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Original article

Effect of polyethylene glycol-6000 on germination and early growth of *Phaseolus vulgaris* L. cv. 'Delicias'

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ABSTRACT

Water stress affects numerous biological processes and crop yield. The present work aimed to evaluate the effect of polyethylene glycol (PEG)-induced water stress on germination and early growth of *Phaseolus vulgaris* cv. 'Delicias 364' seeds. Bean seeds were sown in Petri dishes with different PEG-6000 solutions (0-18 %) for eight days. The following indicators were evaluated: germination percentage, vigor, root length, hypocotyl and epicotyl, root/aerial part ratio, soluble carbohydrate, reducing sugars, protein and soluble phenol contents. A completely randomized design with four replications was used. The results were subjected to a simple analysis of variance and Duncan's test was performed for comparison between means. Polyethylene glycol reduced germination percentage, root length, hypocotyl, epicotyl and leaf structure formation. The content of reducing sugars in roots was higher than the control at 3, 6 and 9 % PEG. Similarly, the concentration of soluble proteins in roots was higher than the control at 3 and 6 % PEG, suggesting the presence of osmotic adjustment mechanisms in this variety. The root to aerial part ratio increased in the presence of PEG. The concentration of soluble polyphenols in the aerial part was similar between the control and treatments 3, 6 and 9 % PEG, which may contribute to reduce the oxidative damage generated under water stress conditions.

Key words: biochemistry, bean, water stress

INTRODUCTION

Beans (*Phaseolus vulgaris* L.) are one of the most important crops for human consumption. It represents one of the main sources of protein and other nutrients such as vitamins, minerals, unsaturated fatty acids and dietary fiber in many countries of the world, especially in underdeveloped countries of Latin America and Africa (1). Because of its importance, in Cuba, substantial resources are devoted annually to the production of this grain; however, in many regions this plant is cultivated under rainfed conditions, which significantly reduces its production.

Worldwide, water deficit reduces bean yields by more than 60 %, with low average values of approximately 0.9 t ha^{-1} (2). This situation is made more complex by the climate change effect on precipitation patterns, which affects the availability of water in agricultural systems, as well as the incidence of intense events such as storms that destroy crops and jeopardize food security in many regions of the planet (3).

Drought has a negative impact on several vital biological processes of plants and in the different stages of their life cycle, especially during germination where they are more vulnerable and where their establishment, subsequent development and yield are defined. This abiotic stress affects different morphological and physiological indicators such as: germination percentage (4), length of vegetative organs, dry and fresh mass, vigor (5), chlorophyll content and photosynthetic activity (6), among others. This has a negative influence on other reproductive indicators such as the number of flowers, yield and its components (7).

Plants possess different mechanisms to face water deficit consequences, which include morphological, physiological and biochemical changes such as: an increase in stomatal conductance (8); the production of osmotically active compounds such as amino acids and sugars, which enable an adjustment of the osmotic potential of tissues (9); and an increase in antioxidant defense (10).

The study of these mechanisms is essential to understand the antistress defense system of plants and to determine their tolerance capacity, which is essential in plant breeding programs (9). *Phaseolus vulgaris* L. has a wide variation in drought tolerance among cultivars, so studies are conducted to identify tolerant genotypes at different stages of development. One of the most widely used methods to estimate plant tolerance to water stress consists of determining the capacity of seeds to germinate and emerge under drought conditions simulated by the polymer polyethylene glycol, since this limits water absorption and can delay and affect the different physiological processes (4,6). This compound is characterized by being highly hydrophilic, inert, nonionic and has no toxic effects on living organisms (11,12). The aim of the present work was to evaluate the effect of polyethylene glycol (PEG)-induced water stress on germination and early growth of *Phaseolus vulgaris* L. cv. 'Delicias 364' seeds.

MATERIALS ANDMETHODS

Plant material

Certified bean seeds of cultivar 'Delicias 364' supplied by the Provincial Seed Company from Jovellanos municipality, Matanzas province were used.

