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# Water Stress Affect Germination, Seed Vigor and Seedlings Growth of *Bidens subalternans*

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**Abstract**

The beggartick (*Bidens subalternans* L.) is one of the main weeds present in agricultural crops, capable of adapting to different environmental conditions. The water stress caused by water deficiency can affect the germination of weed seeds and, consequently, their capacity to colonize the agroecosystem. Knowledge of germination under water stress can be important to obtain an adequate management of the species in the agricultural systems. The objective of this study was to determine the effects of water stress on the germination process and vigor of two batches of *Bidens subalternans* seeds under different osmotic agents. It was completely randomized experimental design was used, with four replicates of 25 seeds. The treatments were arranged in factorial 6 × 2, with the first factor corresponding six levels of osmotic potentials (0.0, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa) and the second the batches (Pernambuco and Ceará). For the simulation of the water stress, the test of germination was installed in substrate paper blotting, moistened with solutions of polyethylene glycol (PEG 6000) and mannitol. The analyzed variables were germination, germination speed index, length and seedling dry mass. Water stress reduced germination, seed vigor and growth of seedlings in all batches of *B. subalternans*. Seeds of *B. subalternans* presented greater tolerance to stress induced by mannitol than to PEG-6000 in terms of germination and germination speed index. Regardless of the osmotic agent used for stress induction, *B. subalternans* seeds did not tolerate water stress higher than -0.4 MPa.

**Keywords:** mannitol, osmotic potential, PEG 6000, beggartick, weed

## 1. Introduction

The occurrence and inadequate management of weeds is one of the main factors responsible for the reduction of the production of crops of economic interest in the agricultural areas. This occurs because, normally, weeds present greater competitive capacity by the same crop growth resources, as water, light and nutrients, moreover the occurrence of allelopathy (Lorenzi, 2000; Zanine & Santos, 2004).

Among the weed species present in the Brazilian semiarid, *Bidens subalternans* L., popularly known as beggartick, is frequently is found year-round, widely distributed in the agricultural areas, disturbed habitats and roadside (Grombone-Guaratini, Solferini, & Semir, 2004). Is a specie invasive highly competitive in annual and perennial crops, with great adaptability on farm soils, which is due to high seed production combined with dormancy mechanisms (Stiegelmeier, Oliveira, Silva, & Karam, 2015). In addition, the beggartick is an alternative host of pests and diseases (Moreira & Bragança, 2011).

In the subtropical and semiarid regions it is common the occurrence of periods without water availability in the soil (Indian summers), due to the lack and irregularity of the rainfall distribution and, consequently are important the effect of the water stress caused by these Indian summers in the species that inhabit this environment (Azeredo, Paula, & Valeri, 2016). For the knowledge of these effects in dynamics of weed populations established in this environment, such as *B. subalternans*, firstly it is important that there is a better characterization of seed germination behavior and establishment of the seedling on water deficit conditions (Pereira, C. C. Martins, D. Martins, & Silva, 2014). This information may be useful to forecast the capacity of *B.*

*subalternans* to invade environments and at the development of adequate management of this species in the agricultural systems.

Water stress may affect the speed and percentage of seed germination. For each specie, there is a value of soil water potential below which germination does not occur (Ávila, Braccini, Scapim, Fagliari, & Santos, 2007). One of the techniques used in laboratory to impose water stress conditions have been the use of aqueous solutions with different osmotic potentials, such as PEG-6000 (polyethylene glycol-6000) and mannitol, because they are chemically inert and non-toxic compounds (Marcos-Filho, 2015).

The effects of water stress induced by osmotic agents on germination and vigor of weed seeds are little understood. Researchers have sought to elucidate these effects in some weeds, such as Pereira et al. (2014) in nabiça (*Raphanus raphanistrum*) and fedegoso (*Senna obtusifolia*) seeds, and Belido et al. (2016) in Mombasa grass seeds (*Panicum maximum* cv. Mombasa). In these studies, the authors verified that water stress impaired seed germination and vigor, reducing seedling initial development, with minimum germinability threshold observed at potential of -0.4 MPa. However, for *B. subalternans*, considerably little attention has been given to study the effects of water stress on seed germination.

In this sense, the objective was to determine the effects of water stress on the germination process and vigor of two batches of *Bidens subalternans* seeds under different osmotic agents.

