Scientific Paper

Effect of hydric stress on the germination of seeds from *Sorghum bicolor* (L.) Moench cv. UDG-110

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Abstract

The objective of this work was to evaluate morphological and biochemical indicators in *Sorghum bicolor* (L.) Moench cv. UDG-110, during the germination process under conditions of hydric stress induced by polyethylene glycol-6000. The seeds were sown on Petri dishes with different concentrations of polyethylene glycol-6000 (0, 3, 6, 9, 12, 15, 18 and 21 %) and were placed in a growth chamber during eight days. The following indicators were evaluated: germination percentage, germination value, length of the root and the aerial part, α-amylase activity and contents of proteins, reducing sugars and soluble phenols. A completely randomized design was used with four replicas per treatment. Polyethylene glycol-6000 affected the germination percentage and the morphophysiological traits, such as length of the roots and aerial parts. The α-amylase activity increased in the variants with presence of the osmotic agent, with values higher than 9 %. In the low and intermediate concentrations of polyethylene glycol-6000 the highest contents of reducing sugars and total soluble proteins in the root and the aerial part were observed, respectively. The concentration of soluble phenols in the aerial part reached high values between 15 and 18 %, which can be related with an antioxidant defense mechanism to face the consequences of the oxidative stress generated under diverse conditions of abiotic stresses. Polyethylene glycol-6000 affected the germination process of *S. bicolor* cv. UDG-110, although there was germination with 21 %, showing the presence of drought tolerance mechanisms, such as the production of osmotically active compounds and the synthesis of antioxidant substances.

Keywords: sorghum, reducing sugars, phenolic compounds, drought

Introduction

Drought is one of the most important environmental stresses that affect crop growth, development and productivity. The understanding of tolerance mechanisms of plants to this stress constitutes one of the most important challenges for agricultural researchers and for plant breeding (Mujtaba *et al*., 2016). This situation is worsened if the consequences of the climate change are considered, which aggravates the abiotic stresses at global scale by increasing the irregularity of meteorological events (Jain and Saxena, 2016). For such reasons, it is necessary to develop strategies that allow crop adaptation to specific environments and to identify at early ages those that can maintain high yields under water deficit conditions (Khaton *et al*., 2016).

Sorghum [(*Sorghum bicolor* (L.) Moench)] is a highly demanded crop at global scale because of its usage in human and animal feeding, as well as its high potential for renewable energy production (Rezende *et al*., 2017). This species was catalogued as moderately tolerant to hydric stress and although water scarcity can exert an adverse effect on the germination process, many sorghum cultivars adapt well to semiarid conditions (Achón-Forno *et al*., 2014).

Germination and the first stages of plant growth constitute the most vulnerable moments within the life cycle of plants. Good germination can contribute remarkably with the transit of the plant through the different phenological stages (Channaoui *et al*., 2017). In this sense works have been conducted about the response of *S. bicolor* to hydric and saline stress during the germination process, in order to understand the tolerance mechanisms that occur in this species (Tsago *et al*., 2013). These studies are fundamental for establishing later successful breeding programs in this crop (Khan *et al*., 2015). The objective of this wok was to evaluate the effect of hydric stress induced by polyethylene glycol-6000 on the germination of seeds from *S. bicolor* cv. UDG-110.

Materials and Methods

Seeds from sorghum cv. UDG-110 were used, supplied by the Pastures and Forages Research Station Indio Hatuey (EEPFIH), of Matanzas province, Cuba: while the studies were conducted in the laboratory of Biotechnology of the School of Agricultural Sciences, University of Matanzas, Cuba.

The essays were developed according to a completely randomized design. In this sense, the seeds were placed on Petri dishes of 5 cm diameter on filter paper moisturized with different concentrations of polyethylene glycol-6000 (0, 3, 6, 9, 12, 15, 18 and 21 %), in a proportion of three times the weight of the substrate which constituted the treatments. Twenty five seeds were placed per Petri dish and four replicas were made per treatment (ISTA, 2010). The germination process was evaluated daily during eight days and the results were expressed in percentage of germinated seeds. For the biochemical analyses five samples were taken per treatment; while for the evaluation of the morphological and physiological indicators 10 seedlings were analyzed.