Germination test

The germination test was carried out in Petri dishes of 9 cm in diameter. Seeds (10 per Petri dish) were placed on filter paper moistened with different concentrations (3, 6, 9, 9, 12, 15 and 18 %) of polyethylene glycol-6000 (PEG-6000) and a control treatment, which did not receive the osmotically active compound. Four Petri dishes per treatment (PEG concentrations) were used. The stressor (PEG) was applied at a rate of three times the mass of the dry substrate. The germination process was evaluated daily for seven days and the results were expressed as percentage of normal seedlings. Petri dishes were placed in a growth room at a temperature of 25 ± 2 °C, with a photoperiod of 16 h (35 µmol $m^{-2}s^{-1}$).

Germination value

The number of germinated and non-germinated seeds was evaluated daily during the seven days of the germination trial. With the data obtained, the germination value (GV) was calculated using the formula of Djavanshir and Pourbeik [13]:

$$\text{VG}= \left(\sum_{i=1}^{n} Vedi\right)\left(\frac{Ef}{10N}\right)$$

where:

Ved = daily emergence rate, calculated as the percentage of cumulative emergence divided by the number of days since the test starting.

N = frequency or Ved number calculated during the test.

Ef = percentage of seedling emergence at the end of the seven-day test.

Peak emergence

It was determined by maximum percentage of emergence in the same day (EP) [14].

Root/ aerial part ratio

It was determined by the ratio of root length (cm) to aerial part length (cm).

Morphological indicators

The following indicators were evaluated: root, hypocotyl and epicotyl length and presence (percentage) of true leaves. Length data were obtained with the use of a millimeter paper and expressed in centimeters.

Biochemical indicators

Extraction and quantification of proteins, total soluble carbohydrates and reducing sugars were performed on roots and aerial part of seedlings at the end of the germination trial. The plant material was cold macerated with sodium phosphate buffer solution 50 mmol L^{-1}, pH 7.0 and in a 25 %:75 % ratio. The homogenate was centrifuged at 10 000 rpm and the supernatant was collected and stored at -20 °C until the time of determinations.

Total soluble protein content

The protein content was determined colorimetrically by the method described by Lowry [15], using bovine serum albumin as a standard. The absorbance values were obtained at 750 nm and the concentrations (mg mL^{-1}) were determined using the standard curve.

Total soluble carbohydrate content

The carbohydrate content of the samples was determined colorimetrically by the phenol-sulfuric method [16]. D-glucose was used as the standard sugar and the absorbance was determined at 490 nm. Concentrations were determined from the standard curve and expressed in mg mL^{-1}.

Reducing sugar content

The content of reducing sugars was quantified by the dinitrosalisylic acid method and D-glucose (Sigma) was used as the standard sugar [17]. The absorbance values were obtained at a wavelength of 456 nm and the concentration was expressed in mg L from the standard curve.

Soluble phenol content

The extraction of soluble phenols was carried out by Friend's method (18). 0.1 g of the plant material was macerated in 1.0 mL of methanol and shaken vigorously. The sample was centrifuged at 12 000 rpm for 10 min and the supernatant was collected for the determination of soluble phenols. Chlorogenic acid (0.05 mol L^{-1}) was used as a standard to determine the concentration of phenols and absorbance values were obtained at 725 nm.

All spectrophotometric measurements described were performed on a UV/VIS Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Sweden).

Experimental design and statistical analysis

A completely randomized design with four replicates was used. For biochemical analyses, five samples were taken per treatment, while for the evaluation of morphological and physiological parameters, 10 seedlings were analyzed.

For the statistical analysis of the experimental data, the SPSS version 18.0 statistical package was used. Normality and homogeneity of variance were tested using the Shapiro-Wilk test and Levene's test, respectively. With the data that met the assumptions of normality and homogeneity of variance, an analysis of variance and Duncan's multiple range test was applied for a confidence level of 95 %. In the case where these assumptions were not met, a non-parametric analysis was performed using the Kruskal Wallis and Mann Whitney tests ($p<0.05$).

The comparison between the percentages of seedlings with true leaves between treatments was carried out by means of an analysis of proportions using the CompaProp program version 3.01 on Windows [(19)].

