

Seed viability of *Dimorphandra gardneriana* subject to water stress in different temperatures

Germinação e vigor de sementes de *Dimorphandra gardneriana* submetidas ao estresse hídrico em diferentes temperaturas

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ABSTRACT

The forest species *Dimorphandra gardneriana* Tul. is widely used for various pharmacological products, yet few basic studies have been undertaken to understand their ecological and physiological attributes under stress conditions. The goal of this research was to evaluate the seed germination and vigor when subjected to different osmotic potentials and temperatures. Water restriction was simulated with polyethylene glycol 6000 solution (PEG 6000) with osmotic potentials of 0; -0,2; -0,4; -0,6; -0,8, and -1,0MPa at temperatures of 20, 25, 30, and 35°C. The effect of the treatment was determined by the germination and vigor (germination speed index, length and phytomass of dry shoot and roots) of the seeds, in an entirely random design with four repetitions. From -0,4MPa it occurs a drastic reduction in germination and vigor of seeds although these factors were less affected at temperature of 25°C up to -0,2MPa.

Key words: Faveira, water restriction, forest species.

RESUMO

A espécie florestal *Dimorphandra gardneriana* Tul. é bastante utilizada para diversos produtos farmacológicos, mesmo assim há poucos estudos básicos para compreender seus atributos ecológicos e fisiológicos sob condições de estresse. Desta forma, o objetivo nesta pesquisa foi avaliar a germinação e o vigor de suas sementes quando submetidas a diferentes potenciais osmóticos e temperaturas. A restrição hídrica foi simulada com solução de polietilenoglicol 6000 (PEG 6000) nos potenciais osmóticos de 0; -0,2; -0,4; -0,6; -0,8 e -1,0MPa nas temperaturas constantes de 20, 25, 30 e 35°C. Na avaliação do efeito dos tratamentos, determinou-se a germinação e o vigor (índice de velocidade de germinação, comprimento e fitomassa seca de parte aérea e raízes) das sementes, em delineamento inteiramente ao acaso, em quatro

repetições. A partir de -0,4MPa ocorre redução drástica da germinação e vigor das sementes, fatores estes menos afetados na temperatura de 25°C até -0,2MPa.

Palavras-chave: faveira, restrição hídrica, espécie florestal.

INTRODUCTION

The species *Dimorphandra gardneriana* Tul., which belongs to the Fabaceae family, is popularly known as *fava d'anta*, *faveiro*, *favela*, and *barbatimão*. It is found mainly in the states of Maranhão, Bahia, Goiás, and Ceará (DÓRES, 2007). Its fruit has a high content of flavonoids such as rutin, quercetin, and rhamnase sugar, which form the basis of many pharmacological products such as eye drops (MENDES et al., 2005). According to the authors, rutin is a substance found in *fava d'anta* seeds and is an important flavonoid due to its vitamin P activity, which decreases the permeability of red blood cells, protects vitamin C against oxidation, and normalizes strength and permeability of capillary walls.

The *faveira* can also be found in the semiarid regions of Brazil - regions characterized by prolonged periods of drought with high temperatures; thus, limiting the development of forest species (FARIAS, 2008). Although typical vegetation of the region has tolerance to water stress, temperature increases due to global warming caused serious shifts in climate,

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especially in regions known for water shortages. Further studies on the behavior of species under these conditions will be necessary (ASSAD & PELLEGRINO, 2007).

The majority of forest species have seeds, which go through periods of dormancy hindering the production of seedlings for nurseries - mainly due to the lack of uniformity and long germination periods (COELHO et al., 2010). Among the various pre-germination treatments that can be used to overcome cutaneous dormancy are mechanical scarification (using friction to rub seeds against abrasive surfaces) and chemical treatment (usually with sulfuric acid) (MARCOS FILHO, 2005).

Water is involved directly or indirectly in all metabolic stages of germination. It plays a decisive role in enzymatic reactions, in the solubilization, and transport of metabolites, and as a reagent in the hydrolytic digestion of storage reserve tissues in seeds, such that very negative osmotic potentials retard and decrease the percentage of seeds that germinate (BRADFORD, 1995).