## 2. Material and Methods

The experiment was conducted in the municipality of Mossoró-RN (5°11′ south latitude and 37°20′ west longitude). The seeds of *Bidens subalternans* were collected in two municipalities of the Brazilian semiarid region (Ceará and Pernambuco). After being collected, the seeds were submitted to asepsis in 1% sodium hypochlorite per minute and washed in running water.

To simulation of water stress, the seeds were conditioned to the osmotic agents polyethylene glycol (PEG 6000) and mannitol, in the following potentials: 0.0; -0.2; -0.4; -0.6; -0.8 and -1.0 MPa. The polyethylene glycol solutions were prepared according to Villela, Doni-Filho, & Sequeira (1991), while those of mannitol according to the equation established by Van't Hoff (Simoni & Chagas, 2007). The experimental design was completely randomized, with treatments distributed in a factorial 6 × 2 (potential osmotic x batches), with four replicates of 25 seeds, evaluated separately in each osmotic agent.

The seeds were seeded in Gerbox® boxes (11 × 11 × 3.5 cm), containing two sheets of blotting paper (sterilized), moistened with PEG solutions, mannitol and distilled water in an amount equivalent to 2.5 times the weight of the dry paper. The gerboxes were placed in 0.05 mm thick plastic bags to conservation the substrate moisture and maintained in a B.O.D. type germinator under constant temperature of 25±2 °C.

The percentage of germination was performed daily, for a period of 14 days. Seeds with a root extension equal to or greater than two millimeters were considered as germinated (Rehman, Harris, Bourne, & Wilkin, 1996). The germination speed index (GSI) was performed in conjunction with the germination test, by means of daily observation of germination of normal seedlings after sowing until the 14th day, when germination was stabilized, with GSI being each determined according to the formula proposed by Maguire (1962).

The seedling length was determined at the end of the experiment, using a ruler graduated in centimeter. The seedlings were measured from the main root to the leaf apex, using all the normal seedlings of each repetition. The seedling measurements were summed and divided by the total of normal seedlings, obtaining average values in cm seedlings$^{-1}$. The seedlings were then packed in Kraft paper bags and placed in greenhouse with forced air circulation at 65±3 °C for 72 hours. After drying, the material was weighed in an analytical balance with an accuracy of 0.0001 g to obtain the dry seedling mass. The results were expressed as g seedling$^{-1}$ (Nakagawa, 1999).

The data were submitted to analysis of variance by the F test ($p \leq 0.05$). In the cases of significance, the averages the means were submitted to regression analysis, using statistical software SISVAR (Ferreira, 2011). In the choice of the regression models, the biological response, the significance of the regression coefficients and the determination coefficients were taken into account. For the variables length and seedlings dry mass it was not possible to perform the regression analysis, because no normal seedlings were observed from the potentials of -0.4 MPa, with this, it was decided to apply the Tukey averages comparison test to 5% probability. The percentage of germination was transformed into an arc sin $\sqrt{x}/100$ when necessary for normality and homogeneity of variances. For GSI, length and seedlings dry mass the analysis were performed with the original data.

**3. Results and Discussion**

There was no interaction between batches and osmotic potentials when submitted to PEG. There was only an isolated effect of PEG potential levels on germination, germination speed index (GSI), length and dry mass seedling. However, when submitted to mannitol, interaction between batches and osmotic potentials was observed for germination and seedling length, and an isolated effect of osmotic potentials for GSI and seedling dry mass.

The percentage of germination was reduced in the PEG osmotic agent from -0.2 MPa. The maximum germination (79%) occurred in the concentration of 0.0 MPa, and the minimum (3%) in -0.6 MPa, with null germination observed in the potential -0.6 MPa (Figure 1A).

