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OSMOTIC POTENTIALS ON WATER UPTAKE AND GERMINATION OF *Guazuma ulmifolia* Lam. (STERCULIACEAE) SEEDS

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ABSTRACT

Osmotic potentials on water uptake and germination of *Guazuma Ulmifolia* Lam. (Sterculiaceae) seeds. This work was carried out in the Germination Lab. of the Department of Botany, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo State, Brazil. The aims of this work were to determine the water uptake curve and to evaluate the germination of *Guazuma ulmifolia* seeds subjected to different water potentials. For the water uptake curve, seven replicates of 50 pre-scarified seeds were placed onto paper moistened with 15 mL PEG 6000 solution under the potentials 0 (control), -0.3 and -0.6 MPa at 25°C in the darkness. For the germination assay, four replicates of 50 seeds were subjected to the same above-described conditions; however, one lot of seeds was modified when there was variation in the refractometric index, whereas the remaining ones were kept in the same solutions until the end of the experiment. All three phases of water uptake were detected under 0 and -0.3 MPa; however, phase II was prolonged under -0.6 MPa and germination was not observed. For 0 and -0.3 MPa, the adopted statistical models consisted of asymptotic (phases I and II) and exponential (phase III) functions, $y = a*[1 - b*\exp(-c*t) + \exp(-d + e*(t - t_0))]$. For -0.6MPa, only the asymptotic function $y = a*[1 - b*\exp(-c*t)]$ was used since there was no evidence of germination. The germination final percentage and speed index were lower under -0.3 MPa, mainly when solutions were not replaced; besides, germination was not detected under -0.6 MPa, with or without solution replacement.

Keywords: model, Imbibition, forest seed, water stress.

INTRODUCTION

Also known as “mutamba”, “embira”, “guaxima-macho”, “guaxima-torcida”, and “pau de macaco”, the species *Guazuma ulmifolia* Lam. (Sterculiaceae) is present in all Brazilian territory, especially in Bahia, Amazonas, Distrito Federal, Goiás, Maranhão, Mato Grosso do Sul, Minas Gerais, São Paulo and Tocantins States (Almeida *et al*., 1998). It has been extensively used as ornamental and medicinal plant and presents properties that are largely appreciated by the industry (Lorenzi and Matos, 2002).

Environmental conditions not always offer all factors necessary for seed development. The desiccation tolerance presented by some species assures their survival during long periods under adverse conditions. Such adaptive mechanism also allows their distribution in climates that are inadequate for germination (Bradford, 1995).

The speed and amount of water imbibed by seeds vary according to tegument nature, seed chemical composition, seed size, and temperature (Coll *et al*., 2001). Carvalho and Nakagawa (2000) stated that water uptake results in the re-hydration of tissues, which intensifies the respiration and other metabolic processes, supplying thus energy and other essential nutrients for the resumption of the embryo axis growth.

Understanding the water uptake phases in different species is important for studies aimed at seed quality improvement by adopting treatments such as osmotic conditioning, pre-hydration and use of plant growth regulators (Albuquerque *et al*., 2000). Water uptake is usually comprised of three phases: Phase I - physical process, in which the water uptake is due to the osmotic potential difference between seed and substrate; such absorption is affected by seed chemical composition, tegument permeability to the water, and presence of liquid or gaseous water in the environment. Phase II - the water uptake by seeds is slow or not all water is absorbed; in addition, the metabolic activation involving the synthesis of nucleic acids, proteins and enzymes can be observed, besides respiratory activity and energy reserve utilization. At the end of this phase, the beginning of germination is evidenced by the root growth. Phase III - the water uptake increases and the emerging radicle grows. The substrate osmotic potential may alter these phases by prolonging phase II (Bewley and Black, 1994; Bradford, 1995; Carvalho and Nakagawa, 2000; Eira and Caldas, 2000; Delachiave and Pinho, 2002).

According to Bradford (1995), seeds kept under osmotic potentials can absorb the minimal water content needed for radicle growth, although imbibition is slower; however, under a extremely low water potential, the critical content may not be reached and no radicle emergence will be detected; thus, phase II will be indefinitely prolonged. In *Senna occidentalis* seeds, the prolongation of phase II was observed under treatment with -0.6 MPa PEG 6000 (Delachiave and Pinho, 2002).

