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Water stress and temperature on germination and vigor of *Handroanthus impetiginosus* (Mart. ex DC)

Paulo C. S. Santos[1], Clarisse P. Benedito[1], Tatianne R. C. Alves[2], Emanoela P. Paiva[3], Erivanessa C. Sousa[2] & Afonso L. A. Freires[2]

[1] Universidade Federal Rural do Semi-Árido/Centro de Ciências Agrárias/Departamento de Ciências Agronômicas e Florestais. Mossoró, RN. E-mail: paulocesaref@hotmail.com - ORCID: 0000-0001-7428-4091; clarisse@ufersa.edu.br - ORCID: 0000-0002-2846-1162
[2] Universidade Federal Rural do Semi-Árido/Centro de Ciências Vegetais/Departamento de Ciências Agronômicas e Florestais, Mossoró, RN. Email: tatianne_rcalves@hotmail.com - ORCID: 0000-0002-5729-3268 (Corresponding author); vanessac.sousa@hotmail.com - ORCID: 0000-0002-3787-8859; afonso_bot@hotmail.com - ORCID: 0000-0002-8711-2202
[3] Universidade Federal Rural do Semi-Árido/Centro de Ciências Agrárias/Programa de Pós-Graduação em Fitotecnia. Mossoró, RN. E-mail: emanuelappaiva@hotmail.com - ORCID: 0000-0003-4510-9205

Key words:
bignoniaceae
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A B S T R A C T

Water availability and temperature are among the main abiotic factors that influence seed germination and vigor, since they act directly on biochemical and physiological processes, which result in the production of the primary root. The objective of this study was to verify the effects of stress on germination and vigor of *H. impetiginosus* ('ipê-roxo') seeds under different temperatures and osmotic agents. The experimental design was completely randomized, in a 6 x 2 factorial scheme (osmotic potentials x temperatures) with four replicates of 25 seeds for each osmotic agent. In order to simulate water stress, polyethylene glycol (PEG 6000) and mannitol solutions were used and distilled water was used as control. Osmotic potentials of 0, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa were evaluated at temperatures of 25 and 30 °C, under 8 h photoperiod. The variables analyzed were: germination, germination speed index, shoot length, root length, and total dry matter of seedlings. Simulated water stress affected seed germination and seed vigor of *H. impetiginosus* at both temperatures and osmotic agents from -0.6 MPa.

Palavras-chave:
bignoniaceae
espécie florestal
estresse abiótico

Estresse hídrico e temperatura na germinação e vigor de sementes de *Handroanthus impetiginosus* (Mart. ex DC)

R E S U M O

A disponibilidade de água e temperatura estão entre os principais fatores abióticos que influenciam sobre a germinação e vigor de sementes, uma vez que atuam diretamente sobre processos bioquímicos e fisiológicos, que resultam na emissão da raiz primária. Dessa forma, objetivou-se verificar os efeitos do estresse hídrico sobre a germinação e vigor de sementes de *H. impetiginosus* (ipê-roxo) sob diferentes temperaturas e agentes osmóticos sob foto-período de 8 h. O delineamento experimental foi o inteiramente casualizado, em esquema fatorial 6 x 2, correspondente a seis níveis de potenciais osmóticos (0; -0,2; -0,4; -0,6; -0,8 e -1,0 MPa) e duas temperaturas (25 e 30 ºC), com quatro repetições de 25 sementes para cada agente osmótico (polietileno glicol-PEG 6000 e manitol). As variáveis analisadas foram germinação, índice de velocidade de germinação, comprimento da parte aérea e da raiz e a massa seca total de plântulas. A germinação e o vigor de sementes de *H. impetiginosus* são comprometidos em condições de estresse hídrico, à medida que o potencial osmótico decresce, em ambas as temperaturas e agentes osmóticos a partir de -0,6 MPa.

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Introduction

Handroanthus impetiginosus (Mart. ex DC) more commonly known as 'ipê-roxo', belonging to the Bignoniaceae family, has economic importance due to the physical-chemical features of its wood, which is appropriate to be used in civil and naval construction (Schulze et al., 2008), besides having ecological importance, being widely used for landscaping and recovery of forest ecosystems (Martins et al., 2012). This species occurs mostly in the Caatinga biome, where saline, sodic and water-deficient soils occur naturally in arid and semi-arid regions (Guedes et al., 2013), and affect all aspects of germination and plant growth, causing anatomic, morphological, physiological and biochemical modifications (Bezerra et al., 2003).

