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RESEARCH ARTICLE

GERMINATION OF SEEDS OF *ASPIDOSPERMA PYRIFOLIUM* MART

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ABSTRACT

Aspidosperma pyrifolium Mart. is native to the Caatinga in the northeast region and has great potential for the recovery of degraded areas, especially riparian forests; however, little is known about the influence of abiotic factors on its germination and vigor. Hence, this study aimed to evaluate the germination *A. pyrifolium* seeds subjected to different conditions of substrate, temperature and water and salt stresses. The experiments were carried out in two steps: the first one evaluated the influence of two temperatures and four substrates, while the second one evaluated the influence of water and salt stresses induced by two levels of NaCl and PEG 6000, plus a control (distilled water). Both experiments used four replicates of 25 seeds. For both experiments, the duration of the germination test was 10 days after sowing, period in which the following variables were evaluated: germination percentage, mean time of germination, shoot and root length, stem diameter, shoot and root dry matter and root/shoot ratio. All substrates at temperature of 25 ºC can be used for the germination test with seeds of *A. pyrifolium*. Water stress from the level of -0.3 MPa on is more severe on the germination and vigor of *A. pyrifolium* seeds in comparison to the salt stress.

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INTRODUCTION

A. pyrifolium Mart. is a regular-sized tree, native to the Caatinga in the northeast region, known as 'pereiro'. It occurs mainly in areas of river floodplains and terrains close to land elevations (mountains and plateaus). The species is one of the few indicated for the recovery of areas under desertification, due to its adaptation and tolerance to drought, salinity and sodicity of the soils (Sá *et al*., 2013). It stands out in areas of riparian forests because of its ecological importance and adaptation to the most severe conditions of drought and shallow or rocky soils (Amorim *et al*., 2005). The propagation of *A. pyrifolium* is mainly through seeds, but various abiotic factors can affect germination, such as: light, temperature, substrate, water availability and salinity. The optimal temperature for the germination of seeds of forest species varies according to its adaptation to the climate of the region of origin, so that, outside this range the seed does not fully express its vigor, leading to low germination index (Carvalho & Nakagawa, 2012). In addition, temperatures below the optimal range, during the development, lead to deformation of leaves and damage the apex of the plant, causing anomalies, such as stem ramification. Besides temperature, the selection of the ideal substrate is crucial for performing the germination test, since it is the physical medium in which the seed is placed with the function of maintaining it under adequate conditions of support and water availability along the germination process; thus, the following substrates are indicated: between sand, on paper (blotting paper) and on paper roll (Germitest® paper) (Brasil, 2013). Water and salt stresses are limiting factors, for decreasing the speed and percentage of seed germination. For each species, there is a value of soil water potential below which germination does not occur (Marcos-Filho, 2015). Germination and the establishment of plantlets of arboreal species are important steps for the survival of forest species, especially in areas with low availability and quality of water (Braga *et al*., 2009), as in the case of areas with salt-affected soils, which affect the development of the plants in different stages (Guimarães *et al*., 2013; Sá *et al*., 2013).

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Recently, studies on the effects of temperature, water stress and salinity on arboreal species have been conducted, such as Freitas *et al*. (2010), with 'jucá' (*Caesalpinia ferrea* Mart. ex Tul.); Guedes *et al*. (2011a), with 'barriguda' (*Chorisia glaziovii O. Kuntze*); Nogueira *et al*. (2012), with flamboyant (*Delonix regia* (Bojer ex Hook.) Raf.); Guimarães *et al*. (2013), with 'mulungu' (*Erythrina vellutina* Willd); Pelegrini *et al*. (2013), with 'corticeira-da-serra' (*Erythrina falcata* Benth., Fabaceae); Almeida *et al*. (2014), with 'cumaru' (*Amburana cearensis* (Allemao) A.C. Smith); Pereira *et al*. (2014), with 'fedegoso' (*Senna obtusifolia* L.); Lopes *et al*. (2015), with 'pau d'alho' (*Gallesia integrifolia* (Spreng.) Harms.) and Azeredo *et al*. (2016), with 'angico-de-bezerro' (*Piptadenia moniliformis* Benth.). However, studies with this focus related to *A. pyrifolium* are still inexistent. Thus, this study aimed to evaluate the germination of *A. pyrifolium* seeds subjected to different conditions of substrate, temperature, water stress and salt stress.