Lopes *et al.* (1996) reported such effect in onion (*Allium cepa*) seeds, which presented lower imbibition speed when subjected to potentials between -0.5 and -1.0 MPa PEG 6000 at 20ºC, delaying radicle emission; besides, no germination was detected until the 12th day under -1.5 MPa.

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Water stress decreases germination percentage and speed (Bradford, 1995; Braccini *et al.*, 1996; Santos *et al.*, 1996). Each species presents a water potential below which there is no germination (Bewley and Black, 1994; Delachiave and Pinho, 2002). For *Stylosanthes guianensis*, this limit was -0.9 MPa and -1.8 MPa under PEG 6000 and mannitol solutions, respectively (Delachiave *et al.*, 1994), whereas for *Senna occidentalis*, it was -0.4 MPa under PEG 6000 and -0.6 MPa under NaCl treatments (Delachiave and Pinho, 2003).

Aqueous solutions of mannitol and polyethylene glycol, which are chemically inert and non-toxic compounds, have been used for mimicking standardized conditions of water stress in germination studies under laboratorial conditions (Bewley and Black, 1994). Bradford (1995) reported that mannitol may be absorbed by seeds, leading to gradient, potential and toxicity alteration; on the other hand, polyethylene glycol (PEG 6000) is a non-toxic substance of high molecular weight which is not absorbed by cell walls or cellular membranes.

Based on the economical and medicinal importance of *Guazuma ulmifolia*, as well as on the scarcity of information regarding seed germination in this species, the present work aimed to determine the water uptake curve and to evaluate the germination of *Guazuma ulmifolia* seeds under different water potentials

MATERIALS AND METHODS

This work was carried out in the Department of Botany, Institute of Biosciences, São Paulo State University (UNESP), Botucatu Campus, São Paulo State, Brazil. *Guazuma ulmifolia* Lam. seeds were collected in such campus near mother plant three.

Previous tests indicated that the seeds presented dormancy related to the tegument; thus, to determine the water uptake curve under different water potentials, seven replicates of 50 seeds were scarified for 90 minutes in sulphuric acid, washed in running water during 4 h and afterwards washed in distilled water. Then, they were placed in gerbox containing filter paper moistened with 15 mL of the osmotic potentials 0 (control), -0.3 and -0.6 MPa polyethylene glycol 6000 (PEG 6000) at 25ºC in continuous darkness. The relation between the concentration and the potential for such solutions was assessed according to Villela *et al*. (1991).

To determine the water uptake curve, seeds were weighed and allowed to imbibe at pre-established intervals; then, they were superficially dried by using a filter paper, weighed and returned to the germinator, according to the method described by Baskin and Baskin (2001). As some authors have reported that PEG 6000 may not be absorbed due to its size (Bradford, 1995), potentials were altered when solutions presented variation in the refractometric index. For data analysis, fresh matter values were transformed into 1.0 gram. Experimental design was completely randomized, including three treatments and seven replicates. A different statistical model was adopted for each potential.

As regards the effect of water potentials on germination, two lots of seeds were pre-treated in order to break the tegument impermeability dormancy. Then, seeds were separated into two lots with four replicates of 50 unities each and allowed to germinate in gerbox containing filter paper moistened with 15 mL 0 (control), -0.3 MPa and -0.6 MPa PEG 6000. One lot had its solutions replaced when there was alteration in the refractometric index, whereas the remaining lot was kept in the same solutions until the end of the experiment (103 h).

Germination was daily evaluated, and seeds presenting at least 2-mm roots were considered germinated (Rehman *et al*., 1996). Such seeds were discarded after evaluation. The assessed parameters were germination final percentage and germination speed index (GSI), according to Maguire (1962). Experimental design was completely randomized, in a 3 x 2, factorial arrangement, with three osmotic potentials (0, -0.3 and -0.6 MPa PEG 6000) and with or without solution replacement.

For statistical analysis, data regarding germination percentage were transformed into arc sen $\sqrt{x/100}$, where X is the germination percentage, according to a completely randomized experimental design. Means were compared by the Tukey's test at 5% significance.