Another abiotic factor that also interferes with seed germination capacity is the temperature of the environment, whose variations affect germination speed, percentage and uniformity (Marcos Filho, 2015). To simulate water stress in the laboratory, different chemical compounds have been used, particularly polyethylene glycol (PEG 6000) and mannitol (Pereira et al., 2012).

Some forest species have already been evaluated regarding water and thermal stresses on seed germination, such as 'jurema-de-embira' (*Mimosa ophthalmocentra* Mart. ex Benth.) (Nogueira et al., 2017), 'catanduva' (*Piptadenia moniliformis* Benth.) (Azerêdo et al., 2016) and 'cumaru' (*Amburana cearenses* Freire Allemão) (Almeida et al., 2014).

Given the relevance of this topic and the scarce information about abiotic stresses on 'ipê-roxo' (*H. impetiginosus*) seeds, the objective was to evaluate the effects of water stress on germination and vigor of seeds of this species, under different osmotic potentials and temperatures.

Material and Methods

The study was carried out at the Seed Analysis Laboratory of the Center of Agricultural Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA) (5° 12' S; 37° 19' W; 20 m). The seeds were purchased at the Forest Garden belonging to the City Hall of Mossoró-RN, in September 2015, and remained in plastic bags stored in cold chamber (17 °C, 50% RH) during the entire experimental period.

Two experiments were conducted, one with the osmotic agent polyethylene glycol (PEG 6000) and the other with mannitol. In both, the experimental design was completely randomized, with treatments arranged in 6 x 2 factorial scheme (osmotic potentials x temperatures), with four replicates of 25 seeds.

To simulate water stress, osmotic solutions were prepared at the following potentials: 0, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa. PEG 6000 solutions were prepared according to Villela et al. (1991), while mannitol solutions were prepared according to the equation established by Van't Hoff (Salisbury & Ross, 1991).

For germination test, the seeds were planted on two Germitest® paper sheets and covered with a third sheet, moistened with the PEG 6000 and mannitol solutions or distilled water (0 MPa) in a volume of three times the dry paper weight, and maintained in Mangelsdorf-type germination chamber at temperatures of 25 and 30 °C, with 8-h photoperiod. Normal seedlings were daily counted until the 14th day after sowing.

Germination speed index (GSI) was calculated using the formula proposed by Maguire (1962), and normal seedlings were daily evaluated from the beginning of germination until the 14th day after sowing. At the end of the germination test, the total length of the normal seedlings was measured with a ruler graduated in millimeter, from the apical meristem to tip of the main root, and the results were expressed in centimeters. Then, seedlings were dried in a forced-air oven at 60 °C, until constant weight.

The data were subjected to analysis of variance by F test at 0.05 probability level and, according to the significance, the data were subjected to regression analysis ($p \leq 0.05$).

Results and Discussion

The interaction between osmotic potentials and temperatures, for both PEG 6000 and mannitol, was significant for all variables analyzed at 0.05 probability level (Tables 1 and 2).

The osmotic agent PEG 6000 caused a significant reduction in germination from -0.4 MPa, and the values were equal to 60 and 64% at temperatures of 25 and 30 °C, respectively, with zero germination from the potential -0.8 MPa on for the temperature of 30 °C (Figure 1A), whereas at 25 °C, germination was null only at 1.0 MPa. Mannitol caused linear reduction as the potentials became more negative and it was more accentuated at the temperature of 25 °C. In addition, differently from what happened with PEG 6000, germination occurred at all potentials and at both temperatures, evidencing higher sensitivity of *H. impetiginosus* seeds to PEG 6000 (Figure 1B).