RESULTS AND DISCUSSION

Germination

Polyethylene glycol affected the germination percentage of *Phaseolus vulgaris* L. cv. 'Delicias 364' (Figure 1). At low concentrations (3 and 6 %) of the osmotic agent, percentages higher than 90 % were obtained from the second day of the experiment. At higher polymer contents (9, 12 and 15 %) the germination percentage decreased significantly with values of 76.7, 73.3 and 20.0 %, respectively, while 18 % of the polymer caused total inhibition of germination.

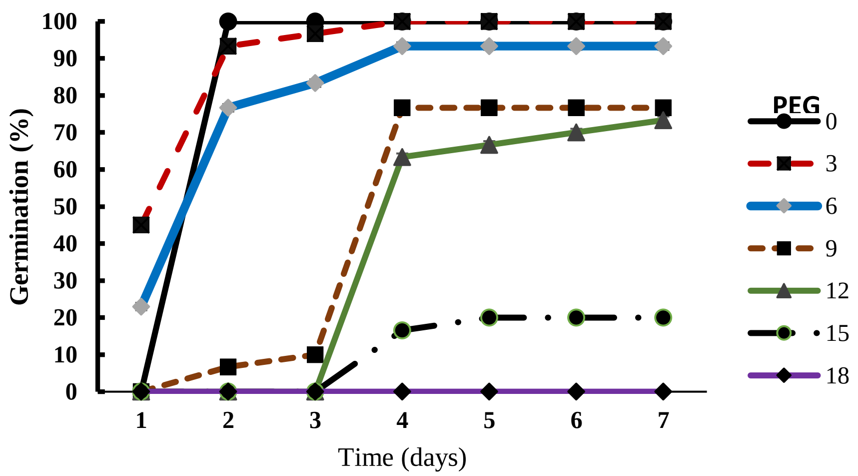


Figure 1. Effect of polyethylene glycol-6000 on the germination percentage of Phaseolus vulgaris L. cv. 'Delicias 364' seeds

The presence of polyethylene glycol at high concentrations also caused a decrease in the germination rate as a function of peak emergence (Table 1). In the control, 3 and 6 % PEG treatments, the highest germination percentage (peak day) was observed on the second day. At higher concentrations of the osmotic agent (9, 12 and 15 %) a delay of the germination process was observed and a maximum germination on the fourth day of the experiment.

Table 1. Peak emergence in seeds of *Phaseolus vulgaris* L. cv. 'Delicias 364' germinated in different concentrations of polyethylene glycol-6000.

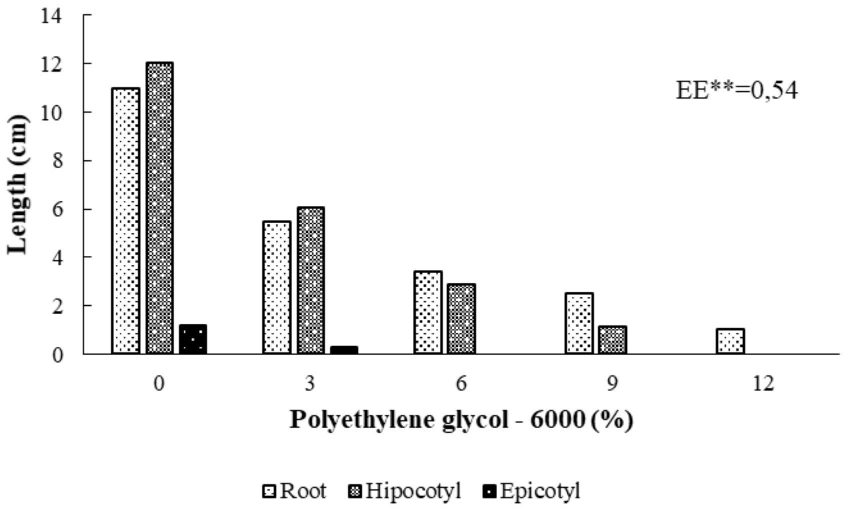
	PEG-6000 (%)					
	0	**3**	**6**	**9**	**12**	**15**
Peak day	2	2	2	4	4	4
Peak emergency (%)	100	53	53	66,6	63,3	16,6