Measurements

Germination value. The quantity of germinated, non-germinated and rotten seeds was determined daily during the experiment. With the obtained data the germination value (GV) was calculated, according to the formula proposed by Djavanshir and Pourbe (1976).

$$\mathrm{GV} = \left(\sum_{i=1}^{n} Rde\right)\left(\frac{Ef}{10N}\right)$$

Where:

Rde: Rate of daily emergence, calculated as the percentage of the accumulated emergence divided by the number of days since the beginning of the test.

N: Frequency or number of Rde which was calculated during the test

Ef: Percentage of seedling emergence at the end of the 8 days of the test.

Morphological indicators. The length of the root and the aerial part of the seedlings eight days after the beginning of the germination experiment was determined, with the use of paper graduated in millimeters and the values were expressed in centimeters.

Biochemical indicators. The root and aerial part of the germinated seedlings were cold macerated with buffer solution of sodium phosphate 50 mmo/L, pH 7,0 and in a proportion 1:3 (w/v). The homogenate was centrifuged at 12 000 rpm and the supernatant was collected, which was preserved at -20 ºC until the moment of the determinations. The biochemical indicators were carried out in all the treatments, except in the highest concentration of polyethylene glycol-6000 (PEG-6000), where the percentage of germinated seeds was very low and the biomass was insufficient to perform the determinations.

α-amylase *enzymatic activity.* The enzymatic extract was cold performed by homogenization of the plant material in a buffer solution of sodium citrate pH 5,0 in a proportion 1:2 (w/v). The mixture was centrifuged during 10 minutes at 10 000 rpm and 4 ºC. The supernatant was collected for determining the α-amylase activity. A quantity of 0,1 mL of the enzymatic extract was added to 0,4 mL, a solution of 1 % starch (w/v) in sodium phosphate buffer 20 mmo/L pH 6,9 and it was left to react during 10 min at 37 ºC. The reaction was stopped with the addition of 0,5 mL of 3,5-dinitrosalicylic acid. Afterwards, the reacting mixture was heated at 100 ºC during 10 minutes and 1,2 mL of distilled water were added to it. The absorbance was determined at 546 nm, and the enzymatic activity was expressed as μmoles/min of glucose released per μg of protein at pH 6,9 and 37 ºC.

The enzymatic activity (EA) was calculated through the following formula:

$$EA = \frac{\mu moles}{t} * \frac{TV}{Ve} * df$$

Where:

t: Time of the essay

TV: Total volume of the essay (9,5 mL)

Ve: Volume of the sample (0,1 mL)

df: Dilution factor of the enzymatic extract

All the described spectrophotometric measurements were made in a UV/VIS Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Sweden).

Content of reducing sugars. The content of reducing sugars was determined by the method of dinitrosalicylic acid and D-glucose (Sigma) was used as pattern sugar (Miller, 1959). The absorbance values were obtained at a wavelength of 456 nm and the concentration was expressed in mg/mL from the pattern curve.

Content of total soluble proteins. The protein content was determined colorimetrically through the method described by Lowry *et al*. (1951), with the use of bovine serum albumin (BSA) as pattern. The absorbance values were obtained at 750 nm and the concentrations (mg/mL) were determined by the pattern curve.

Content of soluble phenols. One hundred milligrams of root and aerial part were homogenized in 1 mL of methanol and were centrifuged at 12 000 rpm. The supernatant was collected for the colorimetric determination of soluble phenolic compounds (Friend, 1992). To determine the concentration of these compounds chlorogenic acid (0,05 mol/L) was used as pattern and the absorbance values were determined at 725 nm.

Statistical analysis. The data were processed with the statistical package SPSS® version 15.0 for Windows. The adjustment of the data to a normal distribution was determined by the Kolmogorov-Smirnov goodness of fit test and variance homogeneity through Bartlett's tests (Sigarroa, 1985). The data were processed through simple classification Anova and Duncan's multiple range test was carried out for the comparison among means ($p \leq 0,05$).

Results and Discussion

Germination percentage. The high concentrations of polyethylene glycol in the medium caused a decrease in the germination percentage of *S. bicolor* (fig. 1). The highest values in this indicator were obtained with 3 % of the polymer, followed by 9, 6 and 0 %. The high contents of PEG-6000 in the medium (18 and 21 %) remarkably decreased the germination percentage. The negative effect of polyethylene glycol on this indicator is related to a reduction of the solute potential of the medium and, consequently, of the hydric potential. The decrease of the hydric potential affects the imbibition process and water availability for the seeds, which is fundamental for the hydration of enzymes and substrates that participate in the diverse biochemical reactions which, in turn, trigger the germination process (Swapna and Rajendrudu, 2015; Fathi and Tari, 2016).

