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# Saline stress and temperatures on germination and vigor of *Piptadenia moniliformis* Benth. seeds

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**Key words:**
Fabaceae
catanduva
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**ABSTRACT**
The knowledge concerning the physiology of germination in saline areas may contribute to the development of more efficient cultural practices and adequate choice of planting areas. Thus, the objective of this study was to evaluate the effects of saline and temperature stress on germination and vigor of *Piptadenia moniliformis* (a species known in Brazil as "catanduva") seeds. The treatments were distributed according to a completely randomized design in accordance with a 3 x 6 factorial arrangement (temperature x osmotic potential), with four replicates of 25 seeds each. The seeds were put to germinate at constant temperatures of 25, 30, and 35 °C on paper towel moistened in distilled water (0.0) and NaCl solutions at 0.2, 0.4, 0.6, 0.8 and 1.0 MPa. The results were evaluated in terms of germination percentage and germination speed index, seedling (root and shoot) length, and total dry matter. *P. moniliformis* seeds were able to germinate under temperatures of 25 and 30 °C and tolerate osmotic potentials of up to -0.6 MPa; from this point on, there is progressive decrease in the physiological quality of seedlings.

**Palavras-chave:**
Fabaceae
catanduva
sementes florestais
salinidade

## Estresse salino e temperaturas na germinação e vigor de sementes de *Piptadenia moniliformis* Benth

**RESUMO**
O conhecimento da fisiologia da germinação em locais salinos pode contribuir para o desenvolvimento do manejo e da escolha adequada das áreas de plantio. Com isto objetivou-se avaliar o efeito do estresse salino e temperaturas na germinação e vigor de sementes de catanduva (*Piptadenia moniliformis* Benth.). O delineamento experimental foi o inteiramente casualizado, com os tratamentos distribuídos em esquema fatorial 3 x 6 (temperatura x potencial osmótico) em quatro repetições de 25 sementes. Para tal, as sementes foram colocadas para germinar nas temperaturas constantes de 25, 30 e 35 °C e semeadas em substrato de papel toalha umedecido com água destilada (0,0) e soluções de NaCl a -0,2; -0,4; -0,6; -0,8 e -1,0 Mpa; as variáveis analisadas foram a porcentagem e o índice de velocidade de germinação, comprimento de plântulas (raiz e parte aérea) e massa seca total; as sementes de *P. moniliformis* germinadas sob temperaturas de 25 e 30 °C suportam concentrações salinas com potencial osmótico de até -0,6 MPa sendo que a partir de então ocorre diminuição progressiva da qualidade fisiológica das plântulas.

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## Introduction

*P. moniliformis* stands out among the trees native to the Caatinga and is known by the popular names of 'catanduva', 'catanduba', 'rama-de-bezerro' and 'angico-de-bezerro' (Lorenzi, 2002; Azerêdo et al., 2010). According to these authors, this species occurs from the state of Maranhão until Bahia and is more frequent in the region of the São Francisco River Valley. It is a species abundant in this biome, with pioneering characteristics, rustic, with fast growth and indicated for reforestation, besides having potential for forage, apiculture, medicine and timber. In general, the salinity of the substrate or irrigation water causes reduction in soil water potential and toxicity to seed cells (Guan et al., 2009). Situations of salinity, of either soil or water, are more common in semiarid regions, since water availability is limited during a certain period of the year, which compromises germination, establishment of seedlings and their survival (Martins et al., 2014).

One of the most widespread methods for the determination of plant tolerance to the excess of salts is the observation of germination percentage in saline substrates (Lima & Torres, 2009). According to Góis et al. (2008), the reduction in germinating power, compared with the control treatment, obtained through seeding in substrate moistened with water, serves as an indicator of the salinity tolerance index of the species. In this method, the ability to germinate also indicates the tolerance of the plants to salts in subsequent development stages (Oliveira et al., 2007).

