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WATER AND SODIUM CHLORIDE EFFECTS ON *Mimosa Tenuiflora* (WILLD.) POIRET SEED GERMINATION

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ABSTRACT - Water shortage and saline soils of the Brazilian semi-arid northeastern region are limiting factors to the development of many plants. Jurema preta (*Mimosa tenuiflora* (Willd.) Poiret) is a small, multiple use tree that abundantly colonizes unfavorable sites, including environments with severe water stress. This work had the objective of investigating the tolerance of jurema preta seeds to water and salt stresses during germination. Seeds germination in polyethylene glycol (PEG-6000) and sodium chloride (NaCl) solutions was analyzed under five different osmotic potentials (0.0; -0.3, -0.6, -0.9 and –1.2MPa), in order to simulate water and salt stress, respectively, in four 100-seed replications for each treatment. Seeds were placed into 10cmx10cmx4cm boxes, and germination accomplished in BOD germinator adjusted to 30ºC. The number of germinated seeds was monitored every 24 hours, and percentage and speed of seed germination were generated from these data. Mean percentage germination in the control treatment was ~95%, reducing to 63-53% at –0.9 to –1.2-MPa PEG solutions, and to 27-9.5% at NaCl solutions at equivalent osmotic potentials. Velocity of germination index was more affected, and decreased up to 1/8 of the control, at –0.6 MPa. Jurema preta seeds showed lower tolerance to NaCl than to water stress, and this species can be classified as a glycophyte.
Key words: Caatinga, jurema preta, speed of germination, osmotic potential.

EFEITOS DO ESTRESSE HÍDRICO E DO CLORETO DE SÓDIO NA GERMINAÇÃO DE SEMENTES DE *Mimosa Tenuiflora* (WILLD.) POIRET

RESUMO - A escassez de água e a salinidade nos solos da região semi-árida do Nordeste do Brasil são fatores limitantes ao desenvolvimento de muitas plantas. A jurema preta (*Mimosa tenuiflora* (Willd) Poiret) é uma pequena árvore de usos múltiplos que coloniza abundantemente sítios desfavoráveis, incluindo aqueles com severo déficit hídrico. Este estudo teve o objetivo de investigar a tolerância das sementes de jurema preta aos estresses hídrico e salino durante a germinação. A germinação das sementes em soluções de polietileno glicol (PEG-6000) e de cloreto de sódio (NaCl) foi testada sob cinco (0,0; -0,3; -0,6; -0,9 e –1,2MPa) níveis de potencial osmótico, para simular estresses hídrico e salino, respectivamente, em quatro repetições de 100 sementes. As sementes foram colocadas em gerboxes de 10cmx10cmx4cm, e a germinação ocorreu em germinador tipo BOD ajustado a 30ºC. O número de sementes germinadas foi anotado diariamente, e a percentagem e a velocidade de germinação foram calculadas a partir desses dados. A percentagem de germinação do tratamento testemunha foi em média ~95%, reduzindo para 63-53% quando submetidas a soluções de PEG com potenciais osmóticos entre –0,9 e –1,2MPa, enquanto em soluções salinas de potenciais equivalentes a germinação se reduziu a 27-9,5% das sementes. O índice de velocidade de germinação foi mais afetado, e no potencial osmótico de –0,6MPa decresceu para até 1/8 do valor

médio observado no tratamento testemunha. As sementes de jurema preta mostraram menor tolerância ao estresse salino do que ao hídrico, e esta espécie pode ser classificada como um glicófita.
Palavras-chave: Caatinga, jurema preta, velocidade de germinação, potencial osmótico.

INTRODUCTION

Jurema preta (*Mimosa tenuiflora* (Willd) Poiret) is a pioneer legume tree, native of the semi-arid area of Brazil's northeast region, which is found in pure stands on shallow soils. It produces forage for cattle, sheep and goats, as well as firewood and charcoal of high calorific power, making it a plant of high potential for exploration in that region (LIMA, 1996). It may also help in alleviating problems of salt and water stresses as its pivotal root system can increase soil permeability, salt lixiviation, and lower water table level (OLIVEIRA, 1997; LEITE, 2002).