The decrease in germination indicators such as germination percentage because of the polyethylene glycol, has been reported in different species, such as: *S. bicolor* (Rezende *et al*., 2017), *Triticum durum* Desf. (Khayatnezhad and Gholamin, 2011), *Pennisetum glaucum* L. (Sani and Boureima, 2014) and *Pongamia pinnata* (L.) (Swapna and Rajendrudu,

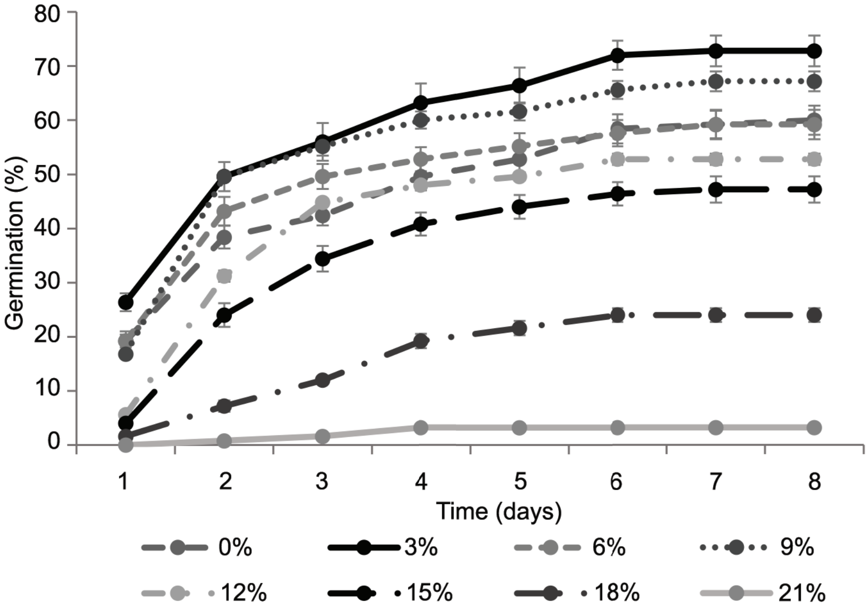

Figure 1. Effect of polyethylene glycol-6000 on the germination of *S. bicolor* seeds germinated on Petri dishes during eight days.

2015). In these works the responses of the seedlings depended on the genotype (variety) in question.

The results also coincide with the ones observed in alfalfa (*Medicago sativa* L.) seedlings, where germination, growth and tolerance to osmotic stress induced by polyethylene glycol-6000 in different concentrations and in three different genotypes: "Dk166", "Verdor", susceptible to water deficit, and "Salina", resistant, were evaluated (Castroluna *et al*., 2014). The results showed a delay in the germination process in all the varieties, although with differences among the evaluated genotypes. In this research, the fact that cultivar *S. bicolor* cv. UDG-110 germinated in a solution of 18 and 21 % PEG-6000 could suggest the presence of drought tolerance mechanisms.

Effect of polyethylene glycol on the germination value of the seedlings. Polyethylene glycol also affected the vigor of *S. bicolor* seedlings (fig. 2). The 3 % PEG-6000 solution significantly increased this indicator with regards to the control; nevertheless, the treatments with high PEG-6000 concentrations significantly affected the germination value. The increase of this indicator in the treatment with 3 % PEG could be related to a higher emergence rate, associated with higher metabolic activity in the root, which is the organ directly in contact with the stressing agent. The increase in the content of polypeptides or other osmotically active compounds can compensate the difference of hydric potential that is generated between the medium and the radicle. On the other hand, low PEG-6000 concentrations could induce the expression of anti-stress genes, as well as many enzymes that accelerate the metabolic reactions during the germination stage.

The osmotic stress caused by polyethylene glycol-4000 (–0,2 MPa) during the germination process of *Vigna unguiculata* (L.) Walp., also decreased several germination indicators, such as germination energy, daily mean germination and vigor index (Jain and Saxena, 2016). Kulkarni *et al*. (2014) referred a decrease of different germination indicators of *Coriandrum sativum* L. under hydric stress conditions with PEG-6000. In this work the vigor index progressively decreased with the increase of the polyethylene glycol concentration: control (883,04), 5 % (396,67), 10 % (92,13) and 15 % (0,00).