Another environmental factor that affects germination performance, especially if associated with salinity, is temperature. These factors in association affect speed and germination, because they influence water absorption speed and biochemical reactions of the germination process (Guan et al., 2009; Betoni et al., 2011).

Given the above, this study aimed to evaluate the influence of saline stress at different temperatures on the germination and vigor of *P. moniliformis* seeds.

## Material and Methods

*P. moniliformis* seeds were harvested from eighteen parent trees located at the Não-Me-Deixes Farm, in Quixadá-CE, Brazil (4° 49' 34" S; 38° 58' 9" W; 210 m) from July to August 2014. The climate of the municipality is classified as semi-arid hot tropical, with mean annual rainfall of 838.1 mm, concentrated in the months of February to April, and mean temperature of 26 to 28 °C (IPECE, 2005).

After fruit harvest, the seeds were extracted, manually processed and dried in the shade, for three days. Then, the seeds were placed in polyethylene bags and stored in a cold chamber (12 °C and 50% RH).

Before the germination test, the seeds were subjected to chemical scarification with concentrated sulfuric acid for 25 min. Then, they were washed in running water for 5 min and dried in the shade on paper towel (Azêredo et al., 2010).

Saline stress was simulated using sodium chloride (NaCl) as the solute, at the following osmotic potentials: -0.2, -0.4, -0.6, -0.8 and -1.0 MPa, diluted in distilled and deionized water. The electrical conductivity in the solutions (5.41, 10.81, 16.22, 21.63 and 27.04 dS $m^{-1}$) was verified using a conductivity meter. For the control, distilled and deionized water was used to moisten the substrate. The electrical conductivity values of the NaCl solutions were obtained through the expression: Yos = - RTC, in which: Yos = osmotic potential (atm); R = universal gas constant (0.082 atm mol. $L^{-1}$ $K^{-1}$); T = temperature (K); C = concentration (mol $L^{-1}$); mol $L^{-1}$ x molar mass of NaCl = g $L^{-1}$ and T (K) = 273 + T (°C) (Salisbury & Ross, 1991).

The experimental design was completely randomized in a 3 x 6 factorial scheme (temperature x osmotic potential), with four replicates of 25 seeds.

The seeds were distributed on two paper towel sheets (Germitest), covered with a third sheet and organized in the form of a roll. The substrate was moistened with distilled water or NaCl solutions using an amount equivalent to 3 times the mass of the paper before hydration. The rolls were placed in 0.04-mm-thick transparent plastic bags to reduce water losses through evaporation. The treatments were evaluated using the following tests:

Germination – conducted in a Biochemical Oxygen Demand (B.O.D.) germinator regulated for constant temperature regimes of 25, 30 and 35 °C. Evaluations were performed by daily counting normal seedlings (Brasil, 2009) for 21 days, when germination stabilized.

Germination speed index (GSI) – conducted along with the germination test, by daily counting normal seedlings, from 3 to 21 days, at the same time, and calculated through the formula proposed by Maguire (1962).

Seedling root and shoot length – the measurements were taken after the final count of the germination test, using a ruler graduated in centimeters, and the results were expressed in cm.

Seedling dry matter – the normal seedlings of each replicate were placed in paper bags and dried in a forced-air oven at 70 °C until constant mass (72 h). After this period, seedlings were weighed on an analytical scale (0.001 g) and the results were expressed in g $seedling^{-1}$.

The data were subjected to analysis of variance by F test and polynomial regression through the program Sisvar, adopting linear and quadratic models with significance below 5% and of greater order ($R^2$), selecting the equation that best fitted the data.

## Results and Discussion

For all temperatures, germination percentage decreased as the osmotic potential decreased and the highest germination percentages (91, 93 and 89%) were obtained when the seeds were germinated in substrate moistened with deionized water (0.0 MPa) at temperatures of 25, 30 and 35 °C, respectively (Figure 1). The temperatures of 25 and 30 °C promoted high seed germination, above 80%, until the osmotic potential of -0.6 MPa, from which there was a sharp decrease in germination until -1.0 MPa; at this point, germination occurred only at temperature of 25 °C.