RESULTS AND DISCUSSIONS

The water uptake speed under 0 MPa PEG 6000 (control) was lower than that detected under -0.3 MPa, and in control seeds germination started after 23h imbibition. When the water potential decreased to -0.3 MPa, such time increased to 27h and under -0.6 MPa there was no germination (Figure-1).

Seeds kept under 0 (control) and -0.3 MPa presented all three germination phases reported in literature (Bewley and Black, 1994; Bradford, 1995; Bewley, 1997; Carvalho and Nakagawa, 2000; Eira and Caldas, 2000; Coll *et al.*, 2001; Delachiave and Pinho, 2002). However, seeds subjected to -0.6MPa had lower water uptake, which was not sufficient to induce germination since phase II was prolonged. This aspect was also reported by Bradford (1995), Lopes *et al*. (1996) and Delachiave and Pinho (2002), who observed that seeds kept under very low potentials, did not absorb the minimal water quantity for the occurrence of germination.

The model adjusted for the osmotic potentials 0 and -0.3 MPa was $y = a^*[1 - b^* \exp(-c^*t) + \exp(-d + e^*(t - t_0)]$. For -0.6 MPa, in which germination was not detected, the model was $y = a^*[1 - b^* \exp(-c^*t)]$, where $a > 0$ is the horizontal asymptote of the asymptotic function; $0 < b < 1$ is the parameter related to the point where the function intercepts the y-axis; $c > 0$ is the reduction parameter of differences between estimated values and the asymptote; d represents the beginning of germination relative to the end of this phase; e is the growth parameter of the germination phase; t is the imbibition time; and t_0 represents the initial time of root emission.

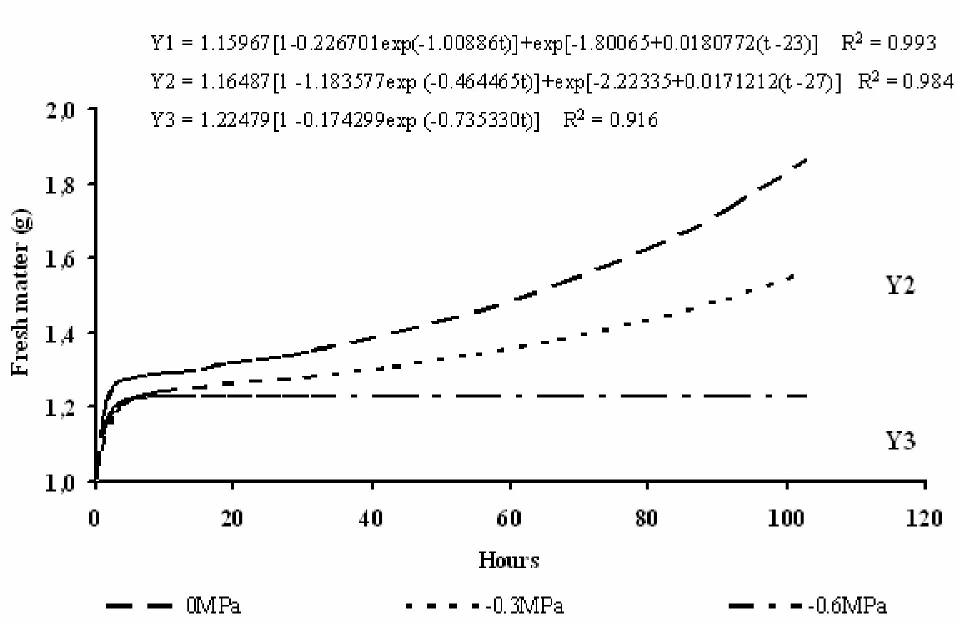

Figure-1. Mean imbibition rate of *Guazuma ulmifolia* seeds subjected to several osmotic potentials (MPa) induced by PEG 6000 at 25⁰C, with solution replacement.

The germination final percentage and speed index decreased from 0 to -0.3 MPa, and no germination was observed under -0.6MPa (Figures 2 and 3). A reduction in germination percentage and GSI was also observed by Nassif and Perez (1997) in *Pterogyne nitens* seeds subjected to water stress with NaCl, $CaCl_2$, KCl, mannitol, and PEG 6000; Albuquerque *et al*. (2000) in *Crotalaria spectabilis* seeds subjected to water stress induced by PEG 6000; and Delachiave and Pinho (2003) in *Senna occidentalis* seeds. In addition, Moraes and Menezes (2003) did not observe soy seed germination under the osmotic potential -0.8 MPa PEG 6000.