Effect of polyethylene glycol on root and aerial part length. The effect of PEG-6000 on sorghum seedling growth is shown in figure 3. The roots as well as the aerial part significantly decreased their length. In the case of the roots there were no differences between the control and 3 %, but the higher concentrations caused an inhibition of growth and the lowest values were observed in 18 and 21 % of PEG-6000.

Similarly, the aerial part length did not show changes between the control and 3 % PEG-6000. The concentrations between 6 and 15 % of the osmotic agent affected the length of this organ, while the higher percentages caused total inhibition of its growth. These results proved that the aerial part was

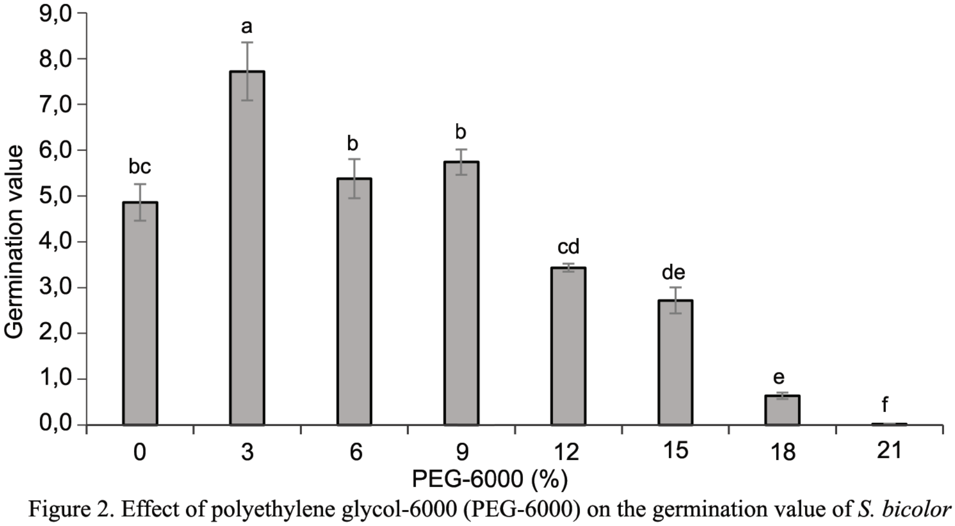

Figure 2. Effect of polyethylene glycol-6000 (PEG-6000) on the germination value of *S. bicolor* cv. UDG-110, eight days after germination.
a, b, c, d, e: Different letters indicate significant differences among treatments for $p \leq 0{,}05$.

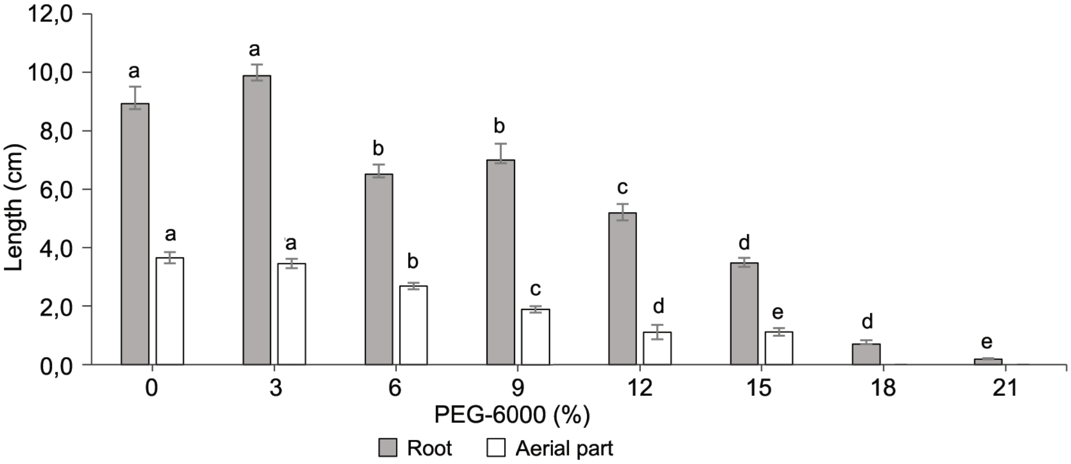

Figure 3. Effect of different concentrations of polyethylene glycol-6000 (PEG-6000) on root and aerial part growth of *S. bicolor* cv. UDG-110 seedlings, after eight days of germination.
a, b, c, d, e: Different letters indicate significant differences among treatments in the same organ for p ≤ 0,05.

more sensitive to the hydric stress than the roots in this cultivar, which is in agreement with the results obtained by Gholami *et al.* (2010) in *Prunus* spp. seeds during the germination process with different PEG concentrations.