Among the various solutions that simulate conditions of water stress, polyethylene glycol (PEG) is widely used as an osmotic agent (HASEGAWA et al., 1984) due to the fact that it is an inert and non-toxic chemical compound with a high molecular weight that is not absorbed by the seed (HARDEGREE & EMMERICH, 1994).

Temperature is another factor that influences the rate of seed germination, dynamics of water absorption, limits and speed of biochemical reactions, in addition to the physiological processes that determine germination (MARCOS FILHO, 2005; CARVALHO & NAKAGAWA, 2012).

Thus, the objective of this research was to evaluate the germination and vigor of *Dimorphandra gardneriana* Tul. seeds that were subjected to different osmotic potentials and temperatures.

MATERIALS AND METHODS

The experiment was conducted at the Seed Analysis Laboratory of the Federal University of Paraíba, in the Center for Agricultural Sciences (UFPB-CCA), with *Dimorphandra gardneriana* Tul. seeds obtained from fruit collected from mother trees in the Araripe *chapada* plateau in the city of Crato, Ceará, Brazil.

To verify the effect of different water potentials on the germination process, polyethylene glycol (PEG 6000) was used as an osmotic agent. Solutions were prepared in accordance with the

procedure described by VILLELA et al. (1991). After PEG 6000 dosages were determined for each osmotic potential, seeds were subjected to tests to evaluate germination and vigor.

The germination test was conducted in a Biological Oxygen Demand (BOD) germination chamber regulated at constant temperatures of 20, 25, 30, and 35°C, with a photoperiod of eight hours of light and 16 hours of darkness using daylight fluorescent lamps (4x20W). For each treatment, 100 seeds were mechanically scarified with 120-grit metal sandpaper on the side opposite to the hilum, in order to overcome cutaneous dormancy. Then seeds were divided into four repetitions of 25 and distributed on two sheets of Germitest paper towel moistened with solutions that had osmotic potentials of 0; -0.2; -0.4; -0.6; -0.8; and -1.0MPa, in an amount equivalent to 2.5 times the mass of the dry paper. They were then covered with a third sheet, arranged in rolls, and packed in plastic bags.

First germination count was made on the thirteenth day and the final germination count was made on the twenty-fifth day after test initiation, which used normal seedlings as its criterion.

The speed of germination index was determined by daily counts of number of germinated seeds, at the same time each day, on days 13 to 25 after sowing. The index was calculated according to the formula ($SGI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n}$), proposed by Maguire (1962), where: SGI = speed of germination index, G_1 , G_2 , and G_n = number of seedlings counted in the first, second and last count; and N_1 , N_2 , and N_n = number of days from sowing until the first, second and last count.

Length shoots and of primary root of normal seedlings after each treatment and repetition was measured at the end of the germination test. Each part was measured using a centimeter ruler and results were expressed in seedling cm^{-1} . Dry phytomass of shoots and roots of the same seedlings that were measured in the previous evaluation were placed in Kraft paper bags and dried in an oven at 65°C until reaching a constant weight (48 hours), and then samples were weighed on an analytical balance with 0.001g precision. Results were expressed in seedling g^{-1} . The experiment and statistical analysis were carried out with a completely randomized design, with treatments distributed in a 4x6 factorial scheme (temperatures and osmotic potentials) in four repetitions; the data were subjected to the analysis of variance and polynomial regression.

RESULTS AND DISCUSSION

Germination of *Dimorphandra gardneriana* Tul. seeds was affected by the water potentials used., the germination percentage was reduced from -0.4MPa onwards, such that germination above this potential occurred only at the temperature of 25°C (Figure 1A). Results observed are consistent with expectations, since ROSA et al. (2005) reported that the osmotic potential of the solution in the germination medium controls the absorption of water by seed tissues;thus, impeding or preventing germination onset.

