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GERMINATION OF *Parkia pendula* (Wild.) Benth. ex Walp. (Fabaceae) SEEDS UNDER DIFFERENT OSMOTIC POTENTIALS

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ABSTRACT
This work aimed to obtain information on the germination behavior of the species *Parkia pendula* ("Angelim-saia" in Portuguese) regarding osmotic potentials, establishing thus its tolerance level to environmental limitations and providing information about innapropriatte areas for its development. "Angelim-saia" seeds were subjected to dormancy break by using H_2SO_4 for 20 minutes and allowed to germinate at 30ºC under 12-hour photoperiod. To evaluate the effect of different osmotic potentials, seed imbibition was assessed using polyethylene glycol 6000 and the salts NaCl and $CaCl_2$ at the following potentials: 0; -0.2; -0.3; -0.4; -0.5; and -0.6 MPa. For each treatment, four replicates of 25 seeds were placed into transparent plastic boxes on filter paper moistened with 15mL of the solutions. Germination percentage and speed were daily calculated during eight days and the seeds that had root length equal to or greater than 2mm were considered germinated. The data were subjected to analysis of variance and means compared by the Tukey's test at 5% probability. Thus, *Parkia pendula* germination is completely inhibited between -0.5 and -0.6 MPa with $CaCl_2$. Osmotic stress was more pronounced with PEG and $CaCl_2$ than with NaCl, and the germination percentage and speed decreased with the reduction in the potential of different solutions.

KEYWORDS: Angelim-saia, forest seed, seed water content, germination.

INTRODUCTION

Water is the factor that starts germination and is both directly and indirectly involved in all the remaining steps of the germinative metabolism. Its participation is decisive in enzymatic reactions, solubilization and transport of metabolits, as well as in hydrolytic digestion of seed reserve tissues. Very low osmotic potentials delay and decrease germination; besides, seed chemical composition and tegument permeability interfere with the minimal water level needed to induce germination (Bradford, 1995). Each species has a critical water level for germination and a specific capability of removing it from the environment (Carvalho and Nakagawa, 2000).

Especially in the beginning of imbibition, very low water potentials influence seed water uptake, preventing the sequence of events in the germinative process. Water stress usually contributes to reduce seed germination speed and percentage, and for each species there is a water potential value in the soil below which no germination occurs. On the other hand, under plenty water availability in the soil, seeds – especially drier ones – can rapidly absorb water, which may rupture their tissues, damaging thus germination (Braga *et al.*, 1999). In tropical regions, groundwater is the main hydric source for forest species. It is subjected to frequent salinization due to the great air evaporationstrength, eliminating water from the soil surface, which becomes more salinized than deeper layers. Salts containing sodium, calcium and magnesium are the most common, and high salinization affects the soil water potential, the pH and the microbial activity. In general, the environment of seeds is more salinized than that of already established seedlings, the roots of which can use the less salinized portion of the soil profile (Agboola, 1998). The osmotic potential of salt solutions can be lower than that of embryo cells, difficulting the water uptake needed to germination. The lower germination of seeds subjected to water stress is due to lower enzymatic activities. Salinity affects germination not only by difficulting the water uptake kinetics, but also by facilitating the entrance of ions at toxic levels into the seeds during imbibition (Tobe *et al*., 2000; TORRES *et al*., 2000).

Studies involving seeds from several species have been carried out under water deficit conditions in order to investigate their germinative behavior (Braga *et al*., 2008; Sousa *et al*., 2008; Jacinto, 2007.; Fazolo, 2006.; Stefanello *et al.,* 2006.; Dickman *et al*., 2005.; Lima *et al.,* 2005; Fanti and Perez, 2004). Thus, several osmotic solutions including PEG, $CaCl_2$, KCl, and NaCl have been used to simulate an environment presenting reduced moisture. To choose a species for reforestation, it is necessary to know its biology and/or ecology. However, there is little information about

native species, which makes difficult the adoption of practices for the conservation or the recovery of degraded areas. Thus, studies concerning germination are needed to improve the scientific knowledge of species that in the future can fulfill the market demand for forest products.

P. pendula is known as "Angelim-saia" and belongs to the family Fabaceae, subfamily Mimosoideae. It is an evergreen heliophyte species characteristic of upland forests in the Amazon and Atlantic Rainforest region, occurring in the states of Amazonas, Pará, Pernambuco, Bahia, and Alagoas. Its wood is moderately heavy, soft, easy to work, and durable in indoor environments. It is a tall tree presenting up to 30m heigth and is considered ornamental especially due to the aspect of its inflorescences. This tree may be used in landscaping for afforestation of public squares, parks and large avenues; besides, it is useful to afforest degraded areas of permanent preservation due to its rapid growth in open environments (Lorenzi, 2002). This species has agronomical interest since it presents characteristics of symbiotic nitrogen fixation, which is important to form silvopasture systems (Souza Filho *et al*., 2005).