Kulkarni *et al.* (2014) observed a similar effect of PEG-6000 (0-20 %) on the root and aerial part length of *Coriandrum sativum* L. seedlings. With 15 % (–30 MPa) of polyethylene glycol root and aerial part growth completely stopped.

Growth inhibition of the seedlings is directly related to the presence of PEG-6000 in the medium, which reduces the input of water to the tissues, which is essential to develop turgor pressure and for cell lengthening to occur. The inhibition of this second process under severe hydric stress conditions was observed in different genotypes of *Oryza sativa* L. by other authors (Pirdashti *et al.*, 2003).

On the other hand, if the hydric status of the embryo is affected the metabolism in general decreases, due to a reduction in the activity of the enzymes that participate in different vital processes, such as degradation of food reserves and cell respiration. The latter is important, because the fast growth during germination and later stages demands high energy intake to perform all the synthesis processes.

A-amylase activity. The effect of different concentrations of PEG-6000 on the α-amylase activity of *S. bicolor* seedlings eight days after germination is shown in figure 4. As can be observed the enzymatic activity in the treatments with PEG-6000 was higher than the control. The highest values were obtained in the treatment with 9 % of the osmotic agent, followed by the medium with 6 % PEG-6000, which was higher than the variants of 3 and 12 %. The lowest values were obtained in the highest PEG-6000 concentrations (15 and 18 %). With 21 % PEG-6000 no results were referred, due to the low germination and seedling growth percentage, which reduced remarkably the quantity of biological material to conduct the biochemical determinations.

Similar results were observed by Li *et al.* (2017) in *Zea mays* L. cv. Zhengtian 68, who determined higher α-amylase activity in seeds germinated in the presence of PEG-6000 compared with the control. On the contrary, in works conducted by Muscolo *et al.* (2014) with varieties of *Lens culinaris* L., a decrease in the α-amylase and α-glycosidase, which participate directly in the germination process, was shown. The inhibition of these enzymatic activities was more remarkable in the sensitive genotypes Ustica and Pantelleria. The decrease in the enzymatic activity in the presence of high PEG-6000 concentrations can be related to the negative effect of the polymer on the enzyme structure, because polyethylene glycol is a highly hydrophilic compound that affects the hydration shell of proteins and protein precipitates are formed (Sim *et al.*, 2012).

The increase of the amylolytic activity in the presence of polyethylene glycol could be related to mechanisms of hydric stress tolerance. The increase in the α-amylase activity was used as a criterion to characterize the response of the genotypes to environmental stresses (Jamil *et al.*, 2006; Othman *et al.*, 2006).

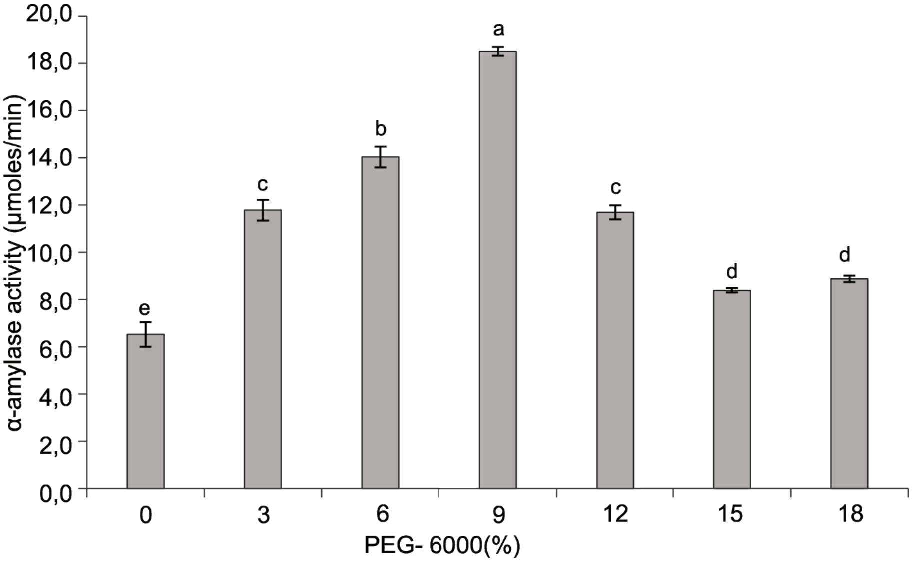

Figure 4. α-amylase activity in seedlings of *S. bicolor* cv. UDG-100 germinated under hydric stress conditions.

a, b, c, d, e: Different letters indicate significant differences among treatments for p ≤ 0,05.