The negative effect of polyethylene glycol on germination may be related to a decrease in the imbibition process of seeds, due to the highly hydrophilic character of the polymer that causes a decrease in the osmotic and hydric potential of the medium [20]. Imbibition is essential for the germination initiation, since the water entry allows the hydration of enzymes and respiratory substrates that activate metabolic processes in the embryo and endosperm of the seed. Among these processes are those of enzymatic hydrolysis that allow the use of food reserves for embryo growth. In addition, a decrease in the imbibition process affects dioxygen input and aerobic respiration, which reduces the metabolic energy required to meet the high cost of germination [21]).

These results coincide with those reported by other authors who have demonstrated the negative effect of polyethylene glycol-induced water stress on germination and early growth of fabaceae such as *Phaseolus vulgaris* L. [22] and *Vigna radiata* L. [12], as well as other economically important non-legume species such as *Sorghum bicolor* (L.) Moench [5], *Brassica napus* L. [4] and *Triticum aestivum* L. [23].

Morphological and physiological indicators

Polyethylene glycol significantly decreased root growth at all concentrations studied (Figure 2). Treatments 6, 9 and 12 % PEG decreased root length by 69.8; 77.6 and 90.4 %, respectively, compared to the control treatment. The concentration of the osmotic agent (15 %) completely inhibited root development. Similarly, the hypocotyl showed a significant reduction of growth in the presence of polyethylene glycol and a greater sensitivity in relation to the root, since at 12 % PEG the growth of this organ was completely inhibited. In relation to the epicotyl, the PEG presence at low concentration (3 %) significantly reduced growth in relation to the control. Higher contents of the polymer in the medium totally inhibited its growth.



Different letters indicate significant differences between treatments for the same organ (Duncan, $p<0.05$). ES=0.54**, n=10

Figure 2. Effect of PEG-6000 on root, hypocotyl and epicotyl growth of *Phaseolus vulgaris* L. 'Delicias 364'

These results coincide with those reported by other authors who observed a decrease in root and aerial growth of seedlings of *Phaseolus vulgaris* L. [2,24], *Vigna unguiculata* cv. 'BRS Tumucumaque' [25], *Glycine max* (L.) Merr. [26], *Brassica napus* L. [4] and *Ocimum basilicum* L. [27] in the presence of PEG-6000. However, the work showed that there were different levels of tolerance to the osmotic agent due to genotypic differences among the species and varieties evaluated.

The decrease in root length and aerial part of the seedlings may be related to osmotic stress of tissues in the stressor presence. Under conditions of low water potential, water leaves plant cells to compensate for the difference in osmotic potential between the plant tissues and the medium. This in turn causes a decrease in the turgor pressure required for cell expansion and growth [28]. Another factor that could affect seedling growth is related to oxidative stress generated under osmotic stress conditions [29]. Under these conditions, the concentration of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion, and the potent hydroxyl anion are exacerbated, which oxidize numerous important macromolecules such as nucleic acids, proteins, and lipids [29,30]. This causes structural and functional modifications in these compounds which affect numerous metabolic processes, cell homeostasis and seedling growth [31].

The values corresponding to the root to aerial part ratio showed an increase in the polyethylene glycol treatments: control (0.83), 3 % (0.86), 6 % (1.18) and 9 % (2.24). Higher PEG values inhibited shoot growth. The increase in root to aerial part ratio indicates that the osmotic agent had a positive effect on root growth compared to aerial structures, which may signify a survival mechanism under water stress conditions. An increase in root to aerial part ratio in the presence of PEG-induced osmotic stress was also observed in *Phaseolus vulgaris* L. [28], *Glycine max* L. [22], *Triticum aestivum* L. [32,33] and can be considered an indicator of tolerance to water deficit [34].

The polyethylene glycol effect on true leaf development in bean seedlings is shown in Table 2. The 3 % PEG treatment decreased by 55 % the percentage of seedlings with true leaves in relation to the control, while in the presence of 6 % of the osmotic agent only 18 % of seedlings developed photosynthetic structures and with a smaller surface area, compared to the leaves of the control and 3 % PEG treatments. At higher concentrations of the polymer there was a complete inhibition of true leaf formation.