When *P. moniliformis* seeds were subjected to the temperature of 35 °C, germination percentage decreased until

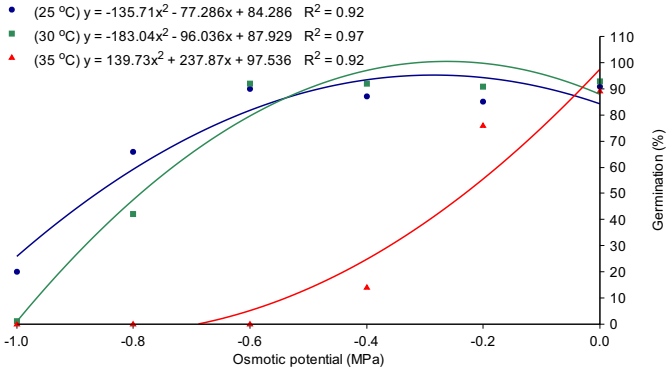


Figure 1. Germination of *Piptadenia moniliformis* seeds subjected to NaCl-induced saline stress at different temperatures

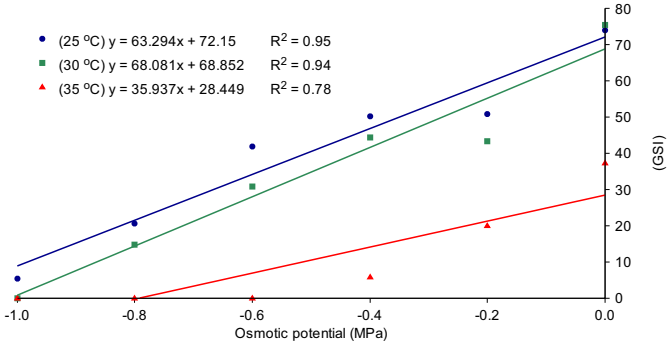


Figure 2. Germination speed index (GSI) of *Piptadenia moniliformis* seeds subjected to NaCl-induced saline stress at different temperatures

the potential of -0.4 MPa, evidencing that this temperature should not be used in the germination test.

As observed in the present study, the reduction in germination percentage of *Caesalpinia ferrea* seeds subjected to different salinity levels also led to a delay in the germination process (Freitas et al., 2010). Irrigation water salinity also decreased significantly the emergence of albizia seedlings as the salinity levels increased (Lima et al., 2015).

The association between environmental factors, such as relatively high temperatures and saline stress, harms in a more intense way the germination performance of the seed, probably for accelerating respiration and metabolic events, thus intensifying the toxic effects of salts and the deterioration (Guedes et al., 2011).

On the other hand, the highest temperature (35 ºC) may have favored the disintegration of protein structures and, along with the toxic effect and the physiological drought caused by the saline osmotic potentials, probably led to reduction in seed germination.

The harmful effects on seed germination, observed under saline stress at 35 ºC, were also reported for seeds of *Peltophorum dubium* (Spreng) Taub. (Alves et al., 2011).

The restriction in water absorption by the seeds occurs due to the reduction in the potential gradient between the soil and their surface, caused by the presence of salts, which interfere with soil water potential. Hence, the presence of salts may reach a high level and significantly harm germination (Martins et al., 2014).

Similar to the result for germination percentage, the germination speed index of *P. moniliformis* seeds was highly affected by the reduction in the osmotic potentials of the NaCl solutions, evidencing the effect of salinity on the delay of germination (Figure 2).

The highest GSI values, 74 and 76, occurred in the absence of salinity (0.0) for seeds incubated at temperatures of 25 and 30 ºC, respectively. Then, there were significant reductions to values lower than 5.0 and 0.0 when the seeds were subjected to saline concentration of -1.0 MPa at the previously mentioned temperatures. In seeds incubated at 35 ºC, the reduction in germination speed was more significant, with index of 37 at the potential of 0.0 MPa, which reached 6.0 at the potential of -0.4 MPa. From this point on, germination did not occur.