Reducing sugars and total soluble proteins. The content of reducing sugars showed variations in the treatments with different polyethylene glycol levels (fig. 5). In the roots of the seedlings treated with 3 and 6 % PEG-6000 a higher concentration was observed compared with the control. In the treatments with 9, 12 and 15 % no differences were observed with regards to the control, while the lowest values were obtained with 18 % of the product.

The concentration of reducing sugars in the aerial part showed higher values in the treatments with 6, 9 and 12 % PEG, and the treatment with 9 % was significantly higher than 6 % of the osmotic agent. The values in these variants were higher than

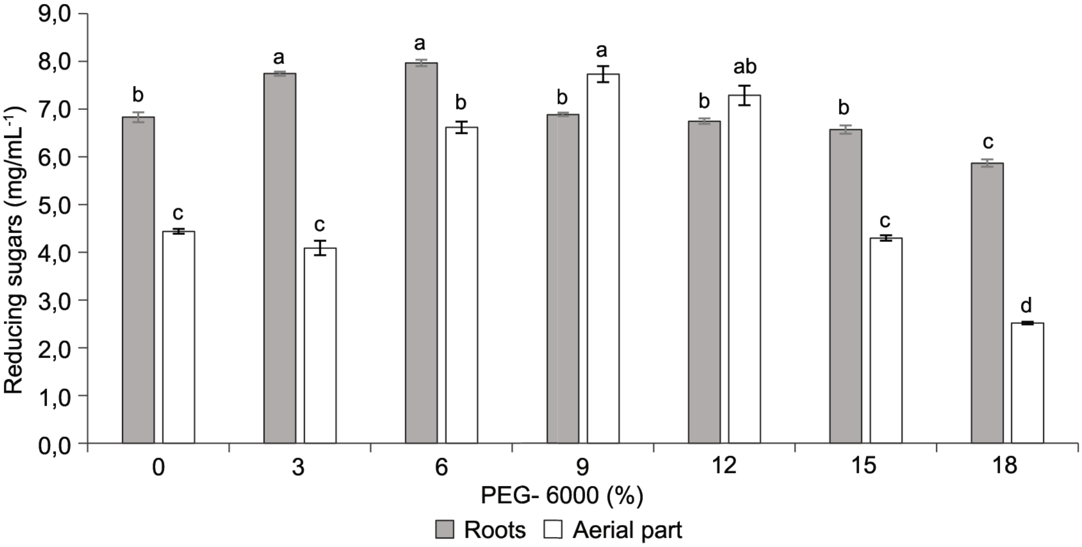

Figure 5. Reducing sugars in the root and the aerial part of the *S. bicolor* cv. UDG-110 seedlings under hydric stress conditions.

a, b, c, d: Different letters indicate significant differences among treatments in the same organ for p ≤ 0,05.

the ones observed in the treatments with 3 and 15 %, and the control (without differences among them), while the lowest values were obtained with 18 % PEG-6000.

These results could be related to the increase of the α-amylase activity in the presence of PEG-6000, as was observed in some treatments such as in 3 and 6 % (aerial part), and 6, 9 and 12 % in the roots. On the other hand, the increase in the levels of reducing sugars can also be associated with the increase in the expression of other hydrolytic enzymes under osmotic stress conditions (Thalmann and Santelia, 2017). The sugars released by the catalyst action of these enzymes may constitute an osmoprotective mechanism to increase osmotic pressure in plant tissues and prevent water loss, as a consequence of the decrease of the hydric potential in the medium imposed by polyethylene glycol. The decrease in the reducing sugar contents of the aerial part, in the treatment with 18 % PEG-6000, can be related to an increase of maintenance respiration in these seedlings; that is, in order to maintain a level of metabolic activity, sugar intake increases for obtaining energy used in cell refill and synthesis of proteins, enzymes and other compounds that participate in anti-stress response.