Based on the importance of the species *P. pendula*, the present work aimed to evaluate its germinative behavior under different osmotic potentials, establishing its level of tolerance to environmental limitations and providing information about areas that are inappropriate to its development.

MATERIAL AND METHODS

This work was carried out in the Germination Physiology Lab, Mato Grosso State University, UNEMAT, Alta Floresta Campus, Alta Floresta Municipality, Mato Grosso State, Brazil.

The *P. pendula* seeds were collected in Cláudia Municipality, Mato Grosso State, after collection, they were manually separated in order to remove impurities; thus, perforated seeds and those presenting abnormal shape were eliminated. The seeds were kept on the laboratory bench for drying; then, they were stored for 18 months (8.4% water content) in closed glass bottles maintained under refrigeration (approximately 10°C) until analysis.

To break the tegument dormancy, seeds were subjected to chemical scarification by immersion for 20 min in sulfuric acid (98%), with occasional shaking. Then, seeds were washed in running water for 10 min, followed by distiled water during the same period. Seeds received antifungal treatment with Ridomil (Metalaxyl) and Cercobin (Thiophanate-methyl) at 0.25% each, relative to weight.

To analyze the effect of different water potentials on the germinative process, polyethylene glycol (PEG 6000) was used at the potentials 0 (control); -0.2; -0.3; -0.4; -0.5; and -0.6 MPa, from which the solutions were prepared based on the calculations mentioned by Villela et al. (1991). To simulate salt stress and establish the maximum tolerance limit of *P. pendula* seeds to NaCl and $CaCl_2$, salt solutions were prepared based on the Van't Hoff equation, mentioned by Braga *et al.* (1999):

$\psi_{os} = -RTC$, where:
ψ_{os}: osmotic potential (atm);
R: gas constant (0.082 atm . l . mol^{-10} k^{-1});
T: temperature (ºK);
C: concentration (mol l^{-1}) (number of moles l^{-1}).

For each treatment, four replicates of 25 seeds were allowed to germinate in gerbox containing filter paper moistened with 15mL of the different PEG, NaCl and $CaCl_2$ solutions; then, seeds were kept in a BOD incubator at 30 ºC in the presence of light with four "daylight" fluorescent white lamps presenting 320W.cm^{-2} mean irradiance under 12h-photoperiod.

Germination counts were daily performed, and seeds presenting primary root ≥ 2mm were considered germinated (Rehman *et al.,* 1996). Calculations regarding germination percentage and speed were done according to Labouriau & Valadares (1976) and Maguire (1962), respectively, following the formules:

$$G\,(\%) = \frac{N}{A} \cdot 100$$

Where: N = number of germinated seeds;
A= total number of seeds allowed to germinate.

$$GSI = \frac{N_1}{D_1} + \frac{N_2}{D_2} + ... + \frac{N_n}{D_n}$$

Where: GSI = Germination Speed Index;
$N_{1:n}$ = number of emerged seedlings on day 1,...., n;
$D_{1:n}$ = days for germination occurrence.

Experimental design was completely randomized, at 6x3 (potentials x agents) factorial arrangement, totalling 18 treatments with four replicates. Means were compared by the Tukey's test at 5% significance.

RESULTS AND DISCUSSION

Based on the analysis of variance, there were significant differences for all factors, and a significant interaction was detected, indicating different behaviors of the levels of a factor inside another one for germination percentage and speed index (GSI) (Table 1).

TABLE 1. F values, general mean, coefficient of variation percentage, and minimum significant difference (MSD) regarding germination percentage and speed index (GSI) for *P. pendula* seeds according to the solution type and osmotic potentials.

Variation causes	Germination (%)	GSI
Solution types	67.85 **	216.09 **
Potentials	159.47 **	932.86 **
Solution types x Potentials	10.34 **	24.36 **
General Mean	5.73	7.28
Coefficient of Variation (%)	14.06	13.92
MSD Solutions	0.5602	0.7053
MSD Potentials	0.9704	1.2218
MSD Pot. (Solutions)	1.6807	2.1162
MSD Solutions (Pot.)	1.3721	1.7276

** indicates significance at 1 percentage, according to the F test.

TABLE 2. Mean values regarding germination percentage of *P. pendula* seeds subjected to different solution types and osmotic potentials.

