

## Ecophysiology of Germination of *Parkia platycephala* Benth. Seeds

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### ABSTRACT

Water is considered the most important environmental resource for the germination process. This study aimed to evaluate the germination performance of *Parkia platycephala* seeds under different conditions of water and saline stress. Seed germination was tested with solutions of poly (ethylene glycol) 6000 and sodium chloride (NaCl) at seven osmotic potentials [0.0, -0.2, -0.4, -0.6, -0.8, -1.0, and -1.2MPa (megapascal)] and four replicates with 25 seeds in a completely randomized design, simulating the water and saline stress levels, respectively. The following seedling variables were evaluated: germination percentage, germination speed index, length, and dry mass. Results showed significant interaction between the agent and the osmotic potential for all evaluated variables. *P. platycephala* seeds presented higher tolerance to saline stress simulated by NaCl than to water stress, showing germination performance appropriate for glycophyte plants.

**Keywords:** forest seeds, salinity, osmotic potential.

## 1. INTRODUCTION

In forest seed technology, germination is considered the emergence and complete development of the embryo essential structures, showing the ability to produce a normal plant under favorable environmental conditions (Brasil, 2009). Many environmental factors interfere with the germination process, such as saline and water stress (Oliveira & Barbosa, 2014).

Water availability is the first condition for germination of a viable and not dormant seed (Carvalho & Nakagawa, 2012; Rego et al., 2011). Water deficit or excess directly influences germination, slowing it down and/or increasing it, and hinders oxygen uptake, reducing seedling survival (Carvalho & Nakagawa, 2012; Marcos, 2015). Therefore, the ability to tolerate water deficit at different stages of plant development, including germination, can be a crucial factor in its establishment and survival in certain environments, depending in large part on the morphological, anatomical, and physiological characteristics of seed and plant (Barbero et al., 2011).

In addition to water availability, high saline concentration is also considered a limiting factor to the germination process (Taiz & Zeiger, 2013), acting to reduce the amount of water available and, consequently, affecting the germination percentage and speed of the seeds and the formation of normal seedlings (Carvalho & Nakagawa, 2012). In addition, salinity can also lead to toxic effects on the seed, which can result in the embryo death (Santos et al., 2016). However, tolerance or resistance to salinity varies according to species (Gordin et al., 2012).

Knowledge about tolerance to water and saline stress of native forest species is still incipient, as in the case of *Parkia platycephala* Benth. Such information is of relevance for species conservation, in addition to serving as subsidy for studies addressing the implementation of forest projects, such as recovery and/or restoration of degraded areas, integration systems, etc. *Parkia platycephala*, popularly known as acorn fava or *faveira*, which belongs to the *Fabaceae* family, occurs in the northeastern part of Brazil in transition areas between the Cerrado (Brazilian savannah biome) or the Atlantic Forest and the Caatinga (Brazilian savannah). The wood of this species is used in the manufacturing of boxes, boards for internal

divisions in small constructions, linings, toys, as well as firewood and coal (Lorenzi, 2002). *Parkia platycephala* also presents a potential use in integrating systems of agricultural, forestry and pastoral activities not only because of its plasticity and rapid growth, but also of its fruits (pods), which are an excellent food source for ruminants when ripe (Alves et al., 2007), an extremely important characteristic especially in regions where herds undergo food shortage during certain periods of the year.

In this context, this study aimed to evaluate the germination performance of *P. platycephala* seeds under different conditions of water and saline stress.

## 2. MATERIAL AND METHODS

This study was conducted at the Forest Ecophysiology Laboratory of the Federal University of Piauí, Professora Cinobelina Elvas Campus (UFPI/CPCE), Piauí state, Brazil. The seeds were obtained from fruits harvested manually from ten mother-trees located in Cerrado-Caatinga transition areas in the rural area of the municipality of Bom Jesus, Piauí state, Brazil.

