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Influence of light, temperature and humidity on substrate and osmoconditioning during the germination of *Mimosa bimucronata* (DC) O. Kuntze.

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Abstract

The maricá (*Mimosa bimucronata* (DC) O. Kuntze) is a forest species, belonging to the family Fabaceae, considered endemic to the Atlantic Forest biome. The present work aimed to study the germinative behavior of M. *bimucronata* seeds under different temperatures and light qualities, as well as to evaluate the effect of the amount of water (humidity) in the substrate and the osmoconditioning during germination. Thus, the experiment was performed using a completely randomized design. The treatments were distributed in a 4 × 4 factorial scheme (temperatures and light qualities), with 4 replicates of 25 seeds each. The means were compared by Tukey's test at 5% probability. The volume of water and osmoconditioning of substrate was evaluated and data were subjected to regression analysis. The following variables were analyzed: first count of germinated seeds, germination, germination speed index, and electrical conductivity. The results revealed that seeds are neutral photoblasts, needing a constant temperature of 30 °C along with a white light for appropriate germination and vigor. Seeding on two sheets of paper towel moistened with water volume (mL) from 2.62 to 2.70 times was more suitable for conducting the germination test. The osmotic conditioning was inefficient in maintaining germination of these seeds.

Keywords: Atlantic forest, Fabaceae, Forest seeds, Seed analysis.
Abbreviations: BOD_Biochemical Oxygen Demand; SISVAR_System for Analysis of Variance; CECA_Center of Agricultural Sciences; UFAL_ Federal University of Alagoas.

Introduction

Popularly known as Marica, *Mimosa bimucronata* (DC) O. Kuntze (Fabaceae) is a short life tree (20–30 years) that can adapt to extreme conditions, such as wet and rocky lands. This plant is known to improve the soil quality and is recommended for the control of erosion and for planting in land subjected to periodic flooding (Carvalho, 2004). In order to recover the degraded forests, it is necessary to use the appropriate species and seeds with good physiological quality. Thus, it is essential to have a good knowledge of the species being used. Among the environmental factors, light and temperature are known to greatly influence germination, affecting both the rate of water absorption and the biochemical reactions that trigger the process. The sensitivity of seeds to light can be altered by several factors such as age, temperature, plant growth conditions, and water (Matos et al., 2015; Silva et al., 2016). Their presence can help reduce the problems caused by low amount of water in the soil and the effect of higher temperatures, promoting or inhibiting the germination process, with varying responses according to the species and depending on the luminous environment surrounding them (Galindo et al., 2012; Pacheco Jr et al., 2013). Another factor that may have a direct influence on the germination process, triggering it, is the humidity of the substrate, in which sowing is carried out. During this process, water absorption is crucial to promote the softening of the seed coat, initiate the embryo growth and reserve tissues, favoring tegument rupture, gas diffusion, and the emergence of the primary root. Water is also essential for protoplasm dilution, allowing the diffusion of hormones and consequently activating the enzymatic systems; thus, digestion, translocation, and assimilation of the reserves, resulting in embryonal growth (Gonçalves et al., 2015; Ramos et al., 2006). An alternative to promote uniform and rapid germination is osmoconditioning. In this technique, the seeds are subjected to the action of osmotic solution to regulate hydration and promote metabolic processes of the initial stages of germination, inhibiting the sprouting of the primary root. The inorganic solutions that can be used are sodium chloride (NaCl) and potassium nitrate (KNO_3), whereas the organic ones are mannitol, glycerol, and polyethylene glycol (PEG) (Oliveira et al., 2014). As aforementioned, the present work aimed to study the germination of *M. bimucronata* seeds under different temperatures and light qualities, besides evaluating the effect of the amount of water in the substrate and the osmoconditioning during the germination.