MATERIALS AND METHODS

The experiments were carried out at the Laboratory of Seed Analysis of the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró-RN, using *A. pyrifolium* from 16 matrix trees situated in five localities of Catolé do Rocha-PB (6°2'38''S, 37°44'48''W and altitude of 275 m). According to Köppen and Geiger, the climate of the region is classified as Aw, with mean annual temperature and rainfall of 26 ºC and 888 mm, respectively. After harvest, the seeds were manually processed and placed to dry in the shade for 3 days. Then, they were homogenized in Gamet-type divider, placed in plastic bag and stored in controlled environment (18 °C and 65% of relative humidity) until the beginning of the experiments. Previously, the germination test was performed in order to detect the presence of seed dormancy; however, there was no need of application of dormancy-breaking treatment. The first experiment used the completely randomized design, in a factorial scheme with two temperatures (25 and 35ºC) and four substrates (Germitest®, blotting paper, sand and vermiculite), constituting eight treatments with four replicates of 25 seeds. For the substrate on paper, the seeds were arranged in a transparent plastic box (11.0 x 11.0 x 3.5 cm), containing two sheets of blotting paper; before sowing, the substrate was moistened using distilled water with twice the weight of the dry paper. For the substrate paper roll, three sheets of paper towel (Germitest®) were moistened using distilled water, at the proportion of 2.5 times the weight of the dry paper, and placed in transparent plastic bags. The substrates sand and vermiculite (100 g of each) were arranged in transparent plastic boxes and moistened to 60% of field capacity. After sowing, the plastic boxes and the paper rolls were transferred to germination chambers (Biochemical Oxygen Demand - B.O.D.), regulated at constant temperatures of 25 and 35 °C and photoperiod of 8 h d^{-1}. The second experiment used a completely randomized design, with five treatments, corresponding to two levels of NaCl and two levels of PEG 6000, both at the osmotic potentials of -0.3 and -0.6 MPa, and the control (distilled water), with four replicates of 25 seeds. The saline solutions with NaCl were prepared using the equation of Van't Hoff, cited by Salisbury & Ross (1992), obtaining electrical conductivities of 8.33 and 16.67 dS m^{-1}, respectively. For the solutions of PEG 6000, the table proposed by Villela *et al*. (1991) was used. For both experiments, the germination test was conducted for 10 days after sowing, considering as normal plantlet the one that showed hypocotyl and protrusion of the primary root, with the results expressed in percentage. For the determination of the mean time of germination, daily counts were performed, at the same time, during the period of the germination test, considering the germinated seeds that met the standards of normality for the germination test. The mean time of germination was calculated using the formula recommended by Schuab *et al*. (2006) and the result was expressed in days after sowing. At the end of the germination test, in both experiments, the primary root and the shoots of normal plantlets of each replicate were measured using a ruler graduated in millimeters and the results were expressed in cm $plantlet^{-1}$. In addition, stem diameter of the plantlets was determined using a digital caliper and the results were expressed in mm $plant^{-1}$. For dry matter of roots (RDM) and shoots (SDM), the plantlets were cut, placed in Kraft-type paper bag, taken to a forced-air oven at 65 ºC until constant weight and weighed on an analytical scale (0.0001 g), with results expressed in mg $plantlet^{-1}$. Based on these data, the root/shoot ratio was determined by dividing RDM by SDM. The data were subjected to analysis of variance by F test and, in case of significance, Tukey test was applied at 0.05 probability level, using the statistical program SISVAR® (Ferreira, 2011).

RESULTS AND DISCUSSION

Experiment I

In general, there was no significant influence between the studied substrates for the variable germination, with similar responses between and at both temperatures used. However, the Germitest® paper promoted shorter time for germination and higher growth and accumulation of root dry matter in 'pereiro' plantlets (Table 1). In a study conducted with *Myracrodruon urundeuva* seeds, evaluating the interaction between temperature and substrate, Guedes *et al*. (2011b) observed that the temperature of 30 ºC combined with the substrates paper towel, arranged in the form of roll, between and on blotting paper, and between the substrates vermiculite, sand, plantmax® and bioplant®, caused high germination percentages. On the other hand, Bassaco *et al*. (2014) observed that the substrate vermiculite promoted higher germination percentage in *Sebastiania brasiliensis* seeds, in comparison to sand, which caused low germination under the same condition of temperature. These results indicate that the plant species has strong influence on the ideal conditions for tests of germination and vigor, including the choice of the substrate.