This was probably due to the high molecular weight of PEG 6000, besides high viscosity. Coupled with low oxygen diffusion

Table 1. Summary of the analysis of variance for germination (G), germination speed index (GSI), shoot length (SL), root length (RL) and seedling dry matter (SDM) relative to *Handroanthus impetiginosus* seeds subjected to different temperatures and osmotic potentials induced by PEG 6000

Sources of variation	F values				
	Temperatures (T)	Potentials (P)	Interaction (TxP)	Mean	CV (%)
DF	1	5	5	-	-
G (%)	1.64^{ns}	344.99*	28.92*	47.52	10.42
GSI	71.61*	25.13*	14.72*	1.03	16.92
SL (cm)	46.03*	162.13*	15.14*	1.23	22.38
RL (cm)	18.84*	171.53*	14.44*	2.90	17.60
SDM (g)	35.44*	139.63*	8.61*	0.0155	15.02

*Significant at 0.05 probability level by F test; ^{ns}Not significant; CV - Coefficient of variation; DF - Degrees of freedom

Table 2. Summary of the F test for germination (G), germination speed index (GSI), shoot length (SL), root length (RL) and seedling dry matter (SDM) relative to *Handroanthus impetiginosus* seeds subjected to different temperatures and osmotic potentials induced by mannitol

Sources of variation	F values				
	Temperatures (T)	Potentials (P)	Interaction (TxP)	Mean	CV (%)
DF	1	5	5	-	-
G (%)	7.44*	5.82*	1.88*	67.75	13.59
GSI	2.76 ns	1.60 ns	2.58*	1.23	17.60
SL (cm)	237.40*	124.52*	8.27*	2.15	10.60
RL (cm)	84.87*	196.87*	13.47*	3.0	14.05
SDM (g)	1558.3*7	36.28*	35.94*	0.130	14.53

*Significant at 0.05 probability level by F test; nsNot significant; CV - Coefficient of variation; DF - Degrees of freedom

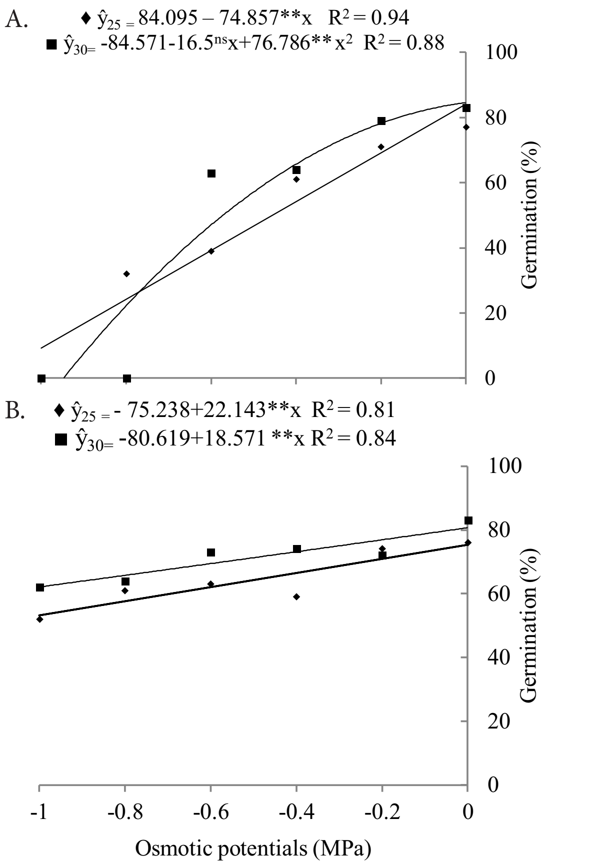

Figure 1. Germination of *Handroanthus impetiginosus* seeds subjected to different osmotic potentials induced by PEG 6000 (A) and mannitol (B) at temperatures of 25 and 30 °C

rate, it may compromise its availability to the seeds during the germination process (Antunes et al., 2011).

In addition, the reduction in germination when the osmotic potential is more negative results from the increase in the time corresponding to phase II of the soaking process, i.e., water absorption occurs more slowly, according to the three-phase pattern proposed by Bewley et al. (2013).

Various species exhibit distinct behavior when seeds are subjected to water stress simulated by PEG 6000, mannitol or any other osmotic agent (Azêredo et al., 2016). In *Erythrina falcata* seeds, germination decreased at potentials lower than -0.4 MPa (Pelegrini et al., 2013), whereas in seeds of *Piptadenia moniliformis* (Azêredo et al., 2016) and *Amburana cearenses* (Almeida et al., 2014) germination decreased at potentials below -0.6 MPa.