Results and Discussion

Germination in different light qualities

The degree of moisture during seed harvest of *Mimosa bimucronata* (DC) O. KTZE. was 16.25%. Table 2 presents the mean values of the germination percentage of *M. bimucronata* seeds subjected to different temperatures and light qualities. It was observed that the highest germination occurred when the seeds were exposed to constant temperatures of 25 and 30 °C and alternated at 20–30 °C, under white light, not statistically different from each other. Similar result was obtained by Rebouças (2009) in seeds of *Anadenanthera colubrina* (Vell.) Brenan., while evaluating the influence of light regimes on the germination and initial seedling growth. The seeds of *M. bimucronata* are germinated both in the presence and absence of light and could be classified as neutral photoblasts, neutral to light in seed germination. According to Silva et al. (2016), neutral photoblasts refers to a behavior commonly described for the understory trees and shade plants.

The ability of seeds to germinate at different temperatures and light qualities is a respectable feature for species survival, as they control colonization events in time and space and simulate forest environments where temperature variations and openings occur (Matos et al., 2015).

The requirement for seeds germination of different species at alternate temperatures was also verified by some authors such as Sales et al. (2011) who stated that germination increased at constant temperatures of 30 °C and alternated at 20–30 °C in all light regimes and for seeds of *Crataeva tapia.* Galindo et al. (2012), found only the alternating temperature of 20-30 ºC under the regimes of white and red light.

The seeds of *M. bimucronata* are germinated at different temperature and luminosity conditions, although oscillations occur due to the temperature and light qualities tested. This characteristic facilitates their dispersion and colonization in a greater diversity of habitats.

The lowest percentages of seed germination were obtained at 20 °C, corroborating the results of Galindo et al. (2012), who reported that a majority of the tropical and subtropical species has maximum germinative potential in the temperature range of 20–30 °C. Low temperatures may reduce the enzyme activities involved in germination metabolism (Larcher, 2004), whereas at higher temperatures, oxygen is less soluble and embryonic tissues would receive insufficient amounts of oxygen to satisfy them in their metabolic requirements. The appropriate quantity of oxygen is essential for the germination process (Oliveira et al., 2016). These reports serve as a basis for explaining the lower percentages of germination obtained from *M. bimucronata* seeds at a temperature of 20 °C in all light qualities.

Temperature and volume of water in the substrate

The results obtained by subjecting the seeds of *M. bimucronata* to different volumes of water in the substrate and temperatures indicated that the interactions between these factors exerted a major influence on germination (Figures 1A and B). The seed vigor of *M. bimucronata* was determined by the initial counting (Figure 1A). We observed that higher percentages of germination are occurred at temperatures of 30 °C and 20-30, according to the quadratic equation. Also, the water volumes of 89.44 and 75.25% equal to 2.67 and 2.65 times of the dry substrate mass are required for germination, respectively. In the water volumes of 2.70 and 2.69, the highest germination percentages were obtained with 81.69% (30 °C) and 97.3% (20–30 °C), respectively (Figure 1).

From these results, it is verified that the water volume of 2.65–2.70 times of the dry substrate mass provided more appropriately moisture required to activate the chemical reactions related to metabolism, triggering the recovery process development of the embryo. An example of such reactions is the hydrolysis of triglycerides by lipases, forming glycerol and fatty acids, part of which is later transformed into sugars, releasing energy for germination (Larcher, 2004).

For *Schizolobium amazonicum* (Huber ex. Ducke), the water contents of 2.5 and 3.0 times of the dry paper mass were the best for germination results (Ramos et al., 2006). Gonçalves (2008) found that the use of water volumes equivalent to 2.0, 2.5, 3.0, and 3.5 times the dry paper mass and temperatures of 25, 30, and 35 °C provided better expression of the physiological potential for seeds of *Parkia platycephala* Benth. (Mimosoideae).

Comparing several temperatures, germination performance was favored when subjected to a temperature of 30 °C. It also expressed a linear increase as a function of the increase in the volume of water used to moisten the substrate (Figure 2), corroborating with results of Rahman et al. (2011), who reported that the hydration temperature can greatly alter the viability and vigor of the seeds. Thus, water presents a key role in the development process, as long as the seeds change from a metabolically active to an inactive state after maturation, as the desiccation returns to the metabolically active state during germination (Guedes et al., 2010).

