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RESEARCH ARTICLE

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EFFECT OF ENVIRONMENTAL FACTORS ON THE EMERGENCY AND GERMINATION OF BITTERGRASS (*Digitaria insularis* (L.) Fedde)

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ABSTRACT

Six experiments were conducted to evaluate the effects of abiotic factors of bittergrass: temperature (15, 20, 25, 30, 35 °C), light (0/24, 8/16, 12/12, 16/8, 24 / 0 hours, being light regimes / dark cycle), pH (4, 5, 6, 7, 8 and 9), osmotic stress (0, - 0.1, -0.3, -0.6, -0, 9 Mpa), salt concentration (0, 20, 40, 80, 160 and 240 mM), conducted in laboratory (DIC) and planting depth (0, 2, 4, 8 and 12 cm), conducted in greenhouse (DBC). The data were submitted to analysis of variance using the program and SigmaPlot e Sisvar. The results showed that germination rate of the seeds was greater than 80% at temperatures of 25 to 35 °C. The maximum germination occurred at 30 °C (92%). In addition, seeds had the tolerance to saline stress at concentrations of 20 (21%) and 40 mM (37%), but the germination was very low at potencies of 80mM (7%), 120mM (2%) and 240mM (0%). When seeds were placed on soil surface emergence was high (91%). The results this study will lead to a better understanding of environmental factors effects of on germination and emergence of bittergrass, will provide information that may contribute to an effective control.

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INTRODUCTION

The bittergrass (*Digitaria Insularis* (L.) Fedde) is a species native to subtropical and tropical regions of America. In Brazil, this species has become a dominant weed in cereal, coffee, orchard and oilseed crops, especially after the advent of the no-tillage system. (LEMES *et al.*, 2010). Among the aggressiveness characteristics that make bittergrass control difficult, the germination capacity stands out. The seeds of this species have mechanisms that allow them to germinate under unfavorable conditions such as different temperatures, burial depth and water stress. Furthermore, the dormancy of its seeds favors a greater seedling emergence flow (VIVIAN *et al.* 2008) which makes the control of bitter grass extremely difficult. Therefore, the knowledge of aspects related to the germination of bittergrass, as well as the environmental factors that affect, such as temperature, light, pH, osmotic pressure, saline concentration, and burial depth, will enable the establishment of an efficient protocol to control this species combined with integrated management (CHACHALIS e REDDY, 2000; SOUZA, FILHO *et al.*, 2001). The aim of this study was to analyze the germination behavior of bittergrass seeds in different environmental factors, namely: temperature, light, pH, osmotic and salt stress, and burial depth.

MATERIALS AND METHODS

Six experiments were conducted, where five experiments were conducted at the Seed Analysis and Research Nucleus Laboratory, namely: different temperatures, different luminosities, different pH and different salines and osmotic stresses. An experiment was conducted in a greenhouse, being the burial depth test, both places located at the University Center of Patos de Minas, UNIPAM, in Patos de Minas, MG, Brazil. The experiments carried out in the laboratory were carried out in a completely randomized design and the experiment was carried out in a greenhouse, conduct in a randomized block design.

Seed collection and storage: To conduct the work, mature seeds of *D. insularis* were randomly collected at the Recanto farm, located on BR365, where those with evidence of malformation or physical damage were discarded. Afterwards, the collected seeds were placed for natural drying and were placed in a tray with paper towels inside the laboratory. After drying, they were stored in paper bags in an acclimatized place (18°C and 20% humidity) until the experiments

were carried out. The seeds went through the dormancy breaking process, which was carried out using solutions of 30 ml of sulfuric acid and 70 ml of distilled water, being immersed in this solution for 20 minutes and soon after surface asepsis was performed by immersing them in a sodium hypochlorite solution 2% for 5 minutes, followed by washing in distilled water.