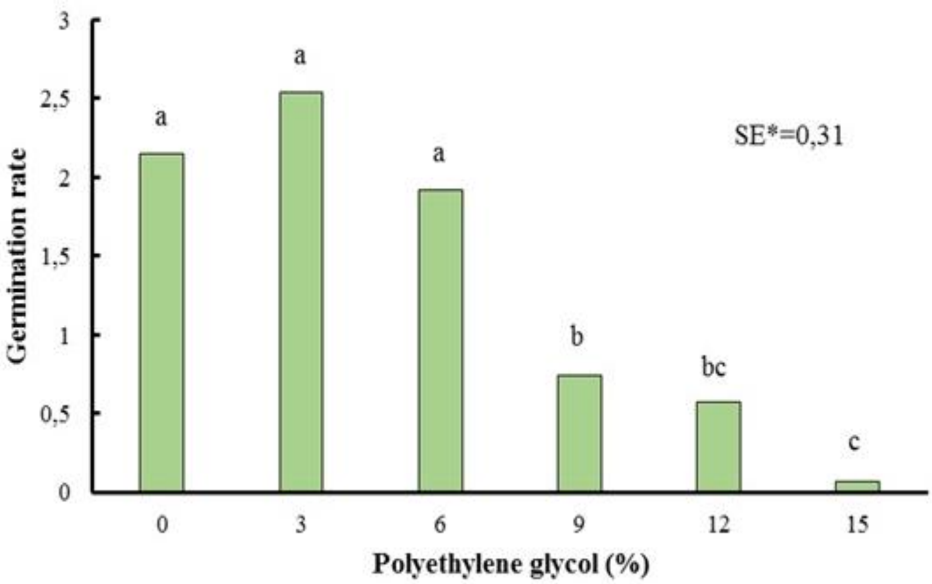
Table 2. Percentage of *Phaseolus vulgaris* L. cv. 'Delicias 364' seedlings with true leaves, germinated in different concentrations of PEG-6000

PEG-6000 (%)	Proportion	%	SE
0	1,00 (a)	100	0,08
3	0,45 (b)	45	0,08
6	0,18 (c)	18	0,08

Different letters indicate significant differences between treatments ($p<0.05$)

The negative effect of water stress on the number of leaves in bean plants has been previously reported by other authors [35]. Osmotic stress considerably inhibits the processes of cell division and elongation, which significantly influence cell number and volume, and consequently, the formation and growth of new leaf structures. In similar studies, a reduction in the leaf area of *P. vulgaris* cv. 'ICA Piajo' seedlings was observed with the application of PEG-6000 at concentrations higher than 6 % of the stress agent [22].

The germination value of bean seedlings germinated in the presence of polyethylene glycol decreased significantly at concentrations equal to or higher than 9 % (Figure 3). In treatments 9, 12 and 15 % of the osmotic agent, the reduction was 66.0, 73.5 and 96.8 %, respectively, in relation to the control; while in treatments 0, 3 and 6 % of PEG, no differences were observed.



Different letters indicate significant differences between treatments according to the Mann Whitney test ($p<0.05$). SE=0.31*, n=10

Figura 3. Effect of PEG-6000 on germination value of *Phaseolus vulgaris* L. cv. 'Delicias 364'

These results coincide with those obtained in *P. vulgaris* cv. 'ICA Pijao', where a significant reduction in germination and vigor of seedlings was observed under conditions of hydric stress induced by PEG-6000 in concentrations higher than 14 % (24). In similar studies carried out with *Vigna unguiclata* L., a reduction in several indicators such as vigor and mean daily germination was observed in seedlings germinated under PEG-4000-induced water stress conditions (36).