At a temperature appropriate to germination (30 °C), there will be a higher water imbibition speed, with rapid softening of the tegument and subsequent protrusion of the radicle, characterizing the ideal condition to trigger the germination process and establishment of the seedlings. The data corroborate with those obtained by Ramos et al. (2006) in which the seed germination rate index of *Schizolobium amazonicum* Huber ex Ducke was influenced by the temperature and volume of water in the substrate. For *Parkia platycephala* Benth., there was a linear increase in germination and germination speed index with increasing amount of water in the substrate (2.0, 2.5, 3.0, and 3.5 times the substrate weight), at 20 °C (Gonçalves, 2008). With *Amburana cearensis* also a linear increase was observed in the germination speed index by increasing the amount of water on the substrate at 30 °C (Guedes et al., 2010).

Osmotic conditioning

The seeds of *M. bimucronata* conditioned in pure saline solution (KNO_3) reached the highest water content (Table 3), followed by descending order of seeds immersed in KNO_3 + PEG solutions and PEG immersion, demonstrating that the imbibition was dependent on the properties of the solute used and also on the conditioning method. The ion NO^{3-} may have been absorbed by the seeds, reducing their osmotic

Table 1. Concentrations of the osmotic solutions at 25 °C, to obtain the osmotic potentials of -0.5 and -1.0 MPa.

Osmotic Solutions	Concentration (g/L of water) (-0.5 MPa)	Concentration (g/L of water) (-1.0MPa)
PEG 6000	141.514	283.029
KNO_3	11.850	23.711
PEG 6000 + KNO_3	70.757 + 5.925	141.514 + 11.850

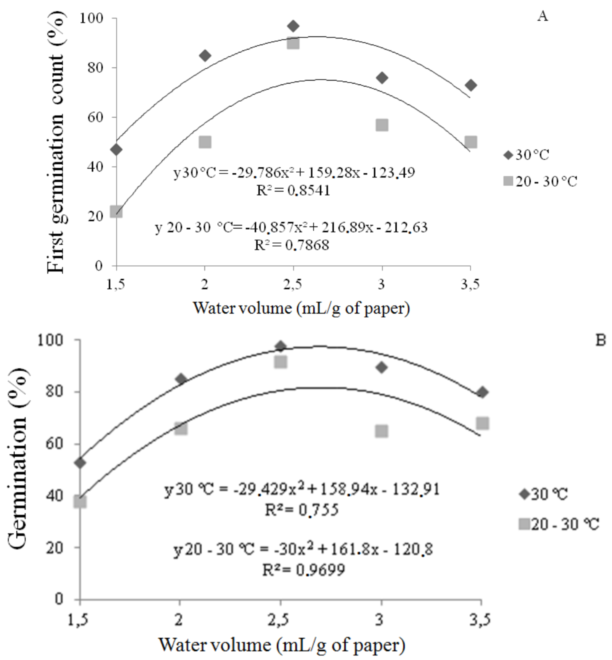

Fig 1. First count (A) and percentage of germination (%) (B) of *Mimosa bimucronata* seeds (DC.) O. Kuntze., submitted to different temperatures and volumes of water in the substrate.

Table 2. Germination (%) of seeds of *Mimosa bimucronata* (DC.) O. Kuntze submitted to different regimes of light and temperatures.

Qualities of Light	Temperatures (°C)			
	20	25	30	20-30
White	78 bA	94 aA	100 aA	94 aA
Dark	66 bB	81 aB	90 aB	81 aB
Red	69 bAB	83 aB	89 aB	85 aAB
Red-distant	70 bAB	85 aAB	89 aB	83 aB
F for temperature (T)	49.39 **			
F for light (L)	17.82 **			
F for interaction (T x L)	3.25 **			
CV (%)	6.15			

Means followed by the same lowercase letter in the row and upper case in the column do not differ by a 5% probability by the Tukey test. (**) Significant at the 1% probability level.