For *Myracrodruon urundeuva* seeds, highest germination rates were found at the potentials of 0, -0.2 and -0.4 MPa. From -0.6 MPa on, germination percentage decreased and, at osmotic potentials lower than -0.8 MPa, a sharp reduction occurred until germination was null (Virgens et al., 2012).

Germination speed index for both osmotic agents at temperature of 30 °C at 0 MPa showed mean value of 1.48, whereas at 25 °C it was equal to 1.26 (Figures 2A and B). This is due to the fact that, at higher temperatures, there is an acceleration of metabolic processes, shortening the phase II of the soaking process. Very low or very high temperatures may alter both germination speed and final percentage. Usually, low temperatures reduce germination speed, while high temperatures increase it (Nascimento, 2005).

For the PEG 6000, as the potentials became more negative the GSI decreased at both temperatures, and from -0.8 MPa at 30 °C it was equal to zero, whereas at 25 °C the index was

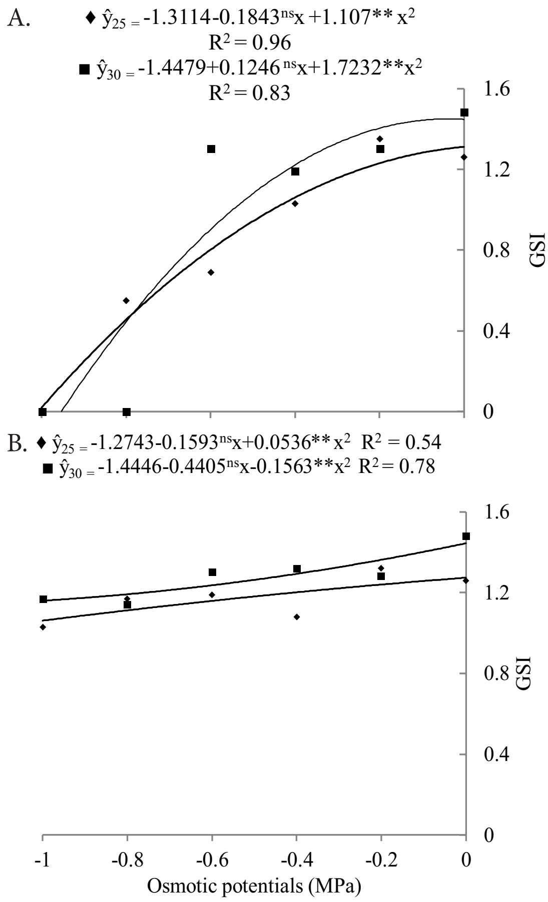

Figure 2. Germination speed index (GSI) of *Handroanthus impetiginosus* seeds subjected to different osmotic potentials induced by PEG 6000 (A) and mannitol (B) at temperatures of 25 and 30 °C

null only at -1 MPa (Figure 2A). For mannitol, there were less accentuated reductions in the GSI as the osmotic potentials decreased at both temperatures, and higher germination speed indices were found at 30 °C at all potentials, compared with the temperature of 25° C (Figure 2B). These results indicate that the temperature may intensify the water stress on *H. impetiginosus* seedlings, as observed for temperature of 30 °C when they were subjected to PEG 6000. Nevertheless, stress severity is lower at the same temperature when seeds are subjected to mannitol.

More negative osmotic potentials reduce water imbibition by seeds and may make the sequence of germination events unviable, acting on the reduction of germination speed and percentage, and each species requires a water potential below which germination does not occur (Stefanello et al., 2008).

Germination speed index was drastically reduced at potentials lower than -0.4 MPa in seeds of *Erythrina falcata* Benth. (Pelegrini et al., 2013), from -0.5 MPa in *Mimosa caesalpiniifolia* Benth. (Moura et al., 2011), in *Plantago ovata* Forsk. at potentials below -0.2 MPa (Sousa et al., 2008) and for *Foeniculum vulgare* Miller at osmotic potential of -0.1 MPa (Stefanello et al., 2006). These results indicate that each species has a maximum level of osmotic potential at which GSI is not affected.