The germination speed index proved to be efficient in the indication of negative effects of both the highest temperature (35 ºC) and the increasing levels of salts in the solution moistening the substrate, because the more negative is the osmotic potential of the environment, the lower is the germination speed of *P. moniliformis* seeds (Figure 2). These results emphasize the importance of water availability in the initial development stages of these seedlings, similar to the results for *Bauhinia cheilantha* (Oliveira et al., 2014) and *Jatropha curcas* (Andréo-Souza et al., 2010).

As observed, the water stress, caused by the increase in salinity, acts on the seed by retarding the absorption of water necessary to germination and, as a consequence, delaying the germination process (Betoni et al., 2011). Thus, germination speed can be considered as the first variable affected by the reduction in water availability due to saline stress (Andréo-Souza et al., 2010; Oliveira et al., 2014).

Seeds of other species from the Caatinga also showed decrease in germination speed due to the increase in the saline concentration of the substrate, such as: *Mimosa caesalpiniifolia* Benth. (Ribeiro et al., 2008), *Guazuma ulmifolia* Lam. (Betoni et al., 2011), *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul.), *Aspidosperma pyrifolium* Mart. and *Erythrina velutina* Willd. (Dantas et al., 2014).

As to the temperatures, they also affected the germination speed of the seeds and the temperature of 35 ºC was the most harmful, regardless of the saline concentrations used, indicating that the deleterious effects of salinity are aggravated in germination under high temperatures. Similar results were also obtained by Guedes et al. (2011) in *Chorizia glaziovii* seeds, for the same temperature at which the increase in the saline concentration of the substrate caused reduction in seed germination and vigor.

As to shoot length (Figure 3), *P. moniliformis* seedlings showed a similar behavior at each temperature, with reduction in the values as the osmotic potential of the solution decreased. Seedlings subjected to temperatures of 25, 30 and 35 ºC, and substrate moistened with water (0.0 MPa), showed the highest values of shoot length (3.08, 2.98 and 3.10 cm, respectively). The increase in salt concentration caused a more pronounced reduction in seedlings at the temperature of 35 ºC. Guedes et al. (2011), working with seeds of *Chorisia glaziovii* O. Kuntze, observed that shoot length was less affected by salinity at temperatures of 25 and 20-30 °C, similar to the result of the present study.

For seeds of *Zizyphus joazeiro* Mart., Lima & Torres (2009) observed significant difference in shoot length between the

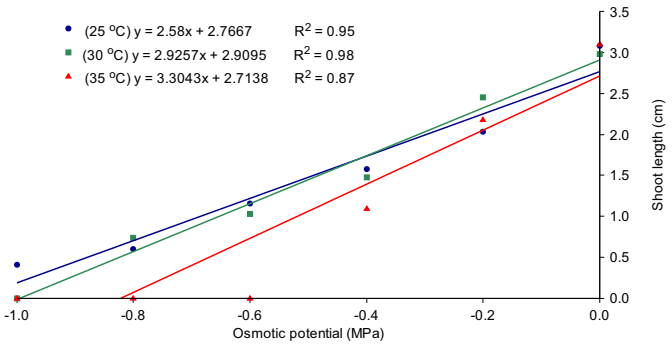


Figure 3. Shoot length of *Piptadenia moniliformis* seedlings subjected to NaCl-induced saline stress at different temperatures

control and the other treatments, reporting that there was no significant growth of seedlings from the osmotic potential of -0.6 MPa of NaCl.

The greatest length in the primary root of the seedlings (3.71 cm) was obtained when the seeds were placed to germinate at 30 ºC in substrate moistened with water (0.0 MPa). As observed for the previous variables, root growth of *P. moniliformis* seedlings was harmed by the increase in the saline concentration of the substrate, at the different temperatures evaluated. Nonetheless, at temperatures of 25 and 30 ºC, root length values remained maximal until the osmotic potential of -0.6 MPa, while the lowest root growth values were observed at 35 ºC, regardless of the osmotic potential (Figure 4).