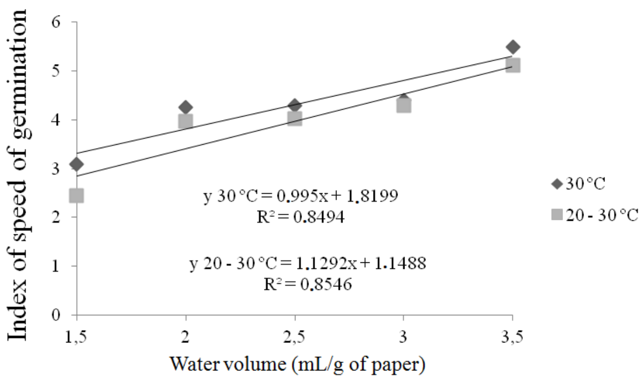

Fig 2. Mimosa seed germination speed index bimucronata (DC.) O. Kuntze, under different temperatures and water volumes on the substrate.

Table 3. Humidity of the seeds of *Mimosa bimucronata* (DC.) O. Kuntze submitted to osmoconditioning, using three solutes in two osmotic potentials, after drying under environmental conditions for 24 hours.

Solutions	Potenciais (MPa)	Time (hour.)			
		24	48	72	96
KNO_3	-0.5	19.76	18.99	17.38	17.01
	-1.0	18.12	18.11	18.11	17.04
KNO_3 + PEG	-0.5	17.78	16.99	16.58	16.48
	-1.0	16.98	16.54	16.50	16.45
PEG	-0.5	15.99	15.92	15.95	15.84
	-1.0	15.97	15.90	15.82	15.80

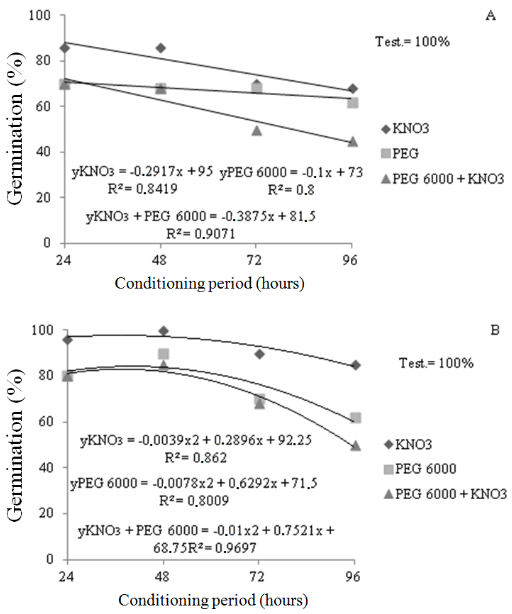

Fig 3. Percentage of germination (%) of *Mimosa bimucronata* (DC) O. KTZE seeds submitted to osmoconditioning in potentials of -0.5 (A) and -1.0 MPa (B).

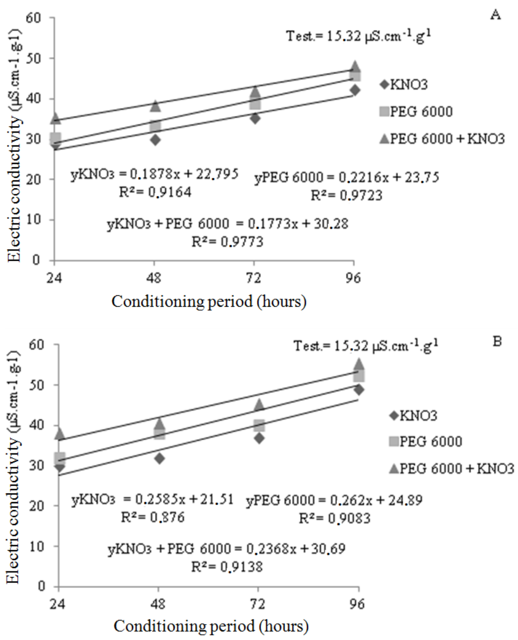

Fig 4. Electrical conductivity ($\mu S.cm^{-1}.g^{-1}$) of *Mimosa bimucronata* (DC) O. KTZE seeds submitted to osmoconditioning at potentials of -0.5 (A) and -1.0 MPa (B).

potential osmotic potential and stimulating the inflow of water, as verified by Frett and Morneau (1991).