Potentials (MPa)	Solutions		
	PEG	NaCl	$CaCl_2$
0	99.00 Aa	99.00 Aa	99.00 Aa
-0.2	50.00 Bb	74.00 Ba	67.00 Ba
-0.3	22.00 CDb	65.00 Ba	25.00 Cb
-0.4	31.00 Ca	41.00 Ca	5.00 Db
-0.5	16.00 DEb	50.00 Ca	2.00 Dc
-0.6	2.00 Ea	11.00 Da	0.00 Da

Means followed by the same uppercase letter in the column and lowercase letter in the line did not differ according to the Tukey's test at 5 percentage significance.

From -0.2 MPa, there was a significant reduction in *Parkia pendula* seed germination under all osmotic agents; at such potential, the lowest percentual value was observed when PEG 6000 was used (Table 2). Such fact could be explained by the high molecular weight of that osmotic agent, which is not absorbed and presents high viscosity; the latter added to the low O_2 difusion rate may damage the oxygen availability for seeds during germination (Braccini et al., 1996).

At -0.3 MPa, there was a negative influence of the $CaCl_2$- and PEG-induced water potentials; besides, from -0.4 MPa, lower percentual values were obtained in the presence of $CaCl_2$ (Table 2). Such results were similar to those described by Jeller & Perez (1997) for *Copaifera langsdorffi* seeds; the above-mentioned authors reported that $CaCl_2$ had a more pronunced inhibitory effect than NaCl. The same behavior observed by Braga *et al.* (2008) for seeds *Schizolobium amazonicum*. Whereas Jacinto (2007),detected significant reduction in the germination percentage of *Acacia mangium* seeds from -0.3 MPa under PEG and NaCl solutions and at -0.1 MPa under $CaCl_2$ solutions.

On the other hand, Fanti and Perez (1998) observed a lower decrease in the germination percentage of *Adenanthera pavonina* seeds under $CaCl_2$ solutions than under NaCl solutions at the same potentials. This behavior was reported by Perez & Tambeline (1995) for *Prosopis juliflora* seeds and by Fazolo (2006) for *Stryphnodrendron barbatiman* seeds.

Santos *et al.* (1992) stated that the lower germination of seeds subjected to water stress is due to the lower enzymatic activity, which leads to a lower meristematic development, whereas salinity affects germination not only by difficulting the water uptake kinetics but also by facilitating ion influx at toxic levels in seeds during imbibition (Bradford, 1995; Braccini *et al.*, 1996), which could explain the $CaCl_2$ effect on *Parkia pendula* seeds from -0.4 MPa.

The lower germinability is due to the higher imbibition time when the osmotic potential decreases, according to the triphasic pattern regarding the imbibition process described by Bewley and Black (1994): phase I is characterized by rapid water uptake as a consequence of the matricial potential of several seed tissues, whereas in phase II there is little or no uptake, followed by phase III, which presents intense water uptake and root protrusion. The duration of each phase depends on substratum hydration level, tegument permeability, seed size, and oxygen uptake.

The maximum tolerance limit of *P. pendula* seeds to the osmotic potential reduction due to $CaCl_2$ was observed at -0.6 MPa. At such potential, germination was completely inhibited. The same was not detected under PEG and NaCl, although they reduced the germination percentage by 97.98% and 88.89%, respectively, at the same potential relative to control (water). According to Larcher (2000), the protoplasm capability of tolerating high salt concentrations depends on the seletive compartmentalization of ions that enter the cell. Most ions from salts accumulate in vacuoles,

decreasing the salt concentration to which cytoplasm and chloroplasts are subjected and providing protection against the effects of salt stress. The compartmentalization in the vacuole occurs due to the action of ATPases. In this context, part of the energy is used to unevenly distribute ions inside the cell. The osmotic equilibrium between cytoplasm and the different cell compartments, such as the vacuole, is kept through the synthesis of organic compounds with osmotic activity. Soluble carbohydrates and amino acids contribute to the protection of biomembranes and proteins against the deletereous effects of high ionic concentration (Larcher, 2000).Germination speed values were significantly lower from -0.2 MPa under all osmotic agents (Table 3). Significant reductions in the germination speed from -0.2 MPa were also observed for *Copaifera langsdorffii* seeds under $CaCl_2$-induced salt stress (Jeller and Perez, 1997), *Senna spectabilis* under NaCl-induced salt stress (Jeller and Perez, 2001) and *Ateleia glazioviana* under PEG-induced water stress (RosA *et al.,* 2005).

TABLE 3. Mean values regarding the germination speed index of *P. pendula*. seeds subjected to different osmotic potentials and agents.