Saline soils are widespread on Earth, and their genesis may be natural or accelerated by incorrect agricultural practices. It is estimated that approximately 10% of the cultivated areas in the world are affected by salt. In the semi-arid area of northeast Brazil, more than 9,000,000 ha (about 10% of the total) show a natural high salt level, and human activity is making things worse, especially in irrigated areas. Salt accumulation may convert agricultural soils in unfavorable environments, reduce local biodiversity and limit growth and reproduction of plants (FLOWERS *et al*., 1977; FONSECA & PEREZ, 1999; OLIVEIRA, 1997).

Most of the cultivated plants are sensitive to salt stress. These plants are known as glycophytes, as is the case of beans (*Phaseolus sp*.) that show a 50% yield reduction at 60mM. In contrast, halophytes are less sensitive to salt stress, as is the case of sugar beet (*Beta vulgaris*) that shows a 50% yield reduction at 260mM (GREENWAY & MUNNS, 1980). Both glicophytes and halophytes, mainly the former one, reduce percentage of germination and speed of emergence when salt concentration is increased (UNGAR, 1982).

High level of salts, especially sodium chloride (NaCl), can inhibit seed germination due to osmotic and toxic effects. Low external water potential can inhibit enzymatic activity of seed and delay radicle development and emergence ((DELL'AQUILLA, 1992; PEREZ & TAMBELINI, 1995).

There are many physiology studies on plant resistance to salts, most of them based on percentage of seed germination and speed of emergence index in saline substrata. Alterations in these parameters compared to a salt-free substratum are normally used as indicators of the degree of tolerance of the species to salt stress (FANTI & PEREZ, 1998).

Water is one of the most important environmental factor influencing seed germination as it triggers the germination process and remains involved, direct or indirectly, in all other subsequent stages of plant metabolism. It decisively participates in enzymatic reactions, metabolite solvency and transportation, and is a reagent itself in the hydrolytic digestion of proteins, carbohydrates and lipids from seed reserve tissues. Water and salt stresses are correlated, as excess of soluble salts reduces soil water potential and, thus, hinders water absorption by seeds and plants in general (CAVALCANTE & PEREZ, 1995).

In seed germination experiments, different osmotic potentials are obtained using aqueous solutions of polyethylene glycol (PEG). It simulates standardized conditions of water stress, as PEG is considered chemically inert and non-toxic (LUCCA & REIS, 1995). In general, salt solutions are considered to have a greater effect on seed germination than PEG solutions of the same osmotic potential (FANTI & PEREZ, 1998).

As a multiple use and widespread tree in the semi-arid area of northeast Brazil, jurema preta assumes a great ecological and economical importance. It is necessary to know its ecophysiologic needs to effectively explore this tree in sites where other species do not thrive. The objective of this work was to verify the effects of salt and water stresses in the germination process of jurema preta seeds.

MATERIAL AND METHODS

Experiments were conducted at the Laboratory of Botany, Department of Forest Engineering of the Federal University of Campina Grande (LB-DEF/UFCG), Patos (PB), Brazil, from October to November 2002. Local coordinates are 7°5'South and 35°15' West.

Jurema preta seeds were collected in July of that year, from native Caatinga forests of two UFCG Experimental Stations, and stored in plastic bags, refrigerated at 5°C.

The seeds were cleaned by immersion in a 2.5% solution of sodium hypochlorite for five minutes and washed five times with distilled water. Seed dormancy was broken by immersion in hot water (85°C) for 30 seconds and, subsequently, in running tap water for one minute.

Salt stress (Experiment 1) was simulated by NaCl solutions, according to J. H. van't HOFF's equation, and water stress (Experiment 2) was simulated by polyethylene glycol-6000 (PEG 6000) solutions, according to tables in Villela *et al*. (1991). Treatments corresponded to five different osmotic potentials (T0= 0.0, T1= -0.3, T2= -0.6, T3= -0.9, and T4= -1.2 MPa), and were replicated four times in a completely randomized design. Each replication consisted of 100 seeds.

The seeds were distributed in 10cmx10cmx4cm plastic boxes (gerbox), on a single layer of germitest paper moistened with 12 ml of the solution of each treatment. Osmotic potential was controlled every two days by replacing germitest paper and solution in each gerbox.