A completely randomized experimental design was used with treatments arranged in 2×7 factorial blocks [two osmotic agents – poly (ethylene glycol) 6000 (PEG 6000) and sodium chloride (NaCl), and seven osmotic potentials: 0.0; -0.2; -0.4; -0.6; -0.8; -1.0; and -1.2MPa] with four replicates of 25 seeds each, totaling 100 seeds per treatment.

Before installing the experiments, the *Parkia platycephala* seeds were submitted to mechanical scarification using 80 grit sandpaper on the side opposite to the micropyle, as recommended by Nascimento et al. (2009). After that, the seeds were disinfected with 5% sodium hypochlorite solution for 5 minutes and then washed with deionized water.

Subsequently, the seeds were sowed on blotter paper substrate and distributed into transparent plastic germination boxes (gerbox, 11×11×3cm) with lid. Next, the substrate was moistened with PEG 6000 and NaCl solutions, in the amount of 2.5 times the mass of the dry paper (Brasil, 2009), at the osmotic potentials of 0.0 (control, distilled water), -0.2, -0.4, -0.6, -0.8, -1.0, and -1.2MPa. The boxes were then placed in a Biochemical Oxygen Demand (BOD) germinator adjusted to the temperature of 30 °C under

continuous light. Every 48 hours, the solutions were replaced when needed, and the substrate (blotting paper) was substituted to avoid changes in the concentrations.

Saline solutions with NaCl were prepared according to the Van'tHoff equation (*apud* Salisbury & Ross, 1991), whereas PEG 6000 solutions were prepared according to Vilella et al. (1991).

When submitted to stress, the germination performance of *P. platycephala* seeds was evaluated, considering germination (%), adopting appearance of hypocotyl as criterion, with consequent emergence of cotyledons, and germination speed index (GSI), a variable determined by the germination test, in which normal seedlings were counted daily at the same time from first count until values became constant. To calculate the GSI, the formula proposed by Maguire (1962) was used for length of primary root and of aerial part - at the end of the experiment, the lengths of the primary roots and of aerial part of normal seedlings of each replicate were measured using a ruler and the results were expressed in cm.seedlings<sup>-1</sup> (Nakagawa, 1999); and for dry mass of the root system and aerial part, the normal seedlings previously measured were packed in identified Kraft paper bags, separated by replicate, and placed in oven at 80 °C for 24h. After that, the seedlings of each replicate were weighed on analytical balance with 0.001g accuracy, and the mean results were expressed in mg.seedlings<sup>-1</sup> (Nakagawa, 1999).

Data were submitted to tests of normality (Lilliefors) and homogeneity of variances (Cochran) and, subsequently, analyses of variance (ANOVA) and regression were performed using STATISTIC 8.0 software (StatSoft, 2008),

and the graphs were edited using Microcal Origin 8.0 software.

### 3. RESULTS AND DISCUSSION

Significant interaction ( $p \leq 0.05$ ) was observed between osmotic agent and osmotic potential for all evaluated variables (Table 1).

A gradual reduction in germination was observed as the osmotic potentials decreased; however, when comparing the osmotic agents (PEG 6000 and NaCl), germination was more affected by poly (ethylene glycol) (PEG 6000) solutions than by sodium chloride (NaCl) solutions (Figure 1). The highest mean germination percentage (84%) was observed in the control treatment (0.0 - distilled water), with reduction of 46% in PEG 6000 solutions at the osmotic potential of -0.2 MPa in comparison with the control treatment, reaching germination null values at the potential of -0.4 MPa, and the tolerance limit for germination ranged from -0.2 to -0.4 MPa, indicating that *P. platycephala* seeds are highly sensitive to water stress.

This fact can be assigned to the higher viscosity of the PEG 6000 solutions (Braccini et al., 1996), which creates a protective film around the seed, compromising moistening and oxygen absorption and reducing enzymatic activity. In contrast, the less drastic effect of NaCl on *P. platycephala* germination can be attributed to a possible mechanism of osmotic adjustment. Thus seed cells would reduce the osmotic potential by accumulating the outer saline ions in the vacuoles (Yamaguchi & Blumwald, 2005) by means of

**Table 1.** Summary analysis of germination variance and vigor of *P. platycephala* seedlings according to the tested treatments.

