1999). The experimental design was completely randomized, with four replicates of 25 seeds. The treatments were distributed in a 4 × 5 factorial scheme (four temperatures and five substrates). The data were analyzed using analysis of variance (ANOVA), and the means were compared by the Tukey test at a 5% probability using the SISVAR statistical analysis program.

RESULTS AND DISCUSSION

For the results on vigor in the first germination count (Table 1), the paper roll substrate exhibited the highest values at 25°C. The temperatures of 30 and 25°C were adequate in expressing the vigor of *C. ferrea* seeds. The

Table 1. First germination count of seed *Caesalpinia ferrea* Mart. Ex Tul. submitted to different temperatures and substrates.

Substrate	Temperatures (°C)			
	20	25	30	35
Paper roll	37 ^{bB}	68 ^{aA}	74 ^{aA}	51 ^{aB}
Between paper	74 ^{aA}	35 ^{bB}	43 ^{cB}	45 ^{abB}
About paper	16 ^{cB}	24 ^{bAB}	37 ^{cA}	15 ^{cB}
Sand	13 ^{cC}	62 ^{aA}	55 ^{bcA}	30 ^{bcB}
Vermiculite	39 ^{bB}	72 ^{aA}	62 ^{abA}	60 ^{aA}
F for interaction	3.25**	-	-	-
CV (%)	6.15	-	-	-

Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other to a 5% probability by the Tukey test.

Table 2. Seed germination of *Caesalpinia ferrea* Mart. Ex Tul. subjected to different temperatures and substrates.

Substrate	Temperatures (°C)			
	20	25	30	35
Paper roll	75 ^{bA}	84 ^{aA}	84 ^{aA}	75 ^{aA}
Between paper	77 ^{bA}	42 ^{bC}	83 ^{aA}	63 ^{abB}
About paper	73 ^{bA}	73 ^{aA}	61 ^{bA}	16 ^{cB}
Sand	85 ^{aBA}	72 ^{aB}	94 ^{aA}	58 ^{bC}
Vermiculite	92 ^{aA}	80 ^{aA}	88 ^{aA}	63 ^{abB}
F for interaction	3.14**	-	-	-
CV (%)	6.73	-	-	-

Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other to a 5% probability by the Tukey test.

combinations of the substrates paper roll at 25 and 30°C, sand at 25°C, paper at 20°C, and vermiculite at 25 and 35°C provided the greatest results, with statistical differences between them. The optimum temperature range for most species is between 20 and 30°C (Marcos Filho, 2015).

Larcher (2000) found that this range can extend up to 35°C. In seeds of *Peltophorum dubium* Sprengel (Taubert), 30°C provided the highest number of seeds germinated in the first count (Oliveira et al., 2008). Brancalion et al. (2008) reported that temperatures of 25 and 30°C are the most favorable for the germination of the species, with a relation between the optimum temperature and the biome occurrence of the species.

The seeds of *C. ferrea* presented low vigor when exposed to 20°C, except for those seeds that were placed on the substrate between paper. Low temperatures decreased the metabolic activity of the seeds, retarding the speed of germination (Sousa et al., 2008) (Table 1).

In this study, the seeds of *C. ferrea* germinated at different temperatures, which allowed their colonization in

highly diverse habitats and facilitated their dispersion. The thermal amplitude for seed germination of a species can indicate the distance of a seed buried in relation to the soil surface because it decreases with increasing depth (Melo, 2011).

Table 2 shows the percentage of germination of the seeds of *C. ferrea*. The seeds placed to germinate on the roll paper towel substrate at 25, 30 and 35°C presented high germination percentages, but they were not statistically different from those in the substrates between paper at 30°C, paper at 25°C, sand at 30°C, and vermiculite at 20, 25, and 30°C. These data demonstrate the range of temperatures in which germination of the seeds of this species may occur, which indicates the high capacity of establishment of the seedlings in the field. Thus, these seedlings can withstand the adverse conditions of the environment.

According to Ramos and Varela (2003), the ideal germination temperature generally varies within the temperature range found at the site and at the ideal time for emergence and establishment of the seedlings. Lopes et al. (2005) studied the seed germination of *Basella*

Table 3. Index of speed seed germination of *Caesalpinia ferrea* Mart. Ex Tul. subjected to different temperatures and substrates.

Substrate	Temperatures (°C)			
	20	25	30	35
Paper roll	4.18 ^{aA}	4.65 ^{aA}	5.03 ^{aA}	4.82 ^{aA}
Between paper	3.86 ^{aAB}	2.28 ^{bC}	4.77 ^{aA}	3.19 ^{bBC}
About paper	2.65 ^{bA}	2.68 ^{bA}	2.73 ^{bA}	0.50 ^{cB}
Sand	3.66 ^{abB}	4.09 ^{aAB}	5.03 ^{aA}	3.09 ^{bB}
Vermiculite	3.87 ^{aAB}	4.65 ^{aA}	4.73 ^{aA}	3.26 ^{abB}
F for interaction	5.20**	-	-	-
CV (%)	8.99	-	-	-

Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other to a 5% probability by the Tukey test.

rubra, and they identified the paper roll as the best substrate because it provides 100% germination compared with seedlings of the substrates sand and vermiculite, which obtained 40 and 53%, respectively (Table 2).