In addition, despite the satisfactory germination in the substrates blotting paper and vermiculite, at 25 ºC, with percentages higher than 90%, the growth of 'pereiro' plantlets was drastically reduced in these substrates in relation to plantlets germinated on Germitest® paper, with reductions of 53 and 48% in shoot length and 59 and 63% in root length, respectively (Table 1). As to the temperature, there were reductions in germination, growth of radicle and shoots, and in the accumulation of root matter in 'pereiro' plantlets

Table 1. Test of means for the variables: germination percentage (GP), mean time of germination (MTG), shoot length (SL), radicle length (RL), stem diameter (SD), shoot dry matter (SDM), root dry matter (RDM) and root/shoot ratio (RSR) of *Aspidosperma pyrifolium* Mart. plantlets for different substrates and temperatures

Substratos/Temperatura	GP (%)		MTG (days)	
	25 ºC	35 ºC	25 ºC	35 ºC
Germitest®	100.00 aA	92.50 aB	4.01 bA	4.17 cA
Blotting Paper	98.80 aA	97.50 aA	4.22 bB	4.74 bcA
Sand	100.00 aA	91.25 aB	4.94 aB	5.46 abA
Vermiculite	90.10 aA	88.75 aA	4.30 abB	5.27 aA
Substratos/Temperatura	SL (cm)		RL (cm)	
	25 ºC	35 ºC	25 ºC	35 ºC
Germitest®	6.26 aA	3.73 aB	11.34 aA	5.67 aB
Blotting Paper	2.92 bA	1.92 bB	4.63 bA	4.11 bA
Sand	5.22 aA	3.88 aB	6.54 bA	6.25 aA
Vermiculite	3.27 bA	2.21 bB	4.13 bA	3.77 bA
Substratos/Temperatura	SD (mm)		SDM (mg)	
	25 ºC	35 ºC	25 ºC	35 ºC
Germitest®	1.88 abA	1.88 aA	114.95 bA	111.95 aA
Blotting Paper	1.94 abA	1.43 bB	125.05 abA	114.50 aB
Sand	2.06 aA	1.89 aA	133.70 aA	117.00 aB
Vermiculite	1.72 bA	1.41 bB	127.55 aA	115.55 aB
Substratos/Temperatura	RDMM (mg)		RSR	
	25 ºC	35 ºC	25 ºC	35 ºC
Germitest®	16.55 aA	10.00 abB	0.14 aA	0.09 abB
Blotting Paper	12.45 bA	7.50 bB	0.10 abA	0.07 bA
Sand	14.55 aA	13.90 aA	0.12 abA	0.12 aA
Vermiculite	12.00 bA	6.40 bB	0.09 bA	0.06 bA

* Equal letters, lowercase in the column and uppercase in the row, do not differ by Tukey test at 0.05 probability level

Table 2. Test of means for the variables: germination percentage, (GP), mean time of germination (MTG), shoot length (SL), radicle length (RL), stem diameter (SD), shoot dry matter (SDM), root dry matter (RDM) and root/shoot ratio (RSR) of *Aspidosperma pyrifolium* Mart. plantlets under conditions of water and salt stresses

Tratamentos	GP	MTG	SL	SR
	(%)	(days)	(cm)	(cm)
Control (0,0 MPa)	98.80 a*	4.14 a	5.60 a	8.46 a
-0,3 MPa (NaCl)	100.00 a	4.93 b	3.79 b	2.89 c
-0,6 MPa (NaCl)	96.20 a	6.50 c	1.97 d	1.16 d
-0,3 MPa (PEG-6000)	93.80 ab	4.60 b	2.95 c	4.96 b
-0,6 MPa (PEG-6000)	80.00 c	7.01 d	1.01 e	1.68 cd
Tratamentos	SD	RDS	RDM	RSR
	(mm)	(mg)	(mg)	------
Control (0,0 MPa)	1.78 a	119.25 ab	10.35 a	0.09 a
-0,3 MPa (NaCl)	1.65 ab	123.40 ab	4.60 c	0.04 b
-0,6 MPa (NaCl)	1.74 a	128.95 a	2.25 d	0.02 b
-0,3 MPa (PEG-6000)	1.54 b	114.95 b	8.20 b	0.07 a
-0,6 MPa (PEG-6000)	1.23 c	122.50 ab	3.60 cd	0.03 b

* Equal letters do not differ by Tukey test at 0.05 probability level.

germinated on Germitest® paper and sand at temperature of 35 ºC, with decreases of 7.5 and 7.8% in relation to the temperature of 25 ºC. For the substrates vermiculite and blotting paper, there was no influence of temperature on germination. However, the increase in temperature from 25 to 35 ºC promoted increment in the mean time of germination of the seeds in these substrates, and also reduced the growth and accumulation of dry matter of the plantlets (Table 1). The results obtained in the present study reinforce the claim that the maximum germination potential of the seeds of most tropical and subtropical species occurs in the temperature range between 20 and 30 ºC (Borges & Rena, 1993). However, the seeds of *A. pyrifolium* are also able to extrapolate this range, as observed in seeds of *M. urundeuva* (Guedes *et al*., 2011b) and *Moringa oleifera* Lam. (Pereira *et al*., 2015).