Variation source	DF	Mean Square					
		G	GSI	RL	APL	DMRS	DMAp
Osmotic agent (Oa)	1	53816.0*	423.4*	172.1*	217.7*	485.5*	3902.8*
Osmotic potential (Op)	6	2637.6*	60.8*	73.7*	45.9*	141.4*	697.9*
Agent × Potential (Oa × Op)	6	1995.3*	20.0*	14.0*	8.6*	30.9*	198.7*
Replicate	3	23.2 <sup>ns</sup>	0.7 <sup>ns</sup>	0.4 <sup>ns</sup>	0.0 <sup>ns</sup>	0.2 <sup>ns</sup>	0.6 <sup>ns</sup>
Residue	39	66.5	0.7	0.3	0.1	0.7	4.5
CV%		17.0	18.8	37.4	8.0	36.2	30.0

CV% = coefficient of variation in percentage; DF = degree of freedom; \*5% significant by the *F* test; <sup>ns</sup>not significant; G = germination; GSI = germination speed index; RL = root length; APL = aerial part length; DMRS = dry mass of root system; DMAp = dry mass of aerial part.

molecular synthesis, enzyme induction, and membrane transport (Henicka et al., 2006).

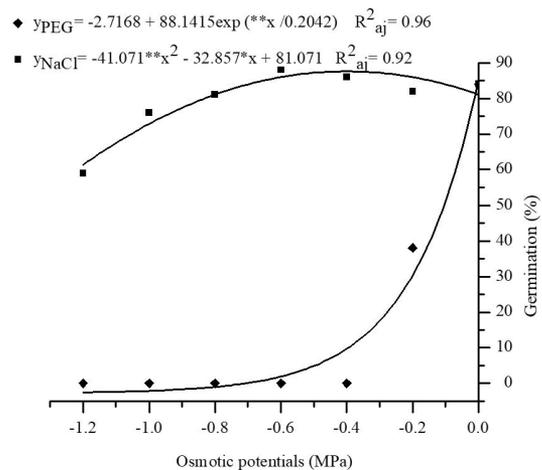
In this perspective, Pequeno et al. (2014) stated that some plant species make an osmotic adjustment to tolerate dehydration and keep the important biological processes active in plant germination and development under stress conditions. Knowledge about the tolerance limits of species is determinative to recommendations for planting in many ecological situations, especially those with high saline levels and low water availability (Rego et al., 2011).

Some research results have shown that seed tolerance to water stress varies according to species - from the most sensitive to the most tolerant, namely, (a) *Anadenanthera colubrina* (Velloso) Brenan., in which reduction in vigor was observed from the potential of -0.6 MPa (Rego et al., 2011); (b) *Piptadenia moniliformis* Benth., in which the seed germination process was compromised from water potentials lower than -0.6 MPa (Azerêdo et al., 2016); (c) *Chorisia glaziovii* O. Kuntze., which presented higher sensitivity and total germination inhibition at the potential of -0.3 MPa (Silva et al., 2016); and (d) *Myracrodruon urundeuva* Allemão. seeds, in which greater reduction in germination was observed from the potential of -0.6 MPa and a sharp reduction until nullity was found at osmotic potentials lower than -0.8 MPa (Virgens et al., 2012).

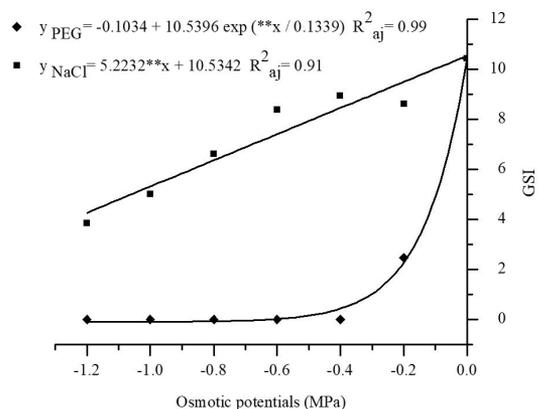
Results similar to those obtained for *P. platycephala* were found by Spadeto et al. (2012) when studying the effect of water and saline stress on seed germination of *Apuleia leiocarpa* (Vogel.) J. F. Macbr.; the authors reported that seed germination was more affected by PEG 6000 than by NaCl. Germination percentage reduction was also affected by increased PEG 6000 concentration in the study by Guedes et al. (2013) on *Apeiba tibourbou* Aubl. seeds.