Germination took place in a BOD Germination Chamber, under constant 30°C temperature. Pathogens were controlled by Nistatina (bactericide) and Captan (fungicide), added to the experimental solutions at 1% (volume/volume) and 2% (weight/volume) rates, respectively.

The number of germinated seeds was counted every day, and each germinated seed was discarded after counting. These data were used to calculate percentage of seed germination and speed of germination index.

Germination was considered to have occurred when a protruded radicle was at least 2mm long. Deteriorated seeds were discarded daily to reduce pathogen contamination. Data collection stopped when no seed germination occurred during four consecutive days.

The remaining pathogen-free seeds were washed in distilled water, placed in gerboxes with germitest paper and 10ml of distilled water, and replaced into the germination chamber to evaluate seed viability after water or salt stress.

Percentage of seed germination ($G_{\%}$) was obtained by $G_{\%} = 100 * A / N$. "A" is the number of germinated seeds, and "N" is the total number of seeds put to germinate, in each replication (FANTI and PEREZ, 1998).

Speed of germination index (S) was calculated according to the following Maguire's formula (JOSÉ *et al*., 1999): $S = \frac{E_1}{N_1} + \frac{E_2}{N_2} + ... + \frac{E_n}{N_n}$. "$E_n$" is the number of emerged seedlings observed in the n^{th} daily counting, and "N_n" is the number of days after the seeds were put to germinate in the n^{th} counting.

Data from experiments 1 and 2 were analyzed separately by the techniques of Analysis of Variance (ANOVA) and Regression Analysis and respective graphs, using the Linear Generalized Models (GLM) subroutine of Statistica 5.0 software (StatiSoft Inc. 1999). Arcsin $\sqrt{\% \ / \ 100}$ and $\sqrt{x}$ transformations were applied, respectively, on G% and S data sets, to satisfy the mathematical model requirements.

RESULTS AND DISCUSSION

Data analyses show a significant reduction (P<0.01) on ARCSIN(sqrt(G%/100)) with increasing NaCl and PEG concentrations (Figure 1), especially under salt stress conditions. Conversion to the original $G_{\%}$ scale shows that a 0.1MPa decrease in the osmotic potential, in the range considered in this study, is expected to negatively affect seed germination by 4.43 to 7.61% or by 1.87 to 5.05%, respectively for NaCl and PEG solutions.

In NaCl solutions at -0.6 and -0.9 MPa (134 and 201 mM), $G_{\%}$ reduced from 60.5% to 27.3%, respectively, while in PEG solutions G% decreased from 70.0% to 63.3% (Table 1).

This NaCl effect implies that jurema preta is comparable to glicophytes such as barley (*Hordeum vulgare*), corn (*Zea mays*), and alfalfa (*Medicago sativa*). These seeds present low germination at 150 mM salt concentration (UNGAR, 1978, 1982; WILLIAN & UNGAR, 1972; PINTO *et al*., 1990). In contrast, *Juncus maritimus* and *Prosopis juliflora* are halophytes, and their seeds are able to germinate normally above that salt concentration (PEREZ 1991; CAVALCANTE & PEREZ, 1995).

Thus, although other environmental factors affect the composition of plant communities, the sensibility to NaCl in the process of seed germination shown by jurema preta and the widespread dominance of this species in the semi-arid region of northeastern Brazil may be an indicative that those areas do not present problems of soil salinity.

Jurema preta seeds at –1.2 MPa-PEG solution reduced germination to 53% (Table 1), probably as a consequence of the reduction of absorbed water by the seeds, as no toxic effect of PEG on seed germination has ever been registered in literature (BRACCINI *et al*., 1998). In addition,