Low performance of seeds in more negative potentials was evident when they were subjected to temperatures of 20, 30, and 35°C, in which germination process was negatively affected, confirming that an ideal temperature of around 20 to 25°C is required for *D. gardneriana* seeds to germinate.

Water stress, as simulated with polyethylene glycol, also negatively impacted the germination of some Fabaceae species, such as *Adenanthera*

pavonina L. (FONSECA & PEREZ, 2003) and *Ateleia glazioviana* Baill. (ROSA et al., 2005).

A marked reduction as levels of osmotic potential of PEG 6000 solutions decreased was observed from results related to vigor as determined by the first germination count(Figure 1B). This sharp reduction in seed germination at higher PEG concentrations may be attributed mainly to a reduction in the amount of water absorbed by seeds, since CAMPOS & ASSUNÇÃO (1990) reported that reductions in seed vigor under water stress might be attributed to apparent inhibition of synthesis and/or activity of hydrolytic enzymes, which are necessary for germination as the concentration of the osmotic solutions increases.

Speed of germination index of seeds was also very negatively affected by decreases in the level of osmotic potential of PEG 6000 solutions (Figure 1C), similar to what occurred with respect to germination and vigor as determined by the first germination count.

Water stress can reduce both the percentage of seeds that germinate as well as the speed of