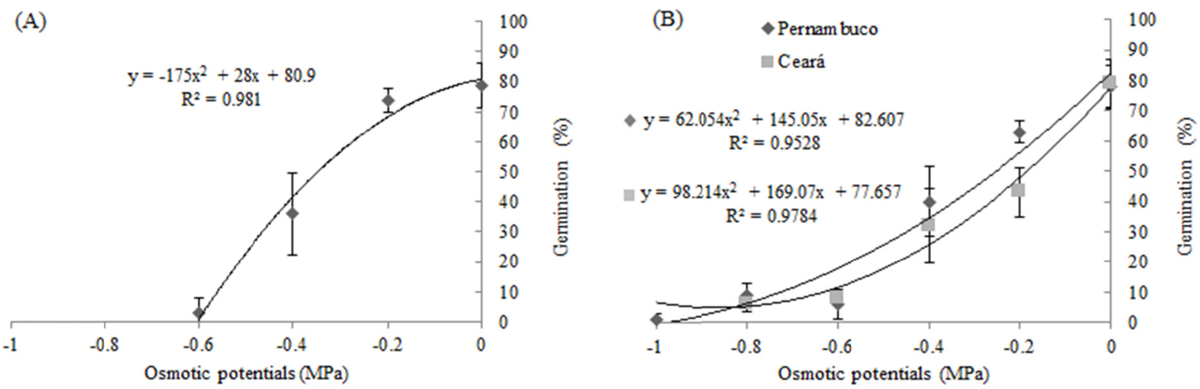


Figure 1. Germination of beggartick (*Bidens subalternans*) seeds submitted to different osmotic potentials induced by PEG 6000 (A) and mannitol (B). Error bars indicate confidence interval of the average at 95% probability

In the presence of mannitol, the seeds of both batches presented maximum germination (79%) at the potential of 0.0 MPa. As the osmotic potential of the solution decreased, there were decreases in the germination values, reaching nullity at -1.0 MPa (Figure 1B).

The explanation for the absence of germination observed in osmotic potential from -0.6 MPa in PEG and -1.0 MPa in mannitol may be due to the water stress caused by the water deficit during the conditioning period, during which it is necessary that the water enter the seeds to start the germination. Once seeds have been exposed to such conditions, they develop an osmotically enforced "dormancy", triggered by insufficient water uptake. This adaptive response prevents seed germination under unfavorable conditions to ensure proper seedling establishment (Masondo, Kulkarni, Finnie, & Staden, 2018). The stress severity was more prominent in the PEG probably due to the high molecular weight of this osmotic agent, which added to the high viscosity and low rate of oxygen diffusion, hindered the entry of water and oxygen into the seeds (Antunes et al., 2011).

The intensity of the germinative response to water stress can vary between seeds of different species or within the same species, since each species requires a minimum water potential value to occur the germination process, below which germination does not occurs (Stefanello, Garcia, Menezes, & Castilhos, 2008). This type of behavior was observed by Azeredo, Paula, and Valeri (2016) in calico-angelfish (*Piptadenia moniliformis*) seeds, which when testing three seed batches induced to stress by PEG 6000 , verified that the seed germination process is compromised from water potentials below -0.6 MPa and that the tolerance to water stress simulated with PEG 6000 is variable between seed batches.

Several studies have reported negative effects in species when submitted to water stress simulated by different osmotic agents, such as in *Panicum maximum* (Belido et al., 2016), *Asphodelus tenuifolius* (Tanveer, Sibtain, Javaid, & Ali, 2014) and *Rhynchosia capitata* (Ali, Tanveer, Nadeem, Asghar, & Javaid, 2013), which presented reduction of germination from potentials lower than -0.8 MPa, under the osmotic agents mannitol and PEG. Similar results were observed to seeds and saplings of fedegoso (Pereira et al., 2014) at potentials lower than -0.4 MPa, and sweet yellow clover (*Melilotus officinalis*) at potential of -1.0 MPa (Ghaderi-Far, Gherekhloo, & Alimagham, 2010), under PEG osmotic agent.

The GSI in both osmotic agents was reduced as the potentials became more negative. The stress promoted by the osmotic agents impaired vigor in a progressive way from the potential of -0.2 MPa, with the greatest decreases in the potential of -0.6 MPa (reduction of 97% in relation to the potential 0.0 MPa), induced by PEG (Figure 2A). For mannitol, the greatest reductions were observed in potential of -1.0 MPa, with reduction of 99% compared to 0.0 MPa potential (Figure 2B).