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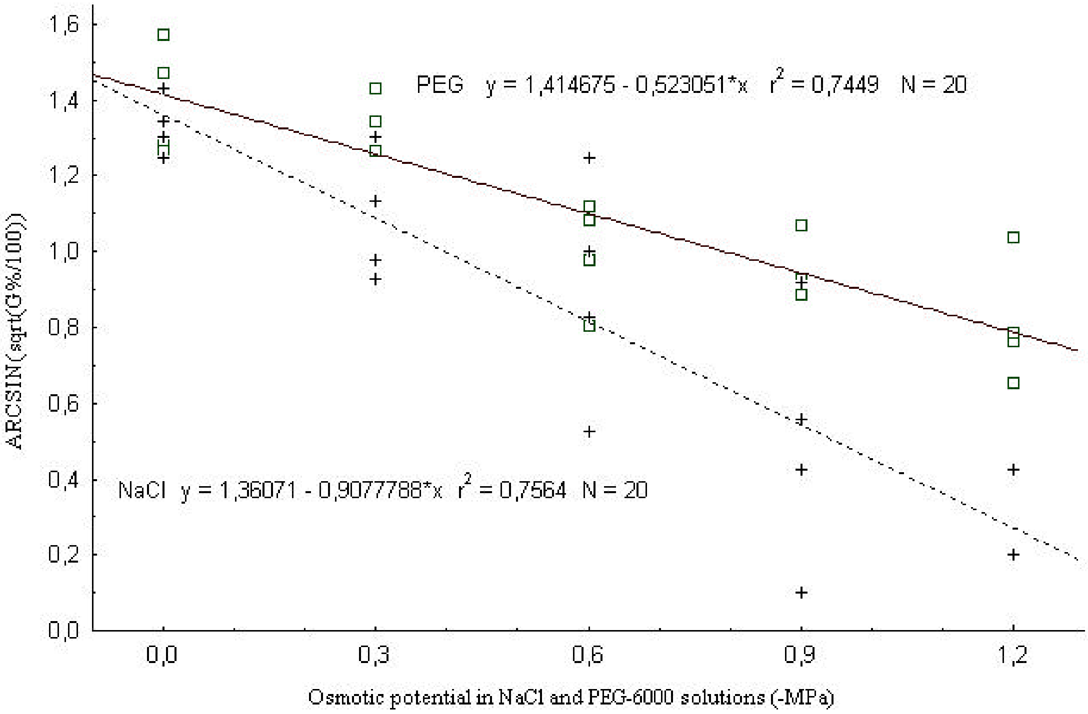

Figure 1: Jurema preta seed germination (G%) under different osmotic potentials of PEG-6000 and NaCl solutions.

PEG-6000 is a viscous substance that allows little oxygen into the seed, so that, probably, water stress and low oxygen availability jointly decreased seed germination (BRACCINI *et al*., 1998; CAMPOS & ASSUNÇÃO, 1990; LUCCA & REIS, 1995; JELLER & PEREZ, 2001). Probably, this can be associated to jurema preta adaptation to water stress, as most of the sites colonized by jurema preta presents severe water deficit during six to eight months of the year.

Speed of germination index was significantly (P<0.01) reduced to about 54% and 73% of the

Table 1. Mean germination (G%) and speed of germination index (S) of jurema preta seeds submitted to different osmotic potentials of NaCl and PEG-6000 solutions.

Osmotic potential	NaCl solutions		PEG-6000 solutions	
(MPa)	$G_{\%}$*	S	$G_{\%}$	S
T0 (0.0)	94.0	45.78	95.5	60.92
T1 (-0.3)	77.0	24.96	95.0	44.52
T2 (-0.6)	60.5	10.13	70.0	8.06
T3 (-0.9)	27.3	3.84	63.3	7.30
T4 (-1.2)	9.5	1.08	53.0	4.13

*Data in the original scale, although analyses were performed on $ARCSIN(\sqrt{G_{\%}/100})$ and $\sqrt{S}$ transformed data

Water stress affects mainly speed and percentage of germination, and depends on plant species (BEWLEY & BLACK, 1994). Those whose seeds germinate satisfactorily under water stress conditions possess the ecological advantage to establish itself in areas where drought-sensitive species cannot make it.

control at –0.3 MPa simulated by NaCl and PEG solutions, respectively (Table 1 & Figure 2). At that potential, G% value was reduced to ~82% in NaCl solution, and was practically not affected by PEG, as reported by Fanti & Perez (1998) to *Adenanthera pavonina*. Salt and water stress interfered more on S than on $G_{\%}$.