The optimum temperature for seed germination is directly associated with the ecological characteristics of the species. Guedes et al. (2010) reported the use of 35°C for the conduction of germination and vigor tests with seeds of *A. cearensis* (Allemão) A.C. Smith. Their results showed that the responses of the seeds to the temperature also vary among the species.

In terms of the germination speed index (IVG; Table 3), the highest index was observed at 30°C, and it was statistically different from those obtained at other temperatures. Silva et al. (2014) studied the effects of temperature on the germination of seeds of *Sideroxylon obtusifolium*. Their findings affirmed that the IVG is linearly dependent on temperature, and it is a good index to evaluate the occupation of a species in a given environment. Rapid germination is a feature of species whose strategy is to establish itself in an environment as quickly as possible by taking advantage of favorable environmental conditions.

Low IVGs were obtained at 5 and 40°C, and the difference was not statistically significant. The small area of contact of the seeds with the substrate at the highest temperature (40°C) may have been responsible for the reduction in substrate moisture, and decrease in the index of germination of *C. ferrea* seeds (Table 3).

An important factor to consider is the interaction between temperature and substrate because the water retention capacity of a substrate is responsible for different responses obtained for the same temperature (Figliolia et al., 1993). In this study, the paper roll substrate provided a higher germination speed than the other substrates at all temperatures. For germination to occur, in addition to temperature, oxygen, and light for some species, the maintenance of moisture in the substrate is fundamental (Ramos and Bianchetti, 1984).

The different water retention capacities between substrates probably also influenced the seed imbibition rate and the mean time to germination. The highest IVG in seeds of *Melocactus bahiensis* Britton and Rose was obtained at 25°C (Lone et al., 2007). The lowest germination speed index was obtained when using the substrate on paper at 35°C. The small area of contact of the seeds with the substrate at the highest temperature may have been responsible for the reduction in substrate moisture and decrease in the rate of germination of *C. ferrea* seeds.

Regarding the initial development of the seedlings, which was evaluated by the length of the primary root and aerial part (Table 4), the highest averages were up to 30°C. At this temperature, efficient degradation of the present reserves in the seeds occurred, which favored the development of the radicles and the aerial part, because all the development of the seedlings at this stage is due to the chemical composition of the seeds (Carvalho and Nakagawa, 2012).

According to Figliolia et al. (1993), in addition to being heavy, the sand substrate may excessively drain water and leave the upper part dry, which impairs germination. In the present work, no surface dryness of this substrate was observed during the germination tests (Table 4).

In *Parkia pendula*, the length of the aerial part and radicle was used to conclude that the temperature of 25°C is the most adequate for the germination test of this species (Rosseto et al., 2009). In the aforementioned study, the radicular length of *C. ferrea* seedlings was strongly influenced by the temperatures studied. The lowest radicle lengths were obtained when exposed to 5, 10, 35 and 40°C, respectively, and these values were not statistically different from one another. In relation to the aerial part, small lengths were obtained at 5, 10, and 40°C, respectively, and the resulting values were not statistically different from one another (Table 5).

For the length of the aerial part of the seedlings (Table 5), the highest values were obtained in the following combinations: vermiculite substrate at 25°C and sand

Table 4. Root length (cm) of seedlings of *Caesalpinia ferrea* Mart. Ex Tul. subjected to different temperatures and substrates.

Substrate	Temperatures (°C)			
	20	25	30	35
Paper roll	9.44 ^{aB}	10.94 ^{bA}	8.96 ^{aB}	4.50 ^{aC}
Between paper	2.76 ^{cA}	7.79 ^{dA}	3.52 ^{bA}	0.90 ^{bC}
About paper	1.08 ^{dBC}	5.37 ^{cA}	2.43 ^{bB}	0.30 ^{cC}
Sand	7.19 ^{bB}	10.48 ^{bA}	9.42 ^{aA}	3.40 ^{abC}
Vermiculite	7.89 ^{bB}	12.49 ^{aA}	8.55 ^{aB}	2.87 ^{bC}
F for interaction	1.90 ^{**}	-	-	-
CV (%)	9.10	-	-	-

Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other to a 5% probability by the Tukey test.

Table 5. Length of aerial part (cm) of seedlings of *Caesalpinia ferrea* Mart. Ex Tul. submitted to different temperatures and substrates.