The optimal temperature for cell division is approximately 30 ºC for most species; therefore, it is close to the optimal temperature for primary root growth (Ferreira & Borghetti, 2004). Results similar to those of the present study were observed for seeds of *Diptychandra aurantiaca* (Mart.) Tul. at temperatures of 25 and 30 ºC (Oliveira et al., 2013).

The highest values of dry matter occurred at temperatures of 25 and 30 ºC, regardless of the saline concentration. Seedlings showed quadratic responses to the increase in saline concentration when subjected to the temperatures of 25 and 30 ºC, while there was a decreasing linear response at the temperature of 35 ºC. The solutions with osmotic potentials of -0.28 and -0.26 MPa promoted higher dry matter accumulation in seedlings at temperatures of 25 and 30 ºC, with value of 0.36 g for both temperatures. From these potentials on, there was a reduction in the dry matter. However, until the potential of -0.6 MPa, there was a slight

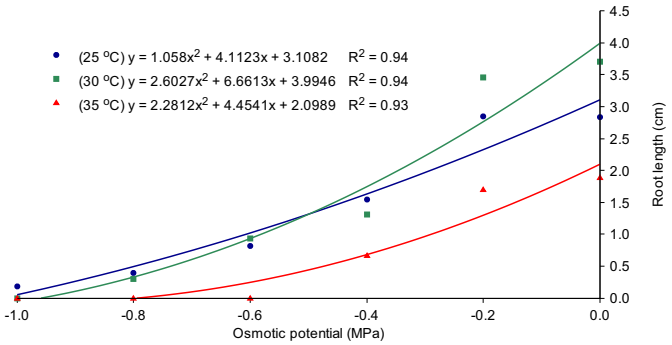


Figure 4. Primary root length of *Piptadenia moniliformis* seedlings subjected to NaCl-induced saline stress at different temperatures

reduction for the use of only deionized water (0.0 MPa) (Figure 5).

Therefore, it is observed that salinity causes changes in the capacity of the plant to absorb, transport and use the ions necessary to growth and also reduces the metabolic assimilation rate and the activity of enzymes responsible for respiration and photosynthesis, thus decreasing the acquisition of energy for cell growth and differentiation in the tissues, and hence reducing embryonic axis elongation and dry matter production (Nobre et al., 2010).

The high limit of tolerance to saline stress, especially in the initial stages of its development, at the temperatures of 25 and 30 ºC, provides an adaptive character to *P. moniliformis*, promoting high capacity of establishment of its seedlings in salt-affected areas.

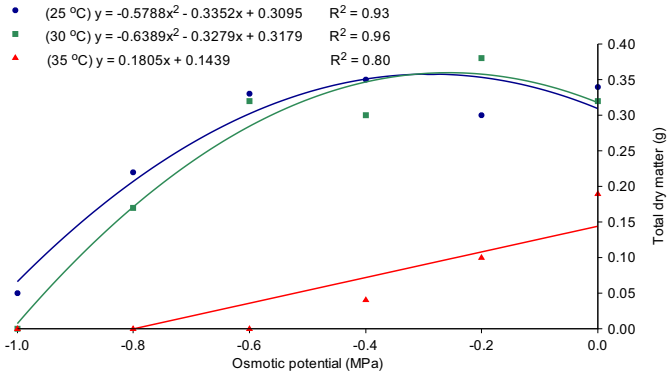


Figure 5. Total dry matter of *Piptadenia moniliformis* seedlings subjected to NaCl-induced saline stress at different temperatures

## Conclusion

*P. moniliformis* seeds germinated at temperatures of 25 and 30 ºC tolerate saline concentrations with osmotic potential of up to -0.6 MPa. From this point on, there is a progressive reduction in the physiological quality of the seedlings.

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