It was verified that the germination of the seeds conditioned with PEG at −0.5 MPa behaved in a linear descending manner (Figure 3A), starting from 70% of germination after 24 h of conditioning, followed by gradual reduction, reaching 60% after 96 h. This fact can be elucidated by the decrease in the seed metabolism due to the lower availability of water for digesting the reserves and translocation of the metabolized products. This may have occurred due to the high molecular weight of polyethylene glycol, which prevents the penetration of water through the cellular membranes, also reducing the availability of oxygen by virtue of its high viscosity, thus affecting the germination process (Lima et al., 2009). For the potential of −1.0 MPa (Figure 3B), the quadratic equation was observed, in which the highest percentage of germination (71.49%) would be reached at a conditioning period of approximately 40 h. Possibly, this potential associated with the conditioning period of 40h, which provided adequate level of hydration during the seed imbibition stage. This allows the reactivation of the metabolic processes, culminating the growth of embryonic axis (Pereira and Lopes, 2011). Kissmann et al. (2010), worked on *Stryphnodendron adstringens* Mart., and reported a reduction in the germination percentage with an increase in the conditioning time under the osmotic potential of −1.0 MPa.

When KNO_3 was used at −0.5 MPa (Figure 3A), the germination was maintained at about 80% during 24 and 48 h time intervals, followed by further reduction. At −1.0 MPa, a quadratic adjustment was found (Figure 3B), reaching a maximum of 100% germination after 48 h of conditioning. The evaluation of nitrate use is important for the germination of certain seeds due to its action similar to nitric oxide and nitrite. Such substances are components of the signaling network that control seed dormancy (Bethke et al., 2006).

The combination of PEG + KNO_3 at −0.5 MPa (Figure 3A), created a linear decrease in behavior, from 70 to 45% germination, after 96 h of conditioning. When PEG + KNO_3 was used at a concentration of −1.0 MPa (Figure 3B), an increase was observed in the germination percentage (82.89%) in lesser time (37 h of imbibition), which was calculated based on the equation regression. Thus, it is evidenced that changes in the osmotic potential of the solutions and in the conditioning periods correspond to variations in seed germination (Reis et al., 2013). These disagreements are due to the fact that the ideal relationship between the osmotic potential and the conditioning period varies according to the species.

The treatments with PEG 6000, KNO_3, and PEG 6000 + KNO_3 (−0.5 and −1.0 MPa) did not reveal increases in the evaluated characteristic (electrical conductivity), exhibiting negative effects with an increase in the conditioning period (Figures 4 A and B). The leakage of solutes from the seeds; and therefore, higher value of electrical conductivity, is associated with the lower vigor of the seeds (Rech et al., 1999). According to Ishida et al. (1988), the reduction of water potential imposed by the osmotic agent reduces speed of seed imbibition and allows the restructuring of the cell membrane by reducing the water inlet and the leakage of solutes; however, Frett et al. (1991) speculated these results, in which solutes can leak from the seeds during the period of osmotic conditioning and interfere with the results of the conductivity.

Among the various laboratory tests that have been developed, the electrical conductivity seems to be mostly preferred due to its simplicity of execution, objectivity, and rapidity in the evaluation (Gonzales et al., 2009). According to Dias and Marcos-Filho (1996), experiments with several species have revealed that the decrease in germination and seed vigor is directly proportional to the increase in the concentration of electrolytes released by the seeds during imbibition.

Materials and Methods

Plant materials

The work was carried out in the Laboratory of Plant Propagation of the Center of Agricultural Sciences (CECA) of the Federal University of Alagoas (UFAL), located in the municipality of Rio Largo, AL, using seeds of *Mimosa bimucronata* (DC) O. Kuntze during March to May 2015. The fruits were harvested using aerial scissors with extensor cable from five matrices located in the municipality of Garanhuns, PE (8°53’25” S, 36°29’34” W and 842 m altitude). Prior to the commencement of the experiment and during the osmoconditioning test, the water content of the seeds was determined by the oven method at 105 ± 3 °C for 24 h (Brasil, 2009), using 4 replicates of 2 g of seeds packed in aluminum containers. The water content was calculated by mass difference based on the wet mass of the seeds.