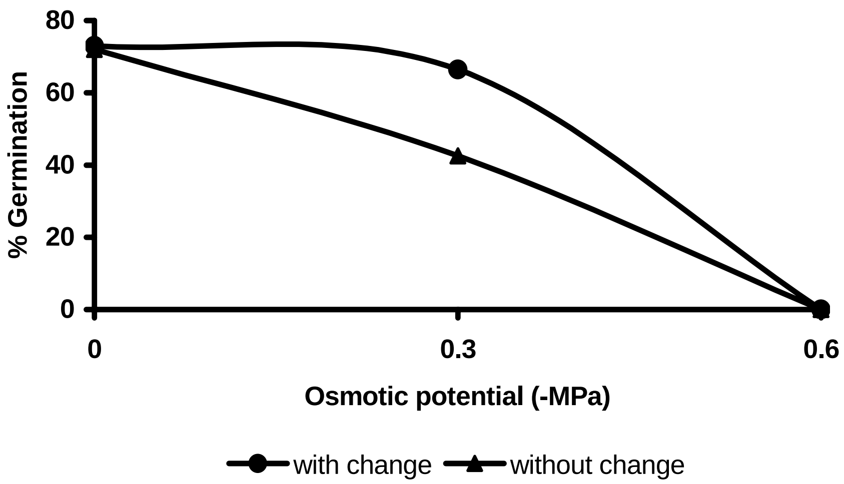

Figure-2. Mean germination percentage of *Guazuma ulmifolia* seeds subjected to different osmotic potentials induced by PEG 6000 at 25⁰C in the darkness, with or without solution replacement.

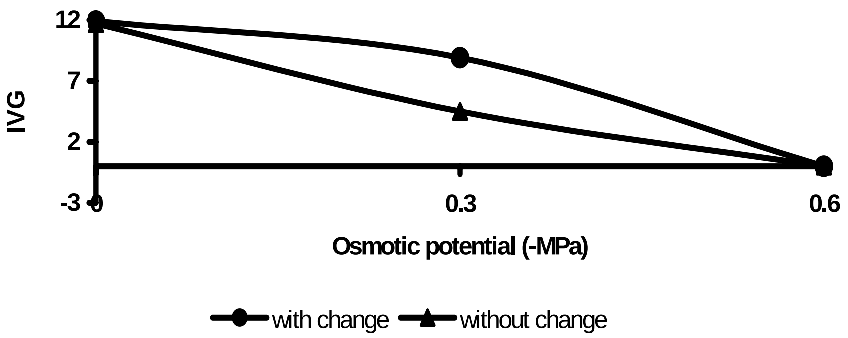

Figure-3. Mean germination speed index (GSI) of Guazuma ulmifolia seeds subjected to different osmotic potentials induced by PEG 6000 at 25°C in the darkness, with or without solution replacement.

This reduction in germination percentage and speed index (Figures 1 and 2) was higher when solutions were not replaced, similarly to what was observed by Delachiave and Pinho (2003) in *Senna occidentalis* seeds. This major reduction probably occurred because PEG 6000 is not absorbed by the seeds, as reported by Bewley and Black (1994) and Bradford (1995), since it presents a high molecular weight; thus, with the water removal by the seeds, the potential becomes lower than the initial one, resulting in more drastic reductions in such indexes.

Therefore, the osmotic potential reduction affects more the germination speed than the germination final percentage, as also observed in seeds of *Prosopis juliflora* (Perez and Moraes, 1990; Perez and Tambelini, 1995); *Glycine max* (Santos *et al*., 1996; Braccini *et al*., 1996); *Pterogyne nitens* (Nassif and Perez, 1997), and *Senna occidentalis* (Delachiave and Pinho, 2003).

CONCLUSIONS

The results obtained in the present study suggest that potentials among -0.3 and -0.6MPa are limiting for *Guazuma ulmifolia* seeds; besides, a greater water potential reduction was detected when solutions were not replaced. In addition, the germination speed index was more drastically reduced, relative to the germination percentage.

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