Experiments: The germination test was standard to evaluate the five experiments: different temperatures, different photoperiod regimes, different pH ranges, different osmotic potentials and different saline concentrations. For each repetition of the germination tests, 50 seeds were placed and distributed in gerbox boxes (11.0 x 11.0 x 3.0 cm) and asepsis was carried out with 70% alcohol, and then three sheets of autoclaved germitest paper were placed inside the boxes, cut in a proportion of 10.5 x 10.5 cm, previously moistened with each experimental solution or distilled water (control), corresponding to two and a half times your total weight (BRAZIL, 2009). For the depth test, 25 seeds were placed per repetition, which were sown in 5 dm³ pots, where the soil for the experiment was collected at SchoolAgrotécnica Afonso Queiroz, located in Patos de Minas - MG, and then sieved through a 3mm sieve and then placed in plastic bags and autoclaved for one hour at a temperature of 120 degrees to avoid any unwanted infestation during the experiment. For the tests carried out in the laboratory, except for the test of different luminosities, the counting was carried out with seven and fourteen days after sowing (BRASIL 2009), where the seeds with visible protrusion of the radicle were considered germinated. For the luminosity test, only one count was made, with seven days. For the test that was carried out in a greenhouse, the IVE (emergence velocity index) where daily counts were made up to 21 days, and seeds with roots of approximately 2 mm were considered germinated (REHMAN *et al.*, 1996).

Effect of temperature on seed germination: To evaluate the effects of temperature on germination, the seeds were incubated at five constant temperatures of 15, 20, 25, 30 and 35°C, totaling like this five treatments, with four replications. The boxes were wrapped in transparent plastic film and randomly distributed inside BOD-type germinators.

Effect of light on seed germination: To determine the effect of light duration on germination, the bitter grass seeds were exposed to 0/24, 8/16, 12/12, 16/8, 24/0 hours, with light/dark regimes at a temperature of 25±1°C in BOD, totaling five treatments, with four replications. In the dark treatment, the boxes were covered with three layers of aluminum foil to avoid any incidence of light.

Effect of pH on seed germination: To evaluate the effect of pH on germination, seeds were placed in buffered solutions at pH values of 4, 5, 6, 7, 8, and 9, totaling six treatments with four replications. Solutions with pH <7 were obtained with sodium hydroxide (NaOH), which is a caustic hydroxide, used mainly as a chemical base, to simulate acidic media, and solutions with pH > 7 were obtained with hydrochloric acid and used to simulate alkaline media. All solutions were checked with a digital peagometer (mPA210) and kept at a temperature of 25±1°C in BOD.

Effect of salt stress on seed germination: To examine the effect of saline stress on the germination process, the bittergrass seeds were incubated in sodium chloride (NaCl) solutions of 0, 20, 40, 80, 160 and 240 mM, thus totaling six treatments, with four replications, kept at a temperature of 25±1°C in BOD.

Effect of osmotic stress on seed germination: To investigate the effect of drought stresses on seed germination, they were tested in aqueous solutions with osmotic potentials of 0, - 0.1, -0.3, -0.6, -0.9 Mpa totaling five treatments, and four repetitions, which were prepared by dissolving 0, 72.5, 143.2, 213.6 and 284.0 g of polyethylene glycol 8000 in 1 L of distilled water, due to this osmotic agent being chemically inert and not toxic (MARCOS FILHO, 2005). Polyethyleneglycol has no penetration into cells, which occurs because its molecular weight is high (HASEGAWA *et al.*, 1984).

Effect of burial depth on seed germination: The seeds were subjected to depths of 0, 2, 4, 8 and 12 cm, where 0 the seed was under the surface of the pot and the rest were placed and covered with the amount of soil at each depth, thus five treatments, with four repetitions. To place the soil in the pots, sheets of newspaper were placed inside them to prevent the soil from coming out through the holes, but without preventing the water from being stored. The pots were irrigated daily to maintain adequate soil moisture. All pots were placed randomly and each treatment was drawn to avoid experimental errors. If the coleoptile was visible above the soil surface, the seeds were considered emergent.

Statistical Analysis: Data were subjected to analysis of variance and regression analysis using the SigmaPlot software, with the exception of the luminosity test that had the aid of the Sisvar software, with the tukey test at 5% probability.