The results of this research are in correspondence with the report by Neto *et al.* (2009). These authors found an accumulation of soluble carbohydrates, sucrose, glucose and fructose in the tissues of *Sorghum* sp. leaves during vegetative development. This suggests that plants are capable of adapting to osmotic changes, due to a fast degradation of starch and formation of soluble sugars during the vegetative stage.

Figure 6 shows the content of total soluble proteins in the seedlings germinated under hydric stress conditions. In the roots the highest values were observed in 3 and 6 % PEG-6000, without differences between both; however, the treatment with 3 % was higher than the control and the variants with 9, 12 and 15 % (without differences among them). The lowest content was obtained with 18 % polyethylene glycol in the medium. Regarding the aerial part, the highest contents were obtained with the variant of 6 % PEG-6000, followed by 9 and 12 %, among which there were no differences. The other treatments showed soluble protein contents lower and similar among them.

The increase in the content of soluble proteins in the aerial part in the presence of 6, 9 and 12 % PEG-6000, can be associated with an increase in the synthesis of specific proteins that participate in the anti-stress response (Lum *et al*., 2014; Shayanfar *et al*., 2015; Chen *et al*., 2018), which could indicate hydric stress tolerance mechanisms, because the seedling is capable of increasing the protein metabolism depending on several possible functions such as cell refill and growth. Nevertheless, in similar trials with *P. pinnata* seeds subject to hydric stress with PEG-6000, a decrease was observed of the content of total soluble proteins with regards to the

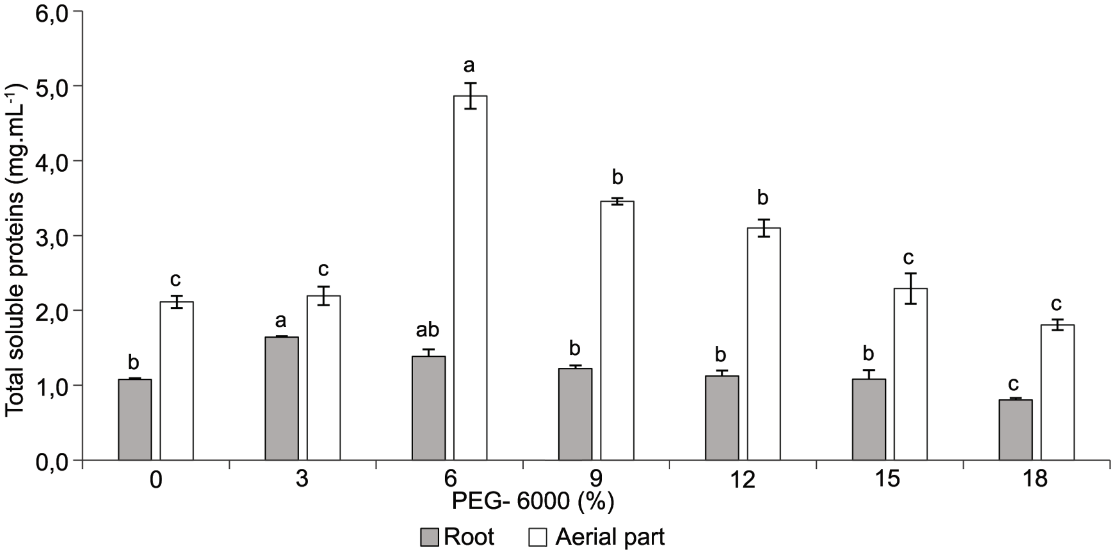

Figure 6. Total soluble protein content in the root and aerial part of the seedlings of *S. bicolor* UDG-110 under hydric stress conditions.

a, b. c: Different letters indicate significant differences among treatments in the same organ for $p \leq 0,05$.

control; although the concentrations of sugars and free proline significantly increased. This last result was associated to defense mechanisms of seedlings to counteract the effect of the osmotic shock exerted by the medium on the plant material (Swapna and Rajendrudu, 2015).

Soluble phenols. The concentration of soluble phenols in the aerial part of the seedlings showed the highest values in the treatment with 18 % PEG-6000, followed by 15 %, which was higher than 12 %. Between this last variant and the concentrations of 9, 6 and 3 % there were no differences; however, the content of polyphenols was higher in 12 % with regards to the control (fig. 7). Regarding the root, the highest values were obtained in 3, 6, 9 and 12 % of polyethylene glycol, among which there were no differences. The polyphenol contents in the treatments of 6 and 9 % were in turn higher than the ones observed in the variants of 15 % and the control (without differences), while the lowest values were obtained with 18 of the polymer.

