Seeds of *Caesalpinia echinata* Lam. Under Water Stress at Different Temperatures

Caroline Marques Rodrigues¹, Edna Ursulino Alves², Rosemere dos Santos Silva², Flávio Ricardo da Silva Cruz², Maria Lúcia Maurício da Silva², Marina Matias Ursulino², Erifranklin Nascimento Santos² & Mácio Farias de Moura¹

¹ Academic Unit of Garanhuns, Federal Rural University of Pernambuco, Garanhuns, Pernambuco, Brazil

² Agricultural Sciences Center, Federal University of Paraíba, Areia, Paraíba, Brazil

Correspondence: Rosemere dos Santos Silva, Graduate Program in Agronomy, Agricultural Sciences Center, Federal University of Paraíba, Campus II, PB. Rodovia 12, PB-079, Areia, Paraíba, 58397-000, Brazil. Tel: 55-83-9.9864-3010. E-mail: rosyufpbio@hotmail.com

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Abstract

The *Caesalpinia echinata* Lam. species, belongs to the Fabaceae family, popularly known as Pau Brazil, is a large tree, native to Brazil, that can be found from Ceará to Rio de Janeiro, with a relevant national historic and economic value. The objective of this study was to evaluate the effects of water stress at different temperatures on germination and seed vigor. The experiment was carried out at the Laboratório de Análise de Sementes of the Centro de Ciências Agrárias of the Universidade Federal da Paraíba, Areia-Paraíba. The water stress was formulated with solutions of polyethylene glycol 6000 (PEG 6000) in different osmotic potentials of 0.0 (control), -0.2; -0.4; -0.6 MPa at constant temperatures of 25, 30 and 35 °C, in a completely randomized design. The percentage of germination, first counting of germination, germination speed index, length and dry matter of seedlings were evaluated to determine the effects of the treatments. Decreases of the osmotic potential negatively influenced the germination and vigor of *C. echinata* seeds at the three evaluated temperatures (25 °C, 30 °C, 35 °C).

Keywords: pau brasil, physiological potential, polyethylene glycol, osmotic potential

1. Introduction

The *Caesalpinia echinata* Lam. species belongs to the Fabaceae family, popularly known as Pau Brazil, pau-rosado or pau-de-pernanbuco, is native to Brazil and can be found from Ceará to Rio de Janeiro in the Atlantic Rainforest (Lorenzi, 2016; Rocha & Barbedo, 2008). The tree is large with trunk and aculeous branches, dehiscent fruits of the pod type containing, on average, five seeds of circular shape.

C. echinata is currently on the list of endangered species due to centuries of exploitation. The wood of this species is very resistant and heavy and was extensively explored for civil and naval construction, and also for production of musical instruments. The dye extracted from wood is widely used for dyeing fabrics and producing paints (Lorenzi, 2016; Rocha & Barbedo, 2008).

Knowledge of ideal conditions for seed germination of each species is essential, since factors such as temperature, light, and salinity can affect the germination process. Besides that, the main factor that influences the germination is the water, which must be in adequate quantity, because when in excess or scarcity, can result in negative effects on the germination (Brasil, 2009; Carvalho & Nakagawa, 2012).

Studies related to the germinative response of seeds under stress conditions are important for the ecophysiology and constitute tools that allow the evaluation of the limits of tolerance, survival and adaptation of the plant species to natural stress conditions (Guedes et al., 2013). The species that tolerate the water stress are more likely to establish themselves in the field (Barbero, Barros, Silva, & Suzuki, 2011), others trigger mechanisms that allow germination (Rosa, Felippi, Nogueira, & Grossi, 2005).

The temperature influences the biochemical reactions that determines germination, because there is a programmed sequence of process in which enzymatic systems have their own thermal requirements (Marcos Filho, 2015). The ideal temperature will provide higher percentage and germination speed index in a short period

(Carvalho & Nakagawa, 2012). For the germination of seeds of tropical species, the optimum temperature varies from 15 to 30 °C (Marcos Filho, 2015).

The water stress is a situation that the plants can find in the field. In a laboratory conditions it is possible to simulate water stress for seed germination (Pelegrini, Borcioni, Nogueira, Koehler, & Quoirin, 2013). For this, the use of osmotically active chemicals as a way of inducing the water stress in the seed has been widely diffused and is usually done by the addition of solutes such as calcium chloride (CaCl₂), sodium chloride (NaCl) and polyethylene glycol (Oliveira & Gomes-Filho, 2009).

For each osmotic solution, there are chemical differences that can lead to different results in seed germination, even in similar water potentials (Souza & Cardoso, 2000). Polyethylene glycol 6000 (PEG 6000) has been used to identify the osmotic potential that is less damaging to the seeds various species. It has a high molecular weight, being non-toxic to the seed (Moraes, Freitas, & Menezes 2003), providing slow and controlled imbibition of the seeds (Villela, Doni Filho, & Siqueira, 1991).