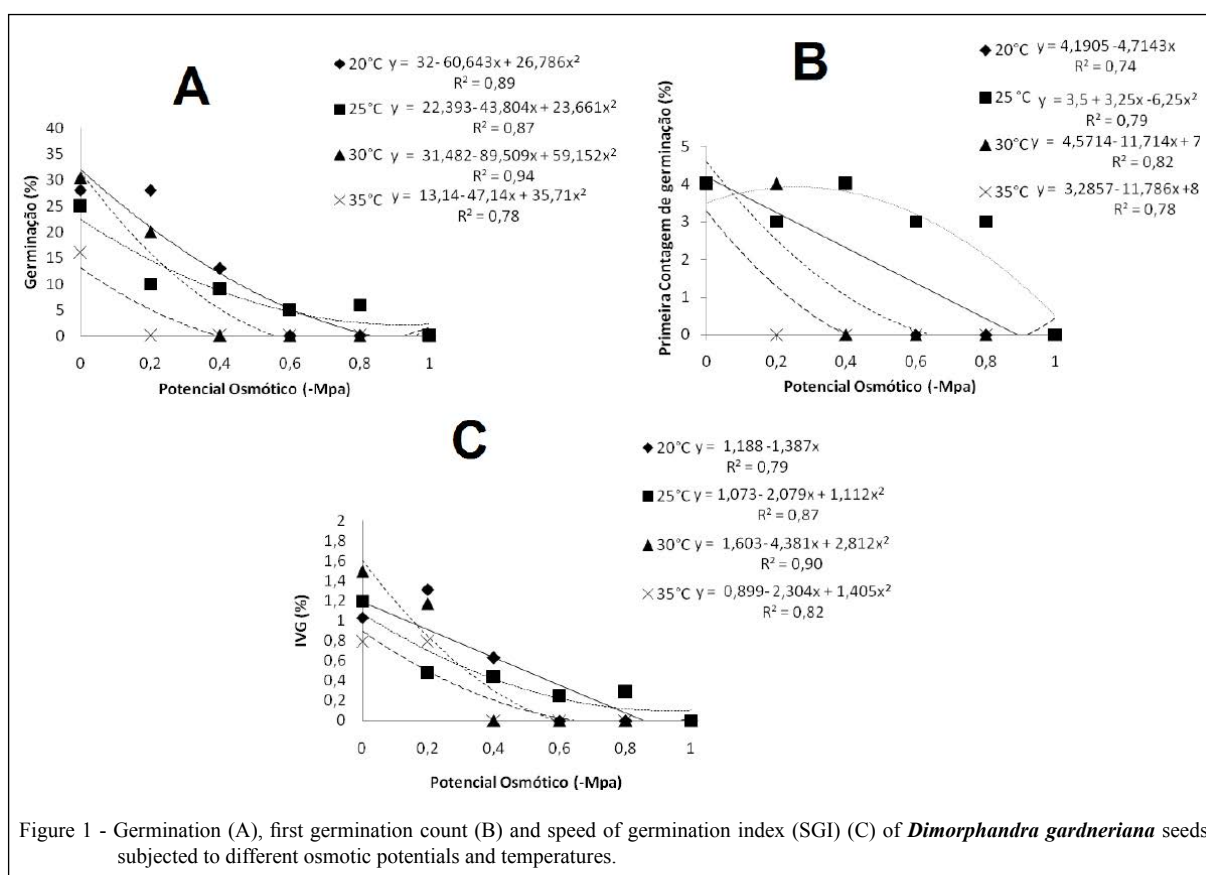


Figure 1 - Germination (A), first germination count (B) and speed of germination index (SGI) (C) of *Dimorphandra gardneriana* seeds subjected to different osmotic potentials and temperatures.

germination (PEREZ et al., 1998). For *Dimorphandra gardneriana* Tul. seeds, it was reported that, as osmotic potential increased, there was a need for more time for seeds to swell and germinate and a slower speed of germination was observed. BRUNI & LEOPOLD (1992) attributed reductions in the speed of germination to a probable loss of protoplasmic swelling, which causes disturbances in cell physiology and damage to systems of biomembranes.

For the *Adenantha pavonina* L. species, FONSECA & PEREZ (2003) observed an increased time required for germination when the osmotic potential of the control increased up to -0.5MPa. The same result was observed for seeds of the species *Senna occidentalis* (L.), H.S. Irwin & R.C. Barneyby (DELACHIAVE & PINHO, 2003) and *Chorisia speciosa*, St. Hil. (FANTI & PEREZ, 2003).

Length of shoots and roots of *D. gardneriana* seedlings was also negatively affected by different osmotic potentials and temperatures, in a way that was more significant and similar to the assessments of previous variables, since there was a decrease in the length of seedlings as the potentials became more negative (Figure 2A and B). Shoots and roots with the greatest lengths (8.5 and 5.5cm,

respectively) were obtained at the temperature of 30°C in the control treatment, when compared to seeds that were subjected to temperatures of 20, 25, and 35°C under the same conditions of stress.

In similar studies carried out with *Peltophorum dubium* (Spreng.) Taub., PEREZ et al. (2001) observed a decrease in the length of seedlings for osmotic potentials from -0.6 to -0.8MPa. ROSE et al. (2005), working with the same concentrations of PEG 6000 used for *D. gardneriana*, obtained similar results for the length of *Ateleia glazioviana* Baill seedlings.

With regard to the dry phytomass content of shoots and roots of *D. gardneriana* seedlings, the influence of different water potentials and temperatures was observed (Figure 2C and D). Dry phytomass content of shoots of seedlings from seeds that were not subjected to water stress was 0.0133g seedling⁻¹ at 20°C (Figure 2C) and, with respect to dry phytomass of roots of seedlings from seeds without water stress, the greatest value (0.0400g seedling⁻¹) was reported at 30°C. Thereafter, the dry phytomass reduced sharply, most notably at the temperature of 35°C (Figure 2D).

Water stress acts to reduce the speed of physiological and biochemical processes in