RESULTS AND DISCUSSION

Effect of temperature on seed germination: Germination of bittergrass occurred at all temperatures of the tests performed. However, the percentage of germination was higher at temperatures of 25 (87%), 30 (92%) and 35°C (86%). The germination of bittergrass had a considerable reduction at temperatures of 15 (5%) and 20°C (53%) (Figure 1).

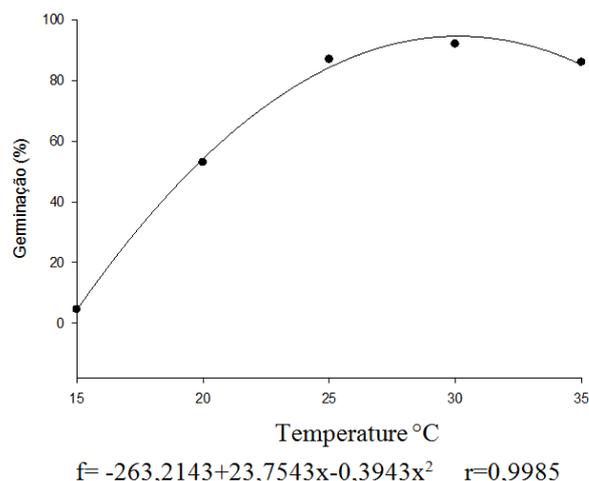


Figure 1. Percentage of bittergrass germination at different temperatures

Mondo et al (2010), analyzing the effect of temperature and light on different species of the *Digitaria* genus, obtained greater germination in *D. insularis* at temperatures above 25°C, matching the results of the present work. According to Mendonça et al (2014), evaluating the effect of different origins, temperatures and light on the germination of bittergrass, observed that temperatures above 30°C, in the presence of light, favor the emergence of the species. The authors also claim that temperatures above 45°C are lethal to seeds. High temperatures allow the plant to express its maximum germination potential in a shorter period of time, and in the case of bitter grass, temperatures above 25°C are favorable for the plant to express maximum vigor. (MONDO et al 2010).

Effect of light on seed germination

Under continuous dark conditions (0/24 h), germination was 31%, while under continuous light exposure (24/0 h) the germination percentage was 36%. The treatment with the best percentage of emergence was 12/12, 76%, followed by 8/16 (47%) and 16/8 (40%) (Figure 2). These values show that the bittergrass has germination potential in a wide range of photoperiods, thus being able to present high germination rates in different periods of the year. Even with significantly lower germination than the other treatments, the absence

of light promoted seed germination, a fact that confers germination potential independent of light.

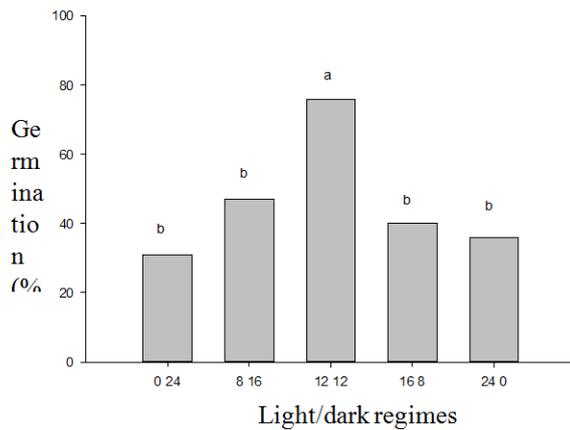


Figure 2. Percentage of bittergrass germination under different light/dark regimes

The lack of light to germinate was also observed by Mondo et al., (2010) in an analysis of the germination of four species of the *Digitaria* genus subjected to alternating temperatures in the presence and absence of light. According to Pyon (1975), seeds of *D. insularis*, when submitted to a photoperiod of 8 to 12 hours of light, have faster germination, with rates close to 70% in up to five days. However, Mayer & Poljakoff-Mayber (1989) and Vázquez-Yanes & Orozco-Segovia (1993), carrying out studies, verified that seeds submitted to 12 hours of light, or in its absence, did not influence the germination of bittergrass, classifying them as neutral photoblastic. Contradicting, Mendonça et al. (2014) observed that the seeds of this species are photoblastic positive, and 35°C, combined with light, is the most favorable condition for their germination.