The evaluation of PEG-induced water stress in seeds has been performed for several forest species, such as Zizyphus joazeiro. Mart. (Lima & Torres, 2009), *Poincianella pyramidalis* (Tul.) L.P. Queiroz, *Anadenanthera colubrina* (Vell.) Brenan (Santos et al., 2016), *Mimosa caesalpiniifolia* Benth. (Sousa et al., 2018), among others.

Based on the above and the importance of this species, the objective of this study was to evaluate the effect of water stress at different temperatures on the germination and vigor of seeds of *Caesalpina echinata* Lam.

2. Method

The experiment was carried out at the Laboratório de Análise de Sementes (LAS) belonging to the Departamento de Fitotecnia e Ciências Ambientais (DFCA) of the Centro de Ciências Agrárias (CCA) of the Universidade Federal da Paraíba (UFPB), located in Areia - Paraíba. The fruits of *C. echinata* were harvested in mother trees located in the CCA/UFPB and then taken to the LAS for processing by manual opening for the extraction of the seeds.

For the formulation of water stress, polyethylene glycol (PEG 6000) was used as the solute, which concentrations were formulated according to the specifications of Vilela et al. (1991) to obtain osmotic potential levels of -0.2; -0.4 and -0.6 MPa, the zero level (0.0) was used as control, using only distilled water to moisten the substrate.

2.1 Germination Test

The test was performed on germinators Biological Oxygen Demand (BOD) type at constant temperatures of 25, 30 and 35 °C with photoperiod of 8/16 hours of light and dark, respectively, using fluorescent lamps day-light type (4×20 W), using 100 seeds divided into four replicates of 25, treated with the fungicide Captan[®] in the dose of 240 g for 100 kg of seeds. The seeds were then distributed over two sheets of germinating paper, covered with a third layer, arranged in a roll, the paper was moistened with the PEG 6000 solutions in the previous mentioned potentials, with an amount equivalent to 2.5 times the paper dry weight without further addition of the solution, only distilled water was used for the control. The rolls were stored in transparent plastic bags to avoid loss of water by evaporation and the evaluations were carried out daily, from the third to the twelfth day after the installation of the experiment, the criteria for evaluation used were normal seedlings, as described by Brasil (2009), with results expressed in percentage.

2.2 First Counting of Germination

Determined concurrently with the germination test, by counting the normal seedlings in the third day after installation of the test, the results were expressed in percentage.

2.3 Germination Speed Index

Performed by the daily counting of germinated seeds, at the same time, for 12 days, the index was calculated by the formula proposed by Maguire (1962).

2.4 Length and Dry Matter of Roots and Shoots

At the end of the germination test, the normal seedlings of each treatment and replication were measured (root and shoot) using a ruler graduated in centimeters, with the results expressed in cm. After the measurements, the roots and shoots of the seedlings, without the cotyledon leaves, were placed in paper bags and placed in a lab stove at 80 °C for 24 hours (Nakagawa, 1999). After this period the samples were weighed on an analytical scale with an accuracy of 0.001 g and the results expressed in grams. Below is a figure containing the total number of

four repetitions in each osmotic potential of normal seedlings, which were put to dry in an oven, thus obtaining their dry mass.

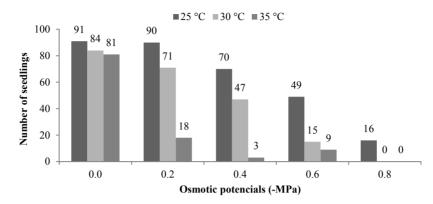


Figure 1. Number of normal seedlings of each osmotic potential at each temperature

2.5 Experimental Design and Statistical Analysis

The experimental design was completely randomized, with the treatments distributed in a 4×3 factorial scheme (osmotic potentials × temperatures). Data were submitted to analysis of variance using the F test ($p \le 0.05$) for comparison of squares. For the quantitative effects, polynomial regression analysis was performed by testing the linear and quadratic models and selecting the one with the highest significant degree. The SISVAR software was used (Ferreira, 2007).

3. Results and Discussion

On Figure 2, it can be verified that the water potentials influenced the germination of *C. echinata* seeds regardless of temperature. At the temperature of 25 °C, the maximum germination (93%) was obtained in the osmotic potential of 0.0, with a decrease following the more negative water potentials. However, at this temperature, seed germination was higher in all osmotic potentials when compared to temperatures of 30 and 35 °C. The most abrupt decrease in germination was observed in seeds submitted to a temperature of 35 °C. Germination and vigor are negatively affected if temperature extremes tolerated by seeds (cardinal temperatures) are exceeded (Felix et al., 2018).