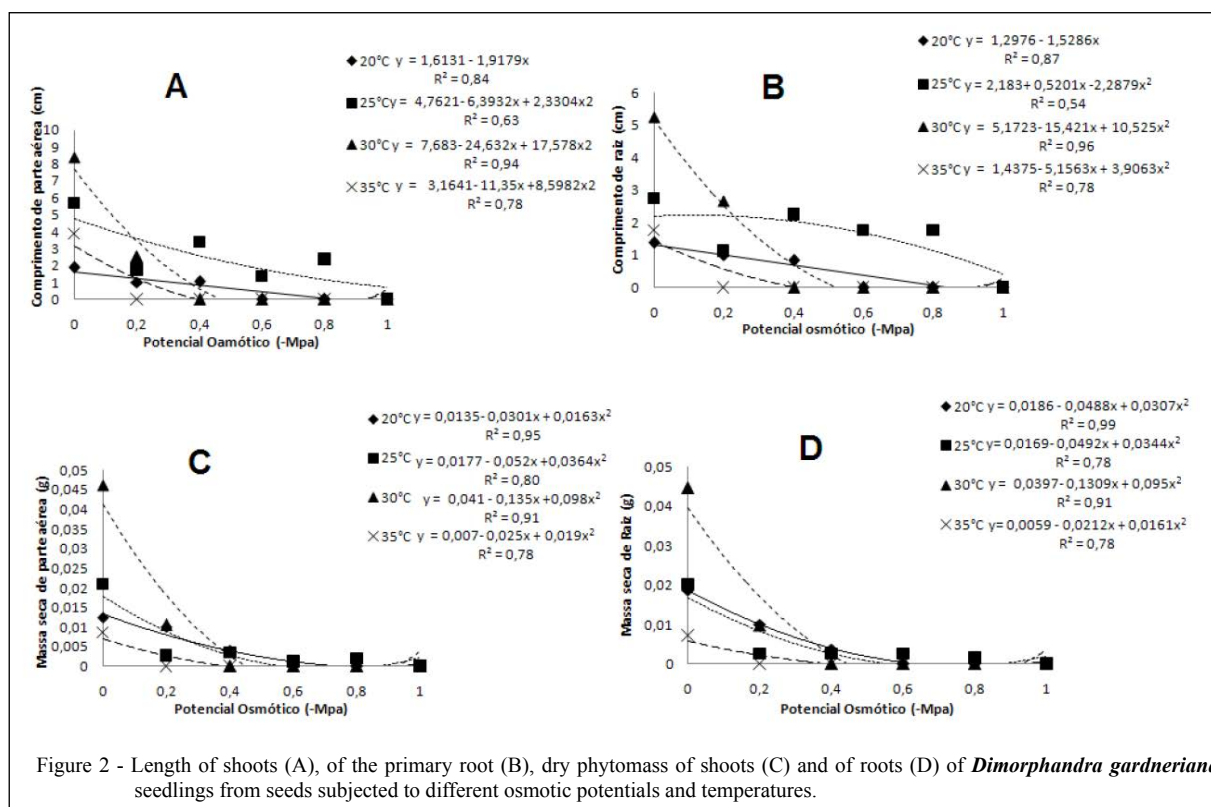


Figure 2 - Length of shoots (A), of the primary root (B), dry phytomass of shoots (C) and of roots (D) of *Dimorphandra gardneriana* seedlings from seeds subjected to different osmotic potentials and temperatures.

the seeds resulting in reduced metabolism, which directly affected the length and accumulation of dry phytomass in *D. gardneriana* seedlings under these stress conditions. BEWLEY & BLACK (1994) noted that the reduction in dry phytomass of seedlings as a function of water restriction occurs due to the difficulty of hydrolysis and mobilization of seed reserves.

In *Ateleia glazioviana* Baill. seeds, a decrease in osmotic potential led to significant reductions in dry phytomass of seedlings (ROSA et al., 2005). Some researchers reported that the germination percentage and speed of germination, as well as the accumulation of dry phytomass in low water potentials, might be preliminary indicators for species selection for colonization in environments that are subjected to water deficiency (PEREZ et al., 1998). The *D. gardneriana* species, due to its high tolerance for water stress, is adaptive because seedlings, which have a greater capacity to tolerate water stress, are able to prevail in dry environments and have a greater ability to establish themselves and survive in these environments.

CONCLUSION

Simulated water restriction with polyethylene glycol 6000 (PEG 6000), starting at -0.4MPa, dramatically reduces the germination and early development of *Dimorphandra gardneriana* Tul. Seedlings.

At a temperature of 25°C, germination and seed vigor are less affected by water stress up to -0.2MPa with regard to all of the characteristics that were assessed.

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