The shoot length of *H. impetiginosus* seedlings was negatively affected by PEG 6000 at all temperatures from the water potential of -0.2 MPa (Figure 3A), whereas for mannitol the lowest values were recorded at both temperatures from -0.6 MPa (Figure 3B).

The difference in length can be explained by the fact that, when the necessary conditions for germination are provided, vigorous seeds originate seedlings with higher growth rate, due to the higher capacity to transform the reserves of the storage tissues and greater incorporation of these reserves by the embryo axis (Nakagawa, 1999).

Reduction in the shoots caused by water deficit induced by PEG 6000 was found by Lima & Torres (2009) in *Ziziphus joazeiro* Mart. seedlings. These authors found reduction in shoot length from the potential of -0.3 MPa. In *Moringa Oleifera* L. seedlings, Rabbani et al. (2012) found reduction in the shoots as water availability decreased, and the development was null from the potential of -0.3 MPa.

Regardless of the temperature studied, as the water potentials decreased, root length in *H. impetiginosus* seedlings was reduced, and the effect was more evident from the osmotic potential of -0.2 MPa for both osmotic agents. However, for mannitol, from -0.6 MPa on, the reduction no longer occurred at both temperatures (Figures 3C and D). The reductions observed in seedling root growth can be attributed to the reduction in the speed of biochemical processes due to water restriction, which interferes with imbibition and cell elongation of the embryo, possibly preventing primary root production by altering the permeability of the plasma membrane (Yamashita & Guimarães, 2010).

Dry matter accumulation in *H. impetiginosus* seedlings was also affected by the different osmotic potentials and temperatures. Using mannitol led to significant reduction from the osmotic potential of -0.6 MPa for the temperatures 25 and 30 °C (Figure 4B). For PEG 6000 at temperature of 25 °C, seedling dry matter fitted to a quadratic equation and, at 30 °C, this variable was less affected. Thus, significant reduction occurred from the potential -0.6 MPa (Figure 4A).