Experiment II

The reduction in the water potentials from 0.0 MPa to -0.3 and -0.6 MPa induced by NaCl did not influence the germination percentage of *A. pyrifolium* seeds, and the results were similar to those in the control treatment. This was also observed in the reduction of the potential until -0.3 MPa induced by the PEG 6000. However, at the level of -0.6 MPa induced by the PEG

6000, there were drastic reductions (18.8%) compared with the control treatment (Table 2).

For the mean time of germination, the reductions of water potentials negatively affected the speed of germination of the seeds, which were more evident in the treatment of -0.6 MPa. Additionally, the potential induced by the PEG 6000 retarded in a more pronounced way the germination of *A. pyrifolium* seeds in relation to the potential induced by NaCl (Table 2). This denotes the more drastic effect of water stress on the germination of *A. pyrifolium* seeds, compared with saline stress, promoting greater limitations to the imbibition of the seed and, consequently, reduction of its germination potential.
The effects of water stress on the growth of *A. pyrifolium* plantlets were more intense in comparison to salt stress. Shoot and radicle length of the plantlets of this species were reduced by the increase in the matric potential induced by the PEG 6000 (Table 2). This phenomenon can be related to the fact that some plants show efficient mechanisms of tolerance to salinity, such as osmotic adjustment, causing salt stress to be milder than water stress (Esteves & Suzuki, 2008; Munns & Tester, 2008). Under the condition of salinity, there is water available in the substrate, but with limitations due to the pressure exerted by the salts, while the contrary is observed for the condition of water stress, where there is no water availability in the substrate, causing the osmotic adjustment to be less efficient (Massetto *et al*., 2014; Lima *et al*., 2015). Similar behavior has been reported by Freitas *et al*. (2010), with 'jucá' (*C. ferrea* Mart. ex Tul.); Guedes *et al*. (2011a), 'barriguda' (*C. glaziovii O. Kuntze*); and Nogueira *et al*. (2012), with flamboyant (*D. regia* (Bojer ex Hook.) Raf.), who also observed reductions in radicle growth as a function of the increase in salinity. The reduction in water potentials induced by NaCl did not affect the growth in stem diameter of *A. pyrifolium* plantlets. However, under conditions of water stress, there was a reduction in stem diameter, possibly due to higher water retention force simulated by the PEG 6000 in relation to NaCl, promoting lower water availability and, thus, compromising cell expansion and turgor (Taiz & Zaiger, 2013). The water stress promoted at the level of -0.3 MPa (PEG 6000) conditioned the *A. pyrifolium* plantlets to a lower shoot phytomass accumulation in relation to the other treatments. However, there was higher accumulation of dry matter in the root system in relation to the saline treatment, denoting that there was stimulation of radicle growth with the increase in water stress (Table 2). This observation was also reported by Pelegrini *et al*. (2013), with 'corticeira-da-serra' (*E.* Benth., Fabaceae); by Almeida *et al*. (2014), with 'cumaru' (*A. cearensis* (Allemao) A.C. Smith) and by Pereira *et al*. (2014), with 'fedegoso' (*S. obtusifolia* L.). However, the reduction of the water potential to -0.6 MPa, regardless of the conditioning factor, promoted reductions in the accumulation of root dry matter (Table 2), which can be related to the lower degradation of reserves of the seed, since the accumulation of shoot dry matter of the plantlets, at this level, was mainly due credited to the cotyledons, because of the low growth of the shoots. Therefore, the stress at the water potential of -0.6 MPa was so severe that it restricted seed imbibition and, consequently, there was lower degradation of reserve tissues. As to the root/shoot ratio of *A. pyrifolium* plantlets, there was a reduction in the distribution of reserves to the root system of the plantlets germinated under saline stress and for plantlets conducted under water stress at -0.06 MPa (Table 2). In regard to the salt stress, precisely at the level of -0.3 MPa, this phenomenon can be related to the expression of the mechanism of tolerance of this species in order to minimize the absorption of specific ions, promoting lower growth the root system and thus stimulating the exclusion of ions by the root (Syvertsen & Garcia-Sanchez, 2014).

Conclusions

- The substrates Germitest®, blotting paper, sand and vermiculite at temperature of 25 ºC can be used for the germination test of *A. pyrifolium* seeds.
- Water stress from the level of -0.3 MPa on is more severe on the germination and vigor of *A. pyrifolium* seeds in comparison to salt stress.
- The reduction in water potential to -0.6 MPa negatively affects germination, growth and dry matter accumulation of *A. pyrifolium* plantlets, regardless of the conditioning factor.

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