Potentials (MPa)	Solutions		
	PEG	NaCl	$CaCl_2$
0	24.75 Aa	24.75 Aa	24.75 Aa
-0.2	4.71 Bb	15.33 Ba	4.44 Bb
-0.3	1.50 CDb	10.69 Ca	1.68 Cb
-0.4	2.83 BCb	6.42 Da	0.40 Cc
-0.5	1.19 CDb	6.29 Da	0.08 Cb
-0.6	0.08 Da	1.17 Ea	0.00 Ca

Means followed by the same uppercase letter in the column and lowercase letter in the line did not differ according to the Tukey's test at 5 percentage significance.

At -0.2, -0.3 and -0.5 MPa, PEG and $CaCl_2$ influenced the germination speed relative to NaCl, whereas at -0.4 MPa, $CaCl_2$ had a more negative effect than PEG and NaCl, which could be related to the germinative vigor characteristics of the used seeds, since at -0.5 MPa the behavior of the osmotic agents PEG and $CaCl_2$ was not significantly different (Table 3). According to Pinã-Rodrigues *et al*. (2004), GSI is considered one of the simpler assays to evaluate vigor and is classified as an indirect evaluation method since it is carried out under laboratorial conditions and evaluates physical, physiological and biochemical characteristics that express the seed quality. Heydecker (1977) stated that the intensity of response to stress is variable among seeds from different species, and what is experienced as stress depends not only on the genetic constitution but also on the physiological condition of the seed. Jeller and Perez (1997) also reported that $CaCl_2$ osmotic solutions had a more pronounced effect on the germination speed reduction. As regards the results obtained by using PEG and $CaCl_2$ (Tables 2 and 3), the pattern of germination speed reduction under both agents was very similar, whereas germination percentage was either lower under PEG at -0.2 MPa or $CaCl_2$ at -0.4 and -0.5 MPa or similar at -0.3 MPa according to the solution type and potential. Such variations were due to the species adaptation to the level of external (water or salt) osmotic stress. Thus, under less negative potential values (-0.2 MPa), *P. pendula* seeds would have lower germination due to the lower water uptake, and under more negative potential values (-0.4 and -0.5), the more pronounced reductions would be due to the salinity nocive effects.

Salts presenting high solubility are the most nocive since seeds absorb salts together with water from the substratum; when excessively absorbed, these salts are toxic and consequently lead to physiological damages to the seeds, which reduces the germination potential (TORRES et al*.,* 2000). Ferreira and Rebouças (1992) also stated that high levels of salt, especially sodium chloride, can inhibit germination, which depends on the level of tolerance and/or resistence to salinity, according to the species and the salt type.

Since NaCl and $CaCl_2$ have similar solubilities, although $CaCl_2$ presents greater mass than NaCl at a certain temperature and solvent volume (100g/100mL and 36.5g/100mL at 30°C, respectively) (ON LINE, 2008; SOUZA, 2000), the size and charge attraction of such ions would probably be the factors that interfere with the uptake and even the toxicity. Na^+ is lower than Ca^{2+} and tends to be more rapidly absorbed. However, Ca^{2+} has two positive charges, which causes higher attractiveness to negative poles (for instance, O_2 from the water or even residual charges in the cell wall), inducing lower water chemical potential. According to Marenco and Lopes (2005), hydrophyllic molecules and ions presenting similar solubilities enter at rates inversely proportional to the size of the hydrated ion. Divalent cations (as Ca^{2+}) link to a larger number of water molecules than monovalent ones and are thus much more slowly absorbed.

In addition, Ashraf & O'Leary (1997) stated that the ion Na is capable of increasing the membrane permeability and reducing the selectivity during uptake. Thus, seeds exposed to NaCl solutions might have presented higher uptake speed

due to permeability alteration relative to $CaCl_2$ in the same period, resulting in differences regarding germination speed. When compared with other species, *Parkia pendula* seeds did not present a high limit of tolerance to stress, since they germinated until 0.6 MPa under PEG and NaCl and between -0.5 and -0.6 MPa under $CaCl_2$ due to the osmotic and ionic effects; thus, such species should be considered glycophilic, presenting moderate tolerance to the salts NaCl and $CaCl_2$. Among the species for which the limit of tolerance to salt stress was similar to that detected in *Parkia pendula* seeds are *Leucaena leucocephala* (Cavalcante and Perez, 1995), *Prosopis juliflora* (Perez and Tambelini, 1995); *Chorisia speciosa* (Fanti and Perez, 2003 and 2004); and *Dinizia excelsa* (Wottrich, 2007).

CONCLUSIONS

Based on the conditions under which the present study was carried out, the following conclusions can be drawn:

- *Parkia pendula* germination is completely inhibited between -0.5 and -0.6 MPa under $CaCl_2$;
- The osmotic stress induced by PEG and $CaCl_2$ was more pronounced than that under NaCl, and lower values of germination percentage and speed were detected with lower potentials of the different solutions.

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