Effect of pH on seed germination: Emergencies were observed in all pH ranges. The bittergrass seeds had $\geq 26\%$ germination in a pH range of 4 to 9. The highest (46%) and lowest (26%) germination percentages occurred at pH 8 and pH 7, respectively (Figure 3).

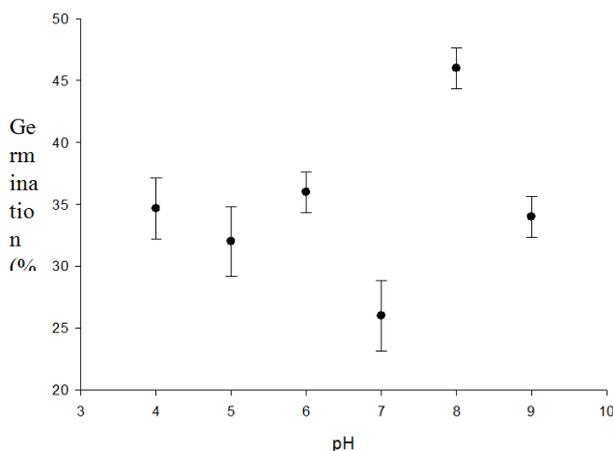


Figure 3. Percentage of bittergrass germination at different pH

These results suggest that bittergrass can germinate at a wide range of pH levels. This feature is common to many weed species such as American barnyardgrass (Rao et al. 2008) and Japanese bromine (Li et al. 2015). The ability of bittergrass to germinate over a wide range of pH levels indicates that soil pH is not a limiting factor on germination and can adapt to a wide range of soil conditions.

Effect of osmotic stress on seed germination: Seed germination in the control treatment was 84%. Only at the potential of -0.1MPa germination percentages (41%) were observed, thus showing a

difference of 43% when compared to the control. At the most negative potentials (-0.6, -0.3, -0.6, -0.9 MPa) the germination percentage of *D. insularis* was inhibited (Figure 4).

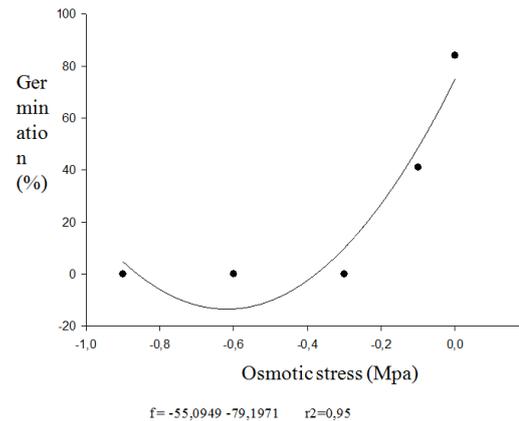


Figure 4. Percentage of bittergrass germination at different osmotic stresses

Similar results were also found by Yamashita and Guimarães (2010) with the species *Conyza canadensis* and *Conyza bonariensis* subjected to PEG-induced water stress, where a significant reduction in germination and germination speed was observed from -0.2 MPa. Water restriction can reduce germination speed (IVG) and germination percentage as the osmotic potential decreases, as it reduces the speed of biochemical and metabolic processes, which thus ends up causing delays or even inhibiting seed germination, consequently interfering in the imbibition and cell elongation of the embryo (BANSAL et al., 1980).

Effect of saline stress on seed germination: High germination was observed in the control treatment (89%) (Figure 5). The bitter grass seeds were sensitive to the salinity conditions imposed by the solutions at the potentials of 80 (7%) and 160 mM (2%), observed by the high reduction in germination and growth during the initial processes of seedling establishment. At the concentration of 20 mM, germination was 42%, and at the concentration of 40 mM the percentage was 37%, with considerable values when compared to the other concentrations.