The increase of polyphenols in the plants in the presence of abiotic stresses, such as the hydric and saline ones, was referred as an antioxidant defense mechanism (Apel and Hirt, 2004). This could be related to the overexpression of transcription factors, which induce the synthesis of enzymes that participate in the production of phenolic compounds. Among them is phenylalanine ammonia-lyase, which constitutes the main enzyme in the metabolic pathway of polyphenolic compounds (Baâtour *et al*., 2013).

These substances have the capacity to eliminate the oxygen reactive species (ROS), which affect drastically cell metabolism and protect the biological membranes from the oxidative damage caused by these radicals (Chernane *et al*., 2015). On the other hand, phenolic compounds can form complexes with the metals that catalyze oxygenation reactions and inhibit the activity of oxidative enzymes. In addition, the antioxidant efficiency is much higher than that of the compounds α-tocopherol and ascorbate, also known for their antioxidant properties (Blokhina *et al*., 2003).

Conclusions

Polyethylene glycol-6000 affected the germination process of *S. bicolor* cv. UDG-110, although the seeds germinated in high concentrations (21 %), proving the presence of drought tolerance mechanisms.

The presence of PEG-6000 in the medium increased the levels of reducing sugars in the seedlings, probably due to the increase of the α-amylase activity in the tissues and other enzymes related to carbohydrate metabolism.

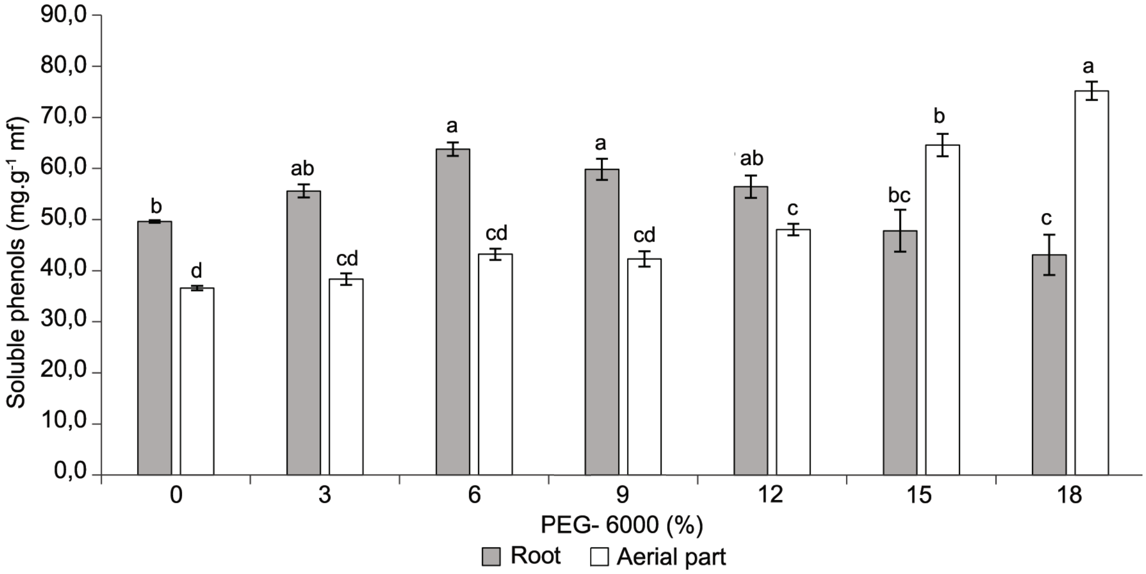

Figure 7. Content of soluble phenols in the root and aerial part of *S. bicolor* cv. UDG-110 seedlings under hydric stress conditions.

a, b, c, d: Different letters indicate significant differences among treatments in the same organ for p ≤ 0,05.

The increase in the content of total soluble proteins in the aerial parts of the seedlings germinated in intermediate PEG-6000 concentrations and the increase in the concentration of polyphenolic concentrations in the treatments with severe hydric stress, suggest the presence of biochemical mechanisms of anti-stress responses to attenuate the damage by osmotic and oxidative stress, respectively.

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