When associated with other factors, such as water deficit, temperature increase may reduce germination, as found for *Caesalpinia echinata*. Unlike *C. echinata*, germination and initial development of *Combretum leprosum* Mart. was impaired already at a temperature of 25 °C in the osmotic potentials from -0.2 to -0.5 Mpa (Leal et al., 2020). The decrease in germination of seeds under water stress is a response of the enzymatic activity (Santos, Calil, Ruiz, Alvarenga & Santos, 1992) and, for each species, there is a critical value of water potential, where in many cases germination does not occur (Carvalho, 2005).

In several studies with species of the Fabaceae family similar results were found. According to Silva, Aguiar & Rodrigues (2001), for Bowdichia virgiloides Kunth. after the potential level of -0.5 MPa of PEG 6000 the germination was reduced in a critical way to a point that in smaller potentials such as -0.9 MPa the seeds did not germinate.

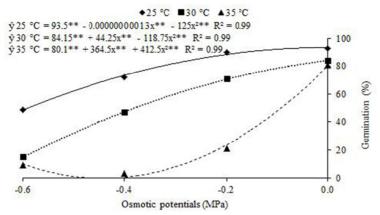


Figure 2. Percentage of germination of *C. echinata* seeds under water stress at different temperatures. Areia-PB, 2017

It can be verified in the first germination counting (Figure 3) that in the 0.0 potential the highest values were observed in all the evaluated temperatures, always above 80%.

As the osmotic potential decreased at all temperatures, there was a reduction in germination percentage, being more expressive at 35 °C.

Temperature, as it influences different metabolic processes, significantly affects seed germination (Oliveira et al., 2015), which tends to be lower with decreasing osmotic potential. One of the reasons that could explain this decrease in germination is the high molecular weight of PEG 6000, which is not absorbed due to the high viscosity, but due to the low rate of diffusion of O_2 can compromise the oxygen availability to the seeds during the germination process (Braccini, Ha, & Braccini, 1996).

In other studies, with seeds of *Erythrina falcata* Benth., Pelegrini et al. (2013) found no germination in the potential of -0.4 MPa, while seed germination of *Erythrina velutina* Willd. (Reis, Dantas, & Pelacani, 2012), and *Dimorphandra gardneriana* Tulasne (Ursulino et al., 2016) were sensitive to water stress, under more negative potentials.

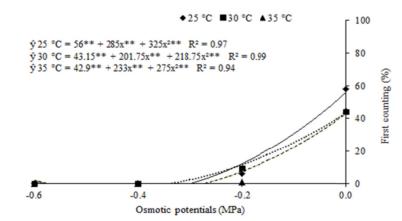


Figure 3. First counting of germination of *C. echinata* seeds under water stress at different temperatures. Areia-PB, 2017

It can be seen on Figure 4 that the germination speed index reduced as the osmotic potentials became more negative. As observed for the germination and first counting, at the temperature of 25 °C the maximum values of germination speed index were verified at the highest osmotic potential. Thus, water stress has the capacity not only to reduce the percentage but also the speed of germination, obtaining different results (Bewley & Black, 1994; Carvalho & Nakagawa, 2012).

Similar results were found by Moura, Lima, Farias, Alves & Silva (2011) evaluating seeds of *Mimosa caesalpiniifolia* Benth. For this species, the germination speed index was also negatively affected by the decrease in the levels of osmotic potential. Rego et al. (2011), evaluating seeds of *Anadenanthera colubrina* (Veloso) Brenan. under different osmotic potentials induced by PEG 6000, obtained decreases of germination at the potential of -0.6 MPa. For seeds of *A. colubrina*, the control provided a higher germination speed index, the lower values were observed in the most negative osmotic potentials, -1.2 MPa of NaCl and -0.8 MPa of PEG (Santos, N. V. Silva, Walter, E. C. A. Silva & Nogueira, 2016).

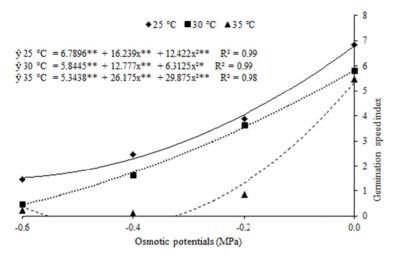


Figure 4. Germination speed index of *C. echinata* seeds under water stress at different temperatures. Areia-PB, 2017

For the root length of *C. echinata* seedlings (Figure 5), the highest value was found in the control (0.0) at 30 °C. For all the evaluated temperatures there was a decrease in the root length with the decrease of the water potential up to -0.4 MPa but was at the temperature of 35 °C that the lowest values were found. Taiz and Zeiger (2009) reported that the decrease in root growth can occur due to the reduction of cellular expansion, so that it does not develop. According to Avila et al. (2007), this effect is due to the fact that plants subjected to water stress have a greater development of the root system in order to improve its water absorption capacity.