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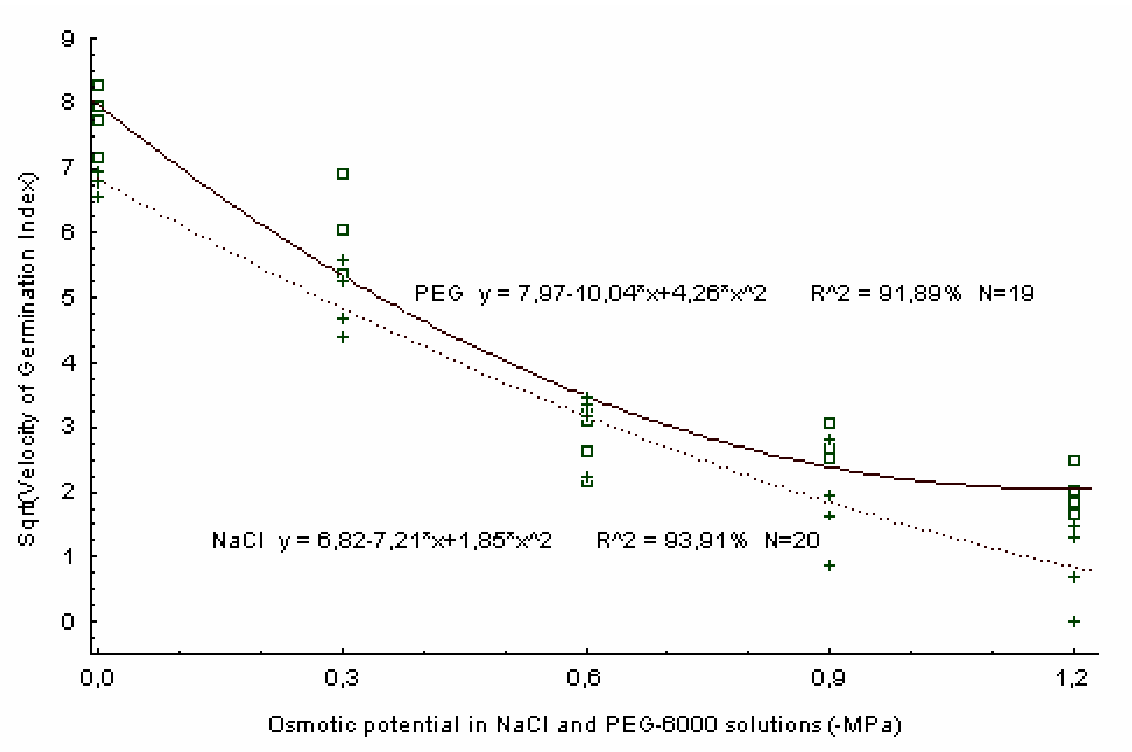

Figure 2: Speed of germination index (S) of jurema preta seeds under different osmotic potentials of NaCl and PEG-6000 solutions

The seeds showed a dark tegument and a gelatinous exudate coat proportional to the negative osmotic potentials in both NaCl and PEG solutions. These characteristics were observed in *Senna spectabilis* seeds under salt stress, probably as an attempt to reduce the direct contact of the seeds with the stressing agent, in order to assure seed viability in those conditions for a certain period of time (JELLER & PEREZ, 2001).

All the remaining jurema preta seeds (*i.e*: those seeds that did not germinate within 28 days in NaCl or PEG solutions) showed radicle emergence within 1 day after being washed and transferred to a gerbox with germitest paper moistened with distilled water. Seeds of a great number of species that do not germinate in NaCl solutions, in concentrations that vary from –0.75 to –2.25 MPa, also resume germination under those conditions (FERREIRA & REBOUÇAS, 1992; KATEMBE *et al*., 1998). That information indicates that the effects caused by NaCl and PEG on jurema preta seeds were just osmotic, causing no physiologic damages to the embryo.

A halt in the process of seed germination, followed by an immediate germination after the stressing factor is removed, may be helpful for species survival, as it makes the seeds viable longer and seedling establishment faster. Consequently, germination and seedling establishment may effectively occur under appropriate environmental conditions (PEREZ *et al.*, 1998).

CONCLUSIONS

Jurema preta showed low tolerance to NaCl-salt stress during seed germination, and can be classified as a glicophyte.

Jurema preta was resistant to water stress, what partially explains its abundance, dominance and pioneer trait in many dry sites of the semi-arid area of northeast Brazil.

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