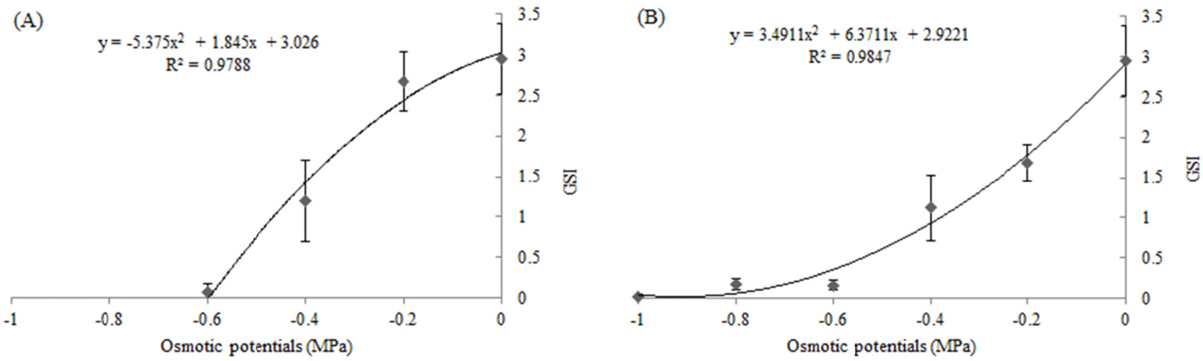


Figure 2. Germination speed index (GSI) of beggartick (*Bidens subalternans*) seeds, submitted to different osmotic potentials induced by PEG 6000 (A) and mannitol (B). Error bars indicate confidence interval of the average at 95% probability

The seeds that were subjected to stress induced by mannitol presented higher GSI in relation to those submitted to PEG, demonstrating that were more sensitive to PEG. This difference can be attributed to the permeability of seed coat to solutes such as mannitol, which presents low molecular weight and thus allows your entry into the seed. This osmotic agent submits the seeds it to the drying effect caused by the solution, unlike PEG which has a high molecular weight and is not absorbed by the seed, causing only a delay in the germination process or decrease in total final germination (Belido et al., 2016; Azeredo et al., 2016).

The water restriction causes a reduction in the speed of the metabolic and biochemical processes, delaying or interfering directly in the germination process of the seeds (Belido et al., 2016). 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 (Bakke, Freire, Bakke, Andrade, & Bruno, 2006). The speed of germination may have implications for weed management. Seeds that germinate later are likely to have greater competition for water, light and nutrients from the crop than the first seeds germinated and may result in less production loss and less weed seed production. This may also mean that late germinating seeds may escape the early application of the post-emergence herbicide (Chauhan, 2012).

The reduction of germination speed in seeds submitted to osmotic agents were also observed in experiments with seeds of *P. maximum* (Belido et al., 2016), in which the GSI reduced as the concentration of mannitol solution decreased, as in the work of Yamashita, Guimarães, Silva, Carvalho, and Camargo (2009) with false-serralha (*Emilia sonchifolia*) seeds, with reduction of GSI from of potential -0.1 MPa and germination null under the potential -0.4 MPa, induced by PEG 6000.

Analyzing the variable seedling length, the lowest values were verified under the potential of -0.2 and -0.4 MPa (5.07 and 3.58 cm, respectively), and the highest in the potential of -0.0 MPa, being statistically higher than the other potentials (Figure 3A). The interaction between the batches and osmotic potentials induced by mannitol shows that the seeds from batch 1 (Ceará) had greater length in relation to batch 2 (Pernambuco), under potential -0.4 MPa, but in both batches as the potentials subsided, the development of normal seedling was impaired (Figure 3B).

For both agents, in potentials lower than -0.4 MPa there were no normal seedlings. Suppressed growth in the seedlings in these osmotic potentials may indicate that, although the seeds germinated, they were still dormant and did not enter phase III, which is the embryonic axes elongation stage (Bewley et al., 2013). Cell elongation is necessary and is generally accepted to be sufficient for the completion of radicle protrusion (Kucera, Cohn, & Leubner-Metzger, 2005). The reduction of the seedling length is a morphological and anatomical alteration of the plants, caused by the reduction of the cellular expansion, being a response of the osmotic effect induced by water stress (Taiz & Zeiger, 2013).