The seeds were scarified manually and subsequently immersed in alcohol (70%) for 1 min and washed with distilled water prior to the experiment.

Light conditions

To simulate the light conditions, cellophane paper filters and fluorescent lamps were combined. For white light, the transparent plastic boxes (gerbox) (11 cm × 11 cm × 3 cm) were placed in clear plastic bags. The red light was simulated with two leaves of red cellophane. For the light regime far-red, two red cellophane sheets and a superimposed blue were used; and for darkness, gerbox black was used. The seeds were wrapped in two overlapping sheets of paper towel (*Germitest®*) and were placed in germinators with *biochemical oxygen demand* (B.O.D.), at constant temperatures of 20, 25, 30 °C and alternating at 20–30 °C, with a photoperiod of 8 h.

Humidity of substrate

To evaluate the adequate moisture of the substrate, two sheets of paper towels moistened with volume of water (mL) equivalent to 1.5, 2.0, 2.5, 3.0, and 3.5 times the mass of the dry substrate without addition of water, were placed in gerbox and maintained in germinators, which were regulated at a constant temperature of 30 °C and alternated at 20–30 °C.

Osmotic conditioning

For the osmotic conditioning of the seeds, the seeds were subjected to imbibition in different conditioning solutions:

PEG 6000, KNO_3, and PEG+KNO_3, under osmotic potential of −0.5 and −1.0 MPa, where they were preserved in a chamber with B.O.D., at a constant temperature of 25 °C, with testing times at 24, 48, 72, and 96 h of imbibition.

The PEG 6000 concentration was obtained according to the equation of Kaufmann (1973), and the concentration of KNO_3, according to the equation of Van't Hoff (Hillel, 1971). For the mixture of PEG 6000 and KNO_3, 50% of the osmotic potential was calculated for each solute, disregarding the interaction between the two products. Table 1 presents the concentrations of the products used to prepare the solutions.

The following variables were analyzed

a) Germination: The number of germinated seeds was recorded daily, where germination criterion was considered to be the initial radicle protrusion of approximately 2 mm in length until the 15th day (stabilization period) after sowing, when the percentages of normal seedlings were calculated (Brasil, 2009).
b) First germination count: The counts were performed collectively with the germination test, computing the normal seedlings of the first germination test count performed on the 3rd day after the test initiation (Brasil, 2009).
c) Index of speed of germination (IVG)**:** This was carried out in conjunction with the germination test, in which the number of seeds germinated daily from the 3rd to the 15th day after sowing, and the index was calculated according to the formula presented by Maguire (1962).
d) Electric conductivity (CE): Four replicates of 25 seeds were used for each treatment. The seeds were weighed to four decimal places and were placed in a disposable plastic cup (200 mL capacity), containing 75 mL of distilled water (EC < $\mu S \cdot cm^{-1} \cdot g^{-1}$) at a constant temperature of 25 °C (Gonzales et al., 2009). Four containers were withdrawn from the chamber at a time, for each treatment. After gently shaking the container, CE soaking solution was evaluated by a digital microprocessor conductivity "Gehaka" model CG 2000 and the results were expressed in $\mu S \cdot cm^{-1} \cdot g$.

Statistical analysis

The statistical analysis was performed through the computer program System for Analysis of Variance-SISVAR (Ferreira, 2011), with trials conducted in a completely randomized design with 4 replicates of 25 seeds, and the data was subjected to analysis of variance (ANOVA). While testing the light quality at different temperatures, the treatments were distributed in a 4 × 4 factorial scheme (temperatures and light regimes), and the means were compared by the Tukey test at 5% probability. The data were subjected to regression analysis for evaluation of substrate water volume and osmoconditioning,

Conclusion

The seeds are neutral photoblasts, being recommended the constant temperature of 30 °C with white light for the test of germination and vigor. Seeding on two sheets of paper, moistened with volume of water (mL) of 2.62–2.70 times the mass of the dry substrate is more suitable for conducting the germination test. The osmotic conditioning in seeds of *M. bimucronata* was not efficient for the maintenaning the germination of these seeds.

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