Germination speed index (GSI) was also affected in greater proportion by PEG 6000 compared with NaCl (Figure 2). For the PEG 6000 solution, gradual reduction in GSI was found with increased concentration, reaching null values at the potential of -0.4MPa.

The decrease in the values of the evaluated characteristics can be associated with reduction in the hydration of seeds, as the water potential decreases, making the sequence of germination processes unfeasible, acting in the reduction of germination speed and percentage, considering that each species



**Figure 1.** Germination (%) of *P. platycephala* seeds under water and saline stress.



**Figure 2.** Germination speed index (GSI) of *P. platycephala* seeds under water and saline stress.

has a critical limit below which germination does not occur (Stefanello et al., 2008). The higher viscosity and molecular weight of PEG 6000 solution slow the speed of tissue hydration and oxygen diffusion, requiring a longer time for the membranes to reorganize and develop the metabolic processes (Antunes et al., 2011), which may result in lower germination speed. Results similar to those obtained by Moura et al. (2011) when working with *Mimosa caesalpiniiifolia* Benth. seeds have been observed in some studies that verified that water stress induced by PEG 6000 provided a greater reduction of GSI in comparison to saline stress simulated by NaCl. Martins et al. (2014) observed that *Eucalyptus camaldulensis* and *E. citriodora* seeds are

more sensitive to water stress simulated by PEG 6000 than to saline stress simulated by NaCl.

Regarding the root and aerial part length of *P. platycephala* seedlings, responses similar to those obtained for the aforementioned variables were also observed, with greater sensitivity to water stress induced by PEG 6000 (Figures 3A and 3B).

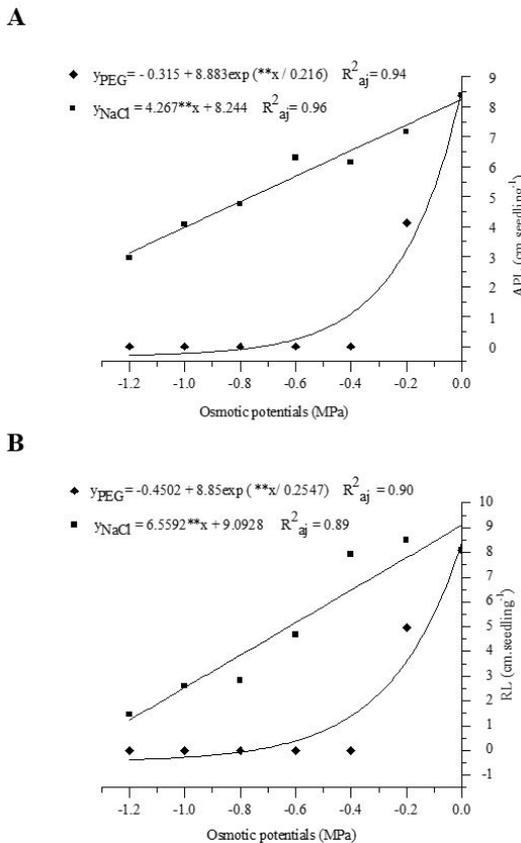
However, regardless of the osmotic agent used, a tendency of reduction in root and aerial part length values of seedlings was observed as the osmotic potential became more negative.

According to Taiz & Zeiger (2013), in addition to inhibiting the synthesis and/or the activity of hydrolytic enzymes needed for germination, water stress affects cell elongation and wall synthesis, compromising the physiological and biochemical processes of seeds, resulting in changes in seedling growth.