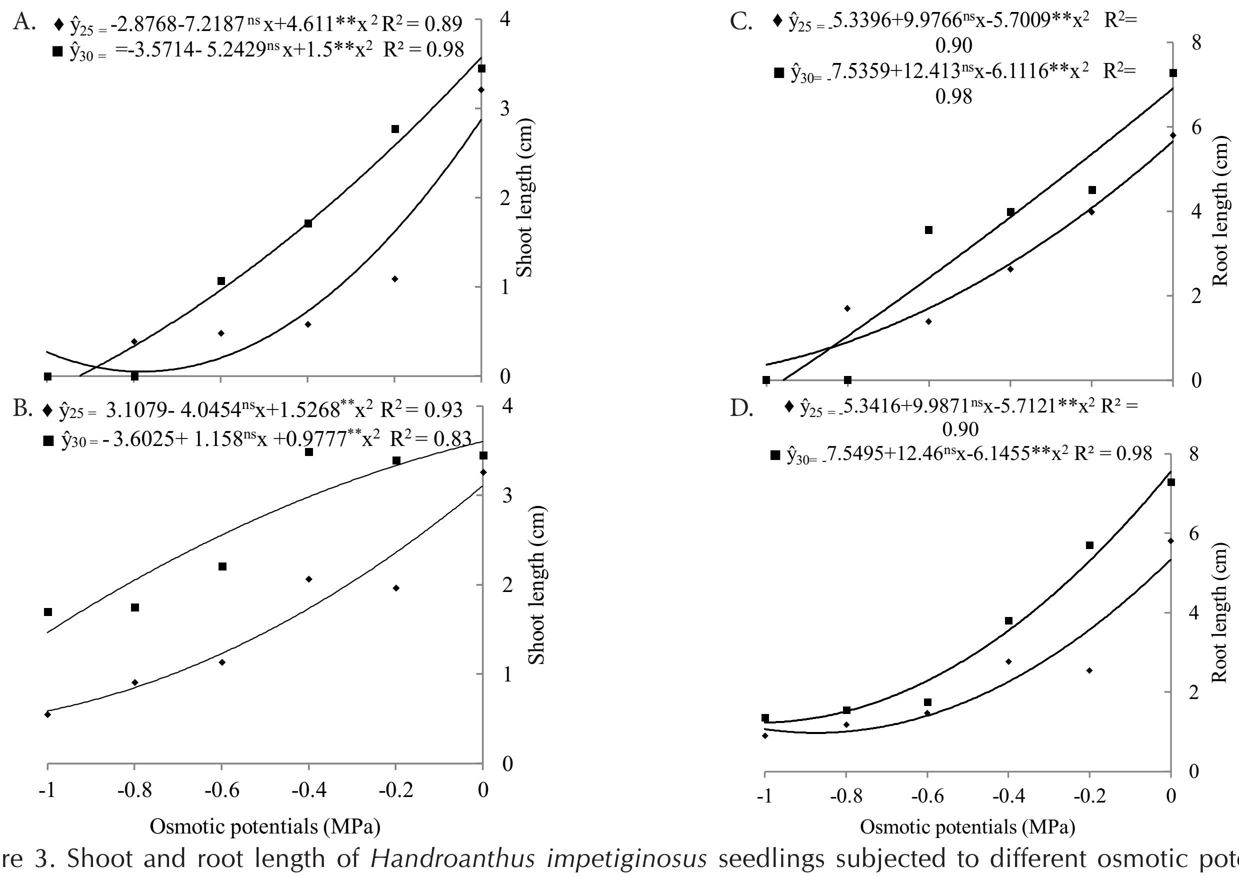

Figure 3. Shoot and root length of *Handroanthus impetiginosus* seedlings subjected to different osmotic potentials induced by PEG 6000 (A and C) and mannitol (B and D) at temperatures of 25 and 30 °C

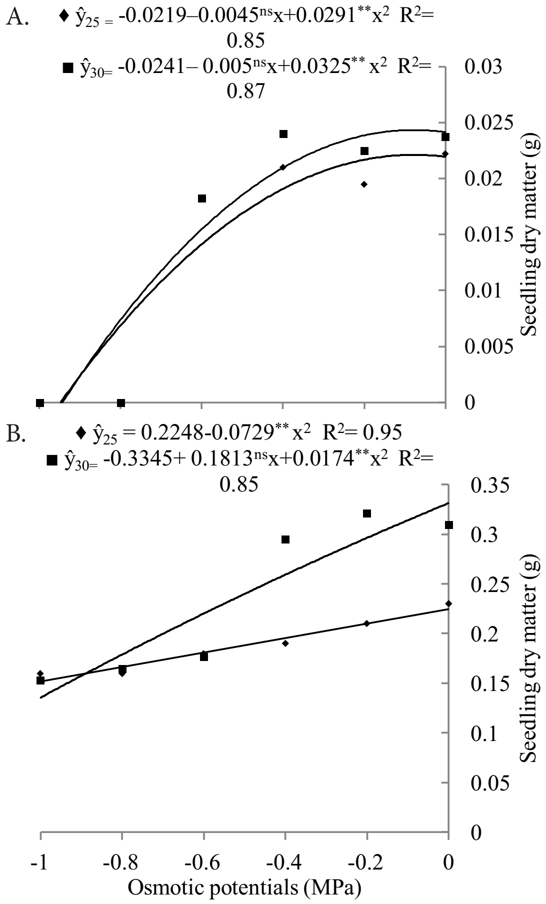

Figure 4. Dry matter of *Handroanthus impetiginosus* seedlings subjected to different osmotic potentials induced by PEG 6000 (A) and mannitol (B) at temperatures of 25 and 30 °C

Water restriction may reduce the speed of physiological and biochemical processes (Rabbani et al., 2012) and, consequently, *H. impetiginosus* seedlings may exhibit lower growth under low moisture conditions. In *Ziziphus joazeiro* Mart. seedlings, Lima & Torres (2009) observed progressive reduction of dry matter with the decline in osmotic potential induced by PEG 6000, from the potential of -0.3 MPa on, and the effects were more severe at -0.9 MPa.

Conclusion

Germination and vigor of *H. impetiginosus* seeds are compromised under water stress conditions as the osmotic potential decreases, for both temperatures and osmotic agents from -0.6 MPa.

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