Substrate	Temperatures (°C)			
	20	25	30	35
Paper roll	5.03 ^{bA}	4.27 ^{cA}	3.77 ^{cA}	1.79 ^{aB}
Between paper	4.36 ^{bA}	1.22 ^{dC}	2.78 ^{cdB}	0.73 ^{abC}
About paper	2.67 ^{cA}	2.05 ^{dA}	1.78 ^{dA}	0.33 ^{bB}
Sand	4.18 ^{bB}	7.69 ^{bA}	8.12 ^{aA}	1.38 ^{abC}
Vermiculite	8.20 ^{aB}	9.86 ^{aA}	5.82 ^{bC}	2.04 ^{aD}
F for interaction	55.42 ^{**}	-	-	-
CV (%)	15.74	-	-	-

Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other to a 5% probability by the Tukey test.

substrate at 30°C. Similar results were found in Table 4 for the seedling root length of *C. ferrea*. Lima and Garcia (1996) observed that seedlings of *Acacia mangium* Willd. showed good development on paper roll substrate at 25, 25 to 35, and 35°C. Kissmann et al. (2008) found that the highest length of the aerial part of *Adenanthera pavonina* L. seedlings is obtained with seeds submitted to 20 to 30, 25 and 30°C, respectively, regardless of the substrate (paper roll and paper).

Determination of the seedling length is important, together with the germination test, because seeds present high germination percentage and low average seedling length, as well as low germination percentage but high average seedling length (Rosseto et al., 2009). When evaluating the dry mass of the root (Table 6), the highest values were obtained when the seed combinations were used in the temperature regime of 30°C. The sand and vermiculite substrates did not yield statistically different results. The highest values were also found at 20, 25 and 35°C, respectively on the paper roll substrate.

Ramos et al. (2004) reported that aerial and root dry mass evaluations are crucial in the evaluation of plant

development. To ensure the establishment of seedlings in the field, the seeds were vigorous in the substrates sand and vermiculite, resulting in high dry mass of seedlings (Table 6). During germination, the vigorous seeds provided strong transfer of dry mass of their reserve tissues to the embryonic axis, leading to seedlings with high weight due to the elevated accumulation of dry matter (Custódio, 2005).

In relation to the dry mass of the aerial part (Table 7), the most vigorous seedlings came from seeds submitted to 30°C with the sand and vermiculite substrates, as well as from seeds subjected to 20, 25 and 35°C, respectively, on the paper roll substrate. The differences were not statistically significant. Alves et al. (2002) used the dry mass to conclude that 25°C is adequate for germination and vigor tests in seeds of *Mimosa caesalpiniaefolia* (Table 7).

According to Hendricks and Taylorson (1996), low temperatures can reduce metabolic rates until the pathways essential to germination can no longer operate. Thus, at high temperatures, the seeds use their reserves more efficiently, resulting in seedlings with high phytomass contents.

Table 6. Root dry mass (g) of seedlings of *Caesalpinia ferrea* Mart. Ex Tul. submitted to different temperatures and substrates.

Substrate	Temperatures (°C)			
	20	25	30	35
Paper roll	0.00897 ^{aA}	0.00763 ^{aA}	0.00557 ^{dA}	0.00318 ^{aA}
Between paper	0.00738 ^{aB}	0.00246 ^{aB}	0.11800 ^{bA}	0.01753 ^{aB}
About paper	0.00653 ^{aB}	0.00621 ^{aB}	0.08898 ^{cA}	0.00345 ^{aB}
Sand	0.00683 ^{aB}	0.01151 ^{aB}	0.17923 ^{aA}	0.02730 ^{aB}
Vermiculite	0.00810 ^{aB}	0.01425 ^{aB}	0.15960 ^{aA}	0.04315 ^{aB}
F for interaction	14.39**	-	-	-
CV (%)	8.62	-	-	-

Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other to a 5% probability by the Tukey test.

Table 7. Dry mass of the aerial part (g) of seedlings of *Caesalpinia ferrea* Mart. Ex Tul. submitted to different temperatures and substrates.

Substrate	Temperatures (°C)			
	20	25	30	35
Paper roll	0.04141 ^{aA}	0.04313 ^{aA}	0.01176 ^{dA}	0.00744 ^{aA}
Between paper	0.01348 ^{aB}	0.02514 ^{aB}	0.44056 ^{bA}	0.05478 ^{aB}
About paper	0.00798 ^{aB}	0.03595 ^{aB}	0.27483 ^{cA}	0.00890 ^{aB}
Sand	0.02310 ^{aB}	0.02396 ^{aB}	0.53785 ^{aA}	0.08210 ^{aB}
Vermiculite	0.02368 ^{aB}	0.06128 ^{aB}	0.56065 ^{aA}	0.09315 ^{aB}
F for interaction	15.29 **	-	-	-
CV (%)	12.92	-	-	-

Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other to a 5% probability by the Tukey test.

Conclusion

The sand substrate and the temperature of 30°C are excellent for evaluating the physiological quality of the pau ferro (*C. ferrea*) seeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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