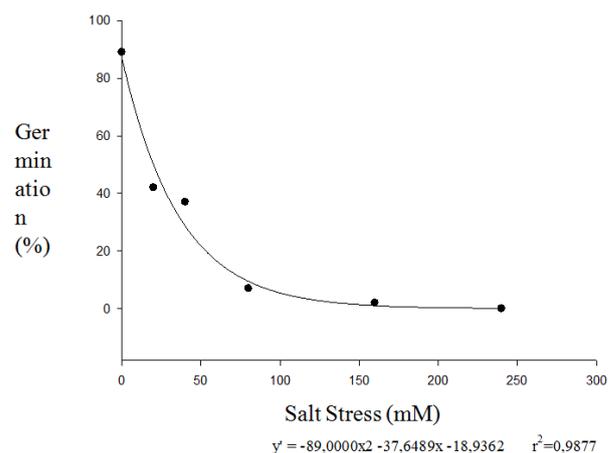


Figure 5. Percentage of bittergrass germination under different salt stresses.

There was no germination at a concentration of 240 mM, thus showing that this species is sensitive to high salinities. The increase in the concentration of salts in the substrate determines a reduction in the water potential, which results in a decrease in the water absorption capacity by the seeds, which generally influences the germination capacity and also the development of the seedlings (Lopes et al 2008). According to Verslues et al. (2006), the presence of salts causes several types of stress, including changes in nutrient absorption, with emphasis on K^+ and Ca^+ ions, accumulation of toxic ions, such as

Na⁺, osmotic and oxidative stress. Salt stress at the beginning of germination is mainly caused by the ionic imbalance and the toxicity caused by excess Na⁺. Water potentials caused by the low presence of salts generally inhibit the growth of the aerial and root parts of the seedling.

Effect of burial depth on seed germination: Only at a depth of 12 cm the emergence of bittergrass seeds was not observed. The highest percentage of emergence and highest IVE occurred at 0 cm depth (91%) (Figure 6). When buried at 8 cm, the bittergrass seeds were able to emerge, but in lower percentages when compared to the lowest depths (2%) (Figure 6).

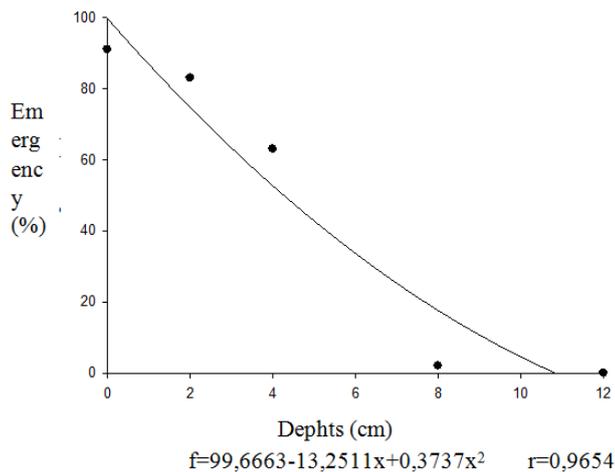


Figure 6. Percentage of bitter-grass germination at different depths

This may have occurred due to the small amount of reserve available in the bitter grass seeds, but also due to the fact that the seeds that were at lower depths were subject to better temperature conditions, which causes a higher germination index, (NETO et al 2016). According to Guimarães et al. (2002), the depth in the soil at which a seed can germinate and thus produce seedlings may vary between species and, in addition, it has agronomic and ecological importance. Barbosa et al. (1991) verified that the levels with the highest percentages and also the emergence speed of seedling species of the *Digitaria* genus were found on the surface and at a depth of 2 cm. Among these species, the seeds of *D. insularis* showed about 80% germination up to 3 cm in depth, and when placed at 4 cm in the soil, the germination percentage was reduced to 10% (Pyon et al., 1977). However, Reinert (2013), when studying the viability of *D. insularis* seeds, found that they only lose viability when they are submitted to burial in the soil, at least 5 cm, for a period longer than 180 days.

CONCLUSION

It can be concluded that the germination behavior of bittergrass seeds showed high germination at temperatures 25 and 30 °C. They can be classified as neutral photoblastic, as they germinated in all light/dark regimes. It has no pH requirements. It has no tolerance to saline and osmotic stress. The adequate depth for the germination of bittergrass is less than 8 cm.

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