Biochemical indicators

The contents of total soluble carbohydrates, reducing sugars and total soluble proteins in roots and aerial part of germinated seedlings at different concentrations of polyethylene glycol are shown in Table 3. With regard to the concentration of soluble carbohydrates, a higher content of these compounds was observed in the root in treatments 6 and 9 % PEG, compared to the control and the rest of the treatments where similar values were obtained. In the aerial part, there were no differences between the control and 3% PEG; however, lower values were observed in treatments 6, 9 and 12 % in relation to the control.

Table 3. Total soluble carbohydrate (TSC), reducing sugars (RS) and total soluble protein (TSP) content in roots and aerial part of Phaseolus vulgaris L. cv. 'Delicias' seedlings germinated in different concentrations of polyethylene glycol-6000

PEG-6000 (%)	TSC ($mg\ mL^{-1}$) Root	TSC ($mg\ mL^{-1}$) Aerial part	RS ($mg\ L^{-1}$) Root	RS ($mg\ L^{-1}$) Aerial part	TSP ($mg\ mL^{-1}$) Root	TSP ($mg\ mL^{-1}$) Aerial part
0	15,49 b	19,80 a	2,16 c	2,99 c	3,89 a	6,18 a
3	15,86 b	19,01 ab	2,32 b	3,51 b	4,23 a	5,44 b
6	18,68 a	18,34 b	2,89 a	3,68 a	3,78 a	3,23 c
9	18,56 a	18,02 b	2,36 b	2,05 d	2,97 b	3,08 c
12	15,13 b	17,87 b	0,84 d	1,75 e	2,60 b	2,42 d
	SE=2,75**; n=5		SE=0,15**; n=5		SE=0,22**; n=5	

Different letters indicate significant differences between treatments for the same organ (Duncan, $p<0.05$). SE= standard error

The highest content of reducing sugars in the roots was obtained in the germinated seedlings with 6 % polyethylene glycol, followed by treatments with 3 and 9 % of the osmotic agent without differences between them, but higher than the control. The lowest values were obtained with 12 % PEG in the medium. In the aerial part, the treatments with 3 and 6 % PEG showed higher reducing sugar contents than the control, while the lowest values were obtained with 9 and 12 % PEG in that order.

Polyethylene glycol affected the protein content in roots and aerial part differently. The aerial part showed greater sensitivity than the roots, since the protein content decreased at the lowest concentration of PEG evaluated (3 %) in relation to the control and the rest of treatments. In the case of roots, there were no differences between the control and the treatments 3 and 6 % polyethylene glycol, while at higher concentrations (9 and 12 %) of the osmotic agent, lower values were obtained without significant differences between them.

The reduction in carbohydrate content may be related to the degradation of these compounds into monosaccharides and their oxidation to obtain ATP for use in the maintenance of metabolic functions under conditions of water stress (maintenance energy).

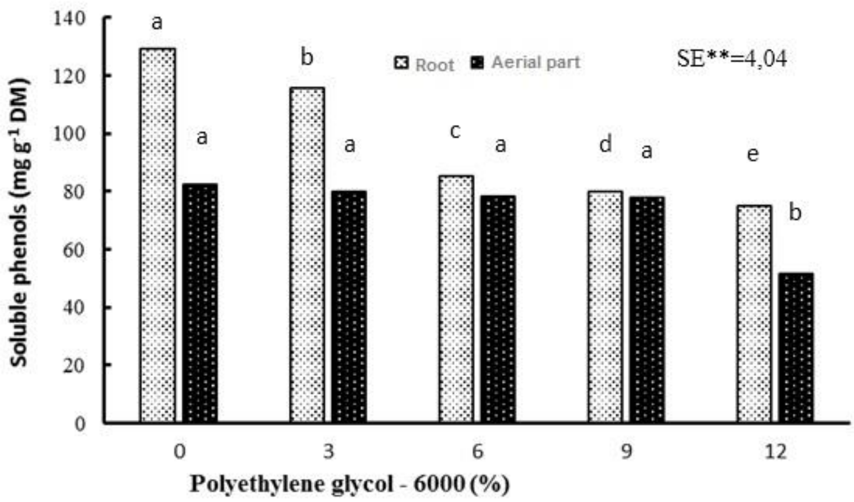
The increase in the content of reducing sugars at low and medium PEG concentrations (3 and 6 %) in roots and aerial part of seedlings may be related to an increase in α-amylase activity in plant tissues. A similar response was observed in *Sorghum bicolor* L. seedlings germinated under PEG-6000-induced water stress conditions [(20)]. The increase in reducing sugar content in the presence of PEG-6000 was also evidenced in seedlings of alfalfa (*Medicago sativa* L.), a species that grows well in drought-affected areas [(30)].