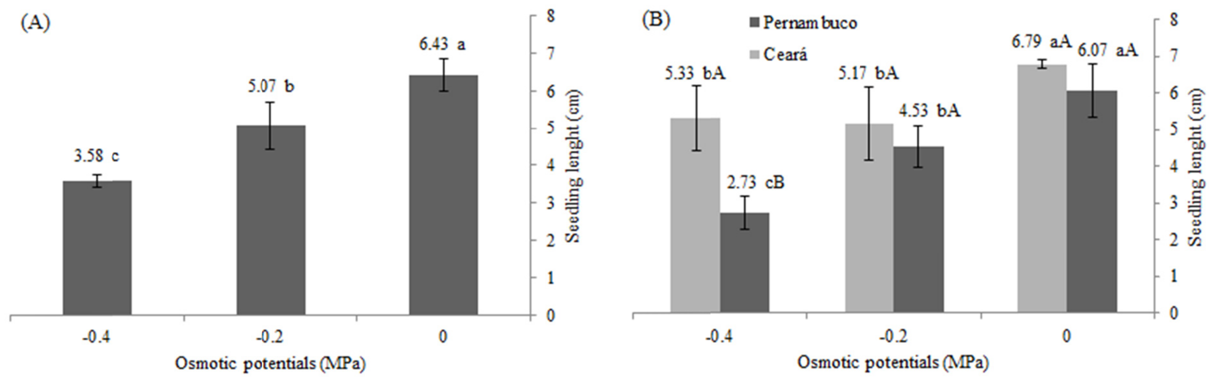


Figure 3. Seedling length of beggartick (*Bidens subalternans*) seeds, submitted to different osmotic potentials induced by PEG 6000 (A) and mannitol (B). Averages followed by the same lowercase letter do not differ between the potentials and upper case between the batches by the Tukey test at 5% probability. Error bars indicate confidence interval of the average at 95% probability

The dry mass production of seedlings was similar for both agents. The highest dry mass accumulation was observed under the potential -0.2 MPa, and the lowest in -0.4 MPa, a difference of 36% between these potential (Figures 4A and 4B). This result can be explained by the fact that water stress reduces the speed of the physiological and biochemical processes in the seeds and, with this, result in a lower development of the seedlings and, consequently, in smaller lengths and accumulations of dry mass of the seedlings (Ursulino et al., 2016). The results of the present study coincide with the findings by Azeredo et al. (2016), that when evaluating the germinative capacity of angico-de-calf seeds submitted to PEG water stress, found progressive reduction in dry mass of seedlings in different plots as the potential became more negative.

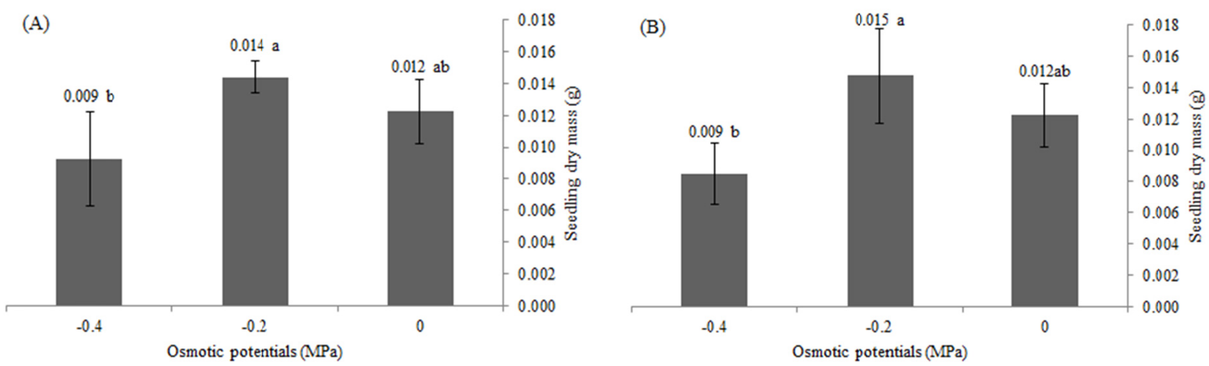


Figure 4. Seedling dry mass of beggartick (*Bidens subalternans*) seeds, submitted to different osmotic potentials induced by PEG 6000 (A) and mannitol (B). Averages followed by the same lowercase letter do not differ from each other by the Tukey test at 5% probability. Error bars indicate confidence interval of the average at 95% probability

The results obtained with water stress on *B. subalternans* seeds are of ecological importance, since they demonstrate that the seeds of this species have low tolerance to water stress, requiring specific water conditions for their twinning. This low tolerance to water stress gives *B. subalternans* a non-adaptive character and indicates that the species presents low establishment capacity in environments with water restriction, due to the narrow limits for germination. Based on this, manipulation of stress to control biological invasions of *B. subalternans* could be an important measure of control of this weed.

## 4. Conclusions

Water stress reduced germination, seed vigor and growth of seedlings in all batches of *B. subalternans*.

Seeds of *B. subalternans* presented greater tolerance to stress induced by mannitol than to PEG-6000 in terms of germination and germination speed index.

Regardless of the osmotic agent used for stress induction, *B. subalternans* seeds did not tolerate water stress higher than -0.4 MPa.

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