Evaluating the root length of seedlings of *Mimosa ophthalmocentra* Mart. ex Benth. (Nogueira, Torres, Freitas, Castro & Sá, 2017) and *Simira gardneriana* M. R. Barbosa & Peixoto (F. N. Oliveira, J. R. Oliveira, Torres, Freitas & Nogueira, 2017) also observed decreases as the water stress became more intense.

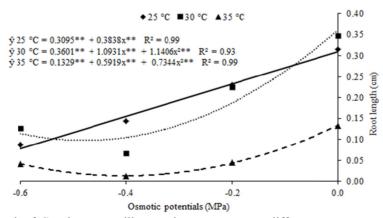


Figure 5. Root length of C. echinata seedlings under water stress at different temperatures. Areia-PB, 2017

For the shoot length of the *C. echinata* seedlings (Figure 6), it was observed that the temperature of 30 °C stood out among the other treatments in the control (0.0), however in the other potentials there was a decrease as the

potentials became more negative and at the temperatures of 25 and 35 °C there was a significant decrease when the potentials became more negative. Similar results were found in *D. gardneriana* (Ursulino et al., 2016) and *M. ophthalmocentra* (Nogueira et al., 2017) where were found decreases in shoot length as the water potential became more negative.

The differences in shoot length can be explained due to the conditions necessary for germination, so that vigorous seeds originate seedlings with higher growth rate, due to the greater capacity of transformation of the reserves of the storage tissues and greater incorporation of these by the embryonic axis (Nakagawa, 1999).

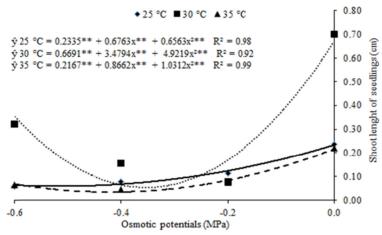


Figure 6. Shoot lenght of seedlings of C. echinata under water stress at different temperatures. Areia-PB, 2017

Figure 7 shows the results for the root dry matter of *C. echinata seedlings*, which shows higher dry matter for the zero level (0.0) at all temperatures, with a subsequent decrease in the other water potentials. At the lower temperature of 25 °C it can be observed that there was a linear decrease of root dry matter. It can be assumed that this temperature favors seed vigor up to the potential of -0.2 MPa without major losses.

Similarly, the root dry matter of *Piptadenia moniliformis* Benth. (Azeredo et al., 2016) and *S. gardneriana* (Oliveira et al., 2017) were also reduced as the osmotic potential of the solutions became more negative.

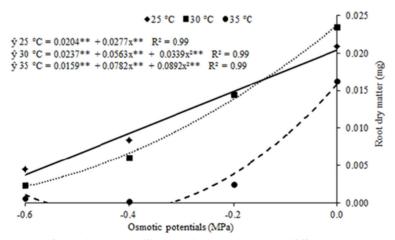


Figure 7. Root dry matter of C. echinata seedlings under water stress at different temperatures. Areia-PB, 2017

For the shoot dry matter (Figure 8), at 25 °C, a quadratic adjustment was observed, decreasing as the potential became more negative. At the temperature of 30 °C, in the potential of 0.0 (control) the highest value was observed, and for potentials below -0.2 MPa lower values were estimated compared to the temperature of 25 °C. The most accentuated decrease was observed at the temperature of 35 °C between the potential 0.0 (control) and

-0.2 MPa. The water stress caused a decrease in the water absorption by the seeds, negatively influencing their germinative performance, and consequently the growth, expressed by the dry matter of shoots.

The low availability of water reduces the speed of the physiological and biochemical processes, causing a lower development of seedlings under stress conditions, consequently having a shorter seedling length and a lower accumulation of dry matter (Sá, 1987). Custódio, Salomão, and Machado Neto (2009) using mannitol to simulate water stress reduced the dry matter of shoots of common bean seedlings (*Phaseolus vulgaris* L.).

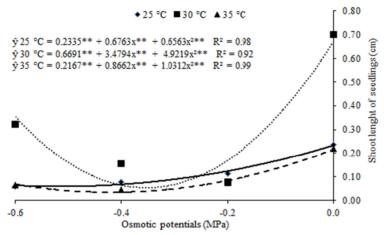


Figure 8. Shoot dry matter of C. echinata seedlings under water stress at different temperatures. Areia-PB, 2017

4. Conclusions

Caesalpina echinata seeds germinate up to the potential of -0.4 Mpa at a temperature of 25 °C.

Temperatures of 30 and 35 °C potentiate the negative effects on the germination and initial development of C. *echinata* seedlings when subjected to water stress.

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