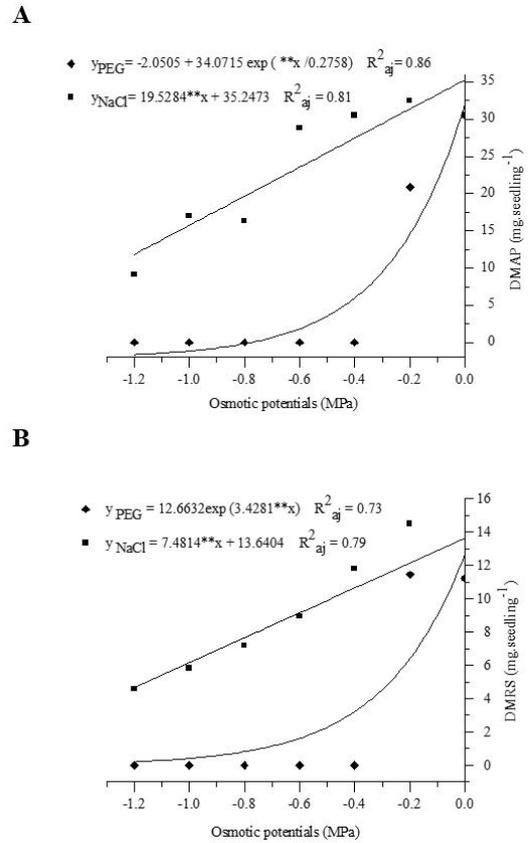
With reduction of hydric potentials, Pereira & Lopes (2011) found a reduction in the length of *A. leiocarpa* seedlings when submitted to PEG 6000 solution. In contrast to results of this study, Alves et al. (2014) found that saline stress induced by NaCl favored greater reduction in root length than water stress (PEG 6000) in *Cucumis anguria* L. seeds.

Figures 4A and B show a similar trend for the results of root and aerial part dry mass of *P. platycephala* seedlings, with more significant reduction observed in PEG 6000 solution. In NaCl solution, reduction was more evident from the potential of -0.8MPa.

Similar results were found by Guedes et al. (2013), who observed a decrease in the dry matter of *A. tibourbou* seedlings when the osmotic potentials in PEG solution presented higher solute concentration. For fennel (*Foeniculum vulgare* MILL.) seeds, reduction in



**Figure 3.** (A) Aerial part length (APL) and (B) root length (RL) of *P. platycephala* seedlings under water and saline stress.



**Figure 4.** (A) Dry mass of aerial part (DMAP) and (B) dry mass of root system (DMRS) of *P. platycephala* seedlings under water and saline stress.

seedling dry mass with decreased osmotic potentials (Stefanello et al., 2006) was also observed.

Water stress acts by reducing the speed of the physiological and biochemical processes, and thus, under conditions of lower water availability, as for solutions with more negative osmotic potentials, seedlings tend to present smaller growth, with consequent smaller seedling sizes and lower dry weight accumulation (Ávila et al., 2007), as found in this study for *P. platycephala* seedlings.

Results obtained in this study show that *P. platycephala* seeds tend to present greater tolerance to saline stress in the germination and initial seedling growth phases simulated by NaCl solution than to water stress simulated by PEG 6000 solution.

Considering these results, it is possible to conclude that *P. platycephala* presents ecological advantages in salinity conditions in relation to more sensitive species; in contrast, it presents low tolerance to environments with water limitations during the germination phase.

In this perspective, studies addressing the ecophysiology of seed germination of native forest species become relevant and needed, considering that research of this nature enable understanding of the adaptation mechanisms, the more compromised morphophysiological characteristics, and the tolerance limits of species to natural conditions, contributing to identification of potentialities and indication of the use of native species in programs for environment recovery and/or restoration. In addition, it contributes to the rational use and exploitation of native species.

#### 4. CONCLUSIONS

*Parkia platycephala* Benth. seeds are more sensitive to water stress induced by poly (ethylene glycol) 6000 than to saline stress simulated by sodium chloride. The germination performance of *P. platycephala* seeds is characteristic of glycoprotein plants, with moderate tolerance to saline levels.

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