The increase in the concentration of reducing sugars may be related to a physiological mechanism to compensate for the difference in water potential between plant tissues and the external environment; since reducing sugars are osmotically active compounds that decrease the solute and water potential of the cells, which allows the retention and/or absorption of water under physiological drought conditions and the osmo-conditioning of plant tissues.

The decrease in the content of reducing sugars in treatments with high PEG concentrations may be related to the inactivation of the enzyme α-amylase, which has the catalytic function of hydrolyzing starch molecules. In similar studies, a positive correlation was observed between α-amylase activity and reducing sugar concentrations in *Zea mays* L. seeds treated with PEG-6000 [(37)]. On the other hand, the decrease in the content of reducing sugars may also be associated with a higher utilization of these compounds, due to an increase in respiration in these seedlings [(38)]. This allows the development of vital processes such as cell turnover and the synthesis of proteins, enzymes, and other compounds involved in the antistress response.

The decrease in protein content may be related to affectations in the protein biosynthesis machinery, as well as in fundamental processes such as cellular respiration, since a decrease in the respiratory rate represents a lower availability of metabolic energy for biosynthetic processes. This is consistent with the observed reduction in reducing sugar content in treatments with the highest concentrations of polyethylene glycol. In addition, a decrease in the cellular respiration process implies a lower concentration of organic acids derived from the Krebs cycle, which can serve as a basis for the synthesis of amino acids used in protein biosynthesis.

The content of soluble polyphenols in the seedlings subjected to water stress is shown in Figure 4. In roots the values decreased with increasing PEG concentration. This result may be related to a reduction in the activity of enzymes involved in the biosynthetic pathway of polyphenols, especially phenylalanine ammoniolase, which plays a central role in the metabolism of these compounds. The polyphenol content in the aerial part showed greater stability with similar values between the control and treatments 3; 6 and 9 % PEG and only decreased by 12 % PEG.



Different letters indicate significant differences between treatments according to Mann Whitney test ($p<0.05$), SE=4.04**, n=5

Figura 4. Soluble polyphenol content in root and aerial part of *Phaseolus vulgaris* L. cv. 'Delicias 364' seedlings germinated under water stress conditions

These results are in correspondence with those observed in other species such as *Solanum lycopersicum* sp. [39], *Sorghum bicolor* (L.) Moench [20] and *Ocimum basilicum* L. [27]. The stability in contents of these compounds in the aerial part may constitute an antioxidant defense mechanism associated with the synthesis of polyphenols and the movement of soluble phenols to the upper tissues of the plant, where a greater susceptibility to water stress was evidenced. In plants, polyphenols may play an important role in the antioxidant system. These compounds decrease the production of reactive oxygen species by reducing the rate of the Fenton reaction by which ROS are formed when hydrogen peroxide reacts with transition metals. This is because phenolic compounds have the ability to donate electrons to the enzyme guaiac peroxidase for the elimination of hydrogen peroxide, and on the other hand, they can also chelate toxic metals [40,41].

CONCLUSIONS

- Polyethylene glycol-6000 affected the germination process and early growth of *Phaseolus vulgaris* L. cv. 'Delicias 364'; however, the results indicate the presence of tolerance mechanisms to water deficit in this variety, such as the increase in levels of reducing sugars and total soluble proteins in the presence of low and medium concentrations of polyethylene glycol (3-9 %), which may constitute an osmotic conditioning mechanism.
- The significant increase in the root to aerial part ratio at 9 % PEG and the stable production of polyphenolic compounds in the aerial part of seedlings subjected to water stress also suggest the presence of survival mechanisms and pathways to attenuate the oxidative stress generated as a consequence of osmotic stress.

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