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Evaluation Effect of Putrescine Treatment on Growth Factors of Soybean (*Glycine max* L.) Under Drought Stress Induced by Polyethylene Glycol

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ABSTRACT

The present study aimed at evaluation of putrescine treatment on growth factors of soybean (*Glycine max* L.) under drought stress induced by polyethylene glycol (PEG). Soybean seeds were planted and irrigated with five solutions (control, drought, PEG, Put, and PEG-Put). Then, growth factors (length and weight) of seedlings, root, shoot, stem, hypocotyl, first internode, and plant were measured. Statistical analyses were performed by one-way ANOVA through Duncan Test at $p \leq 0.05$ in SPSS (Version 21) in three iterations. Graphs were drawn in Excel Software (Microsoft Office, 2010). The results obtained from the present study showed that putrescine could not have considerable effect to alleviate adverse effect of drought stress caused by PEG in soybean.

KEYWORDS: Putrescine, PEG, Soybean, Length, Weight, Polyamine.

1- INTRODUCTION

Drought is an important limitation for achieving high yield all around the world. By changing a series of physiological, biochemical, and molecular responses, plants adapt to drought stress. Polyamines are aliphatic amines with low molecular mass in all plant cells and they play important roles in plants' responses to drought stress. Common polyamines in higher plants are putrescine, spermidine, and spermine. Because of their cationic nature in physiological pH, the polyamines can interact with negatively-charged macromolecules such as membrane phospholipids, DNA, and proteins. Therefore, polyamines can stabilize DNA structure and protein configurations and regulate activities of several enzymes such as antioxidant enzymes and H^+-ATPase during stress conditions. Putrescine can be decomposed by di-amine oxidase and used for yielding H_2O_2 leading to elevation of Ca^{2+} in guard cells and induction of stomata closure. Stomata closure by various polyamines leads to reduction of water loss in primary stages of drought stress which is very crucial for drought tolerance [1].

Putrescine, or tetramethylenediamine, is a foul-smellingorganic chemical compound $NH_2(CH_2)4NH_2$ (1,4-diaminobutane or butanediamine) that is associated withcadaverine; both are formed by the breakdown of amino acids in living and dead organisms and both are toxic in large doses. The two compounds are mostly in charge of the foul odor of putrefying flesh, but also contribute to the odor of such processes as bad breath and bacterial vaginosis. They are also found in semen and some microalgae, together with related molecules like spermine and spermidine. Table 1 summarizes properties of putrescine and Figure 1 depicts its chemical structure[2].

Table 1: Properties of the polyamine putrescine

Molecular formula	$C_4H_{12}N_2$
Molar mass	88.15 $g.mol^{-1}$
Appearance	Colorless crystals
Odor	strong, piperidine-like
Density	877 mg.ml-1
Melting point	27.5°C (82°F)
Boiling point	159°C (317°F)

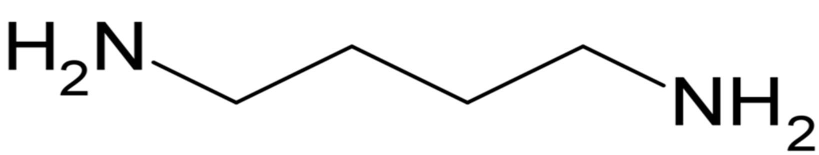

Figure 1: Chemical structure of putrescine

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Putrescine is formed either directly from ornithine by ornithine decarboxylase (ODC) or indirectly from arginine by arginine decarboxylase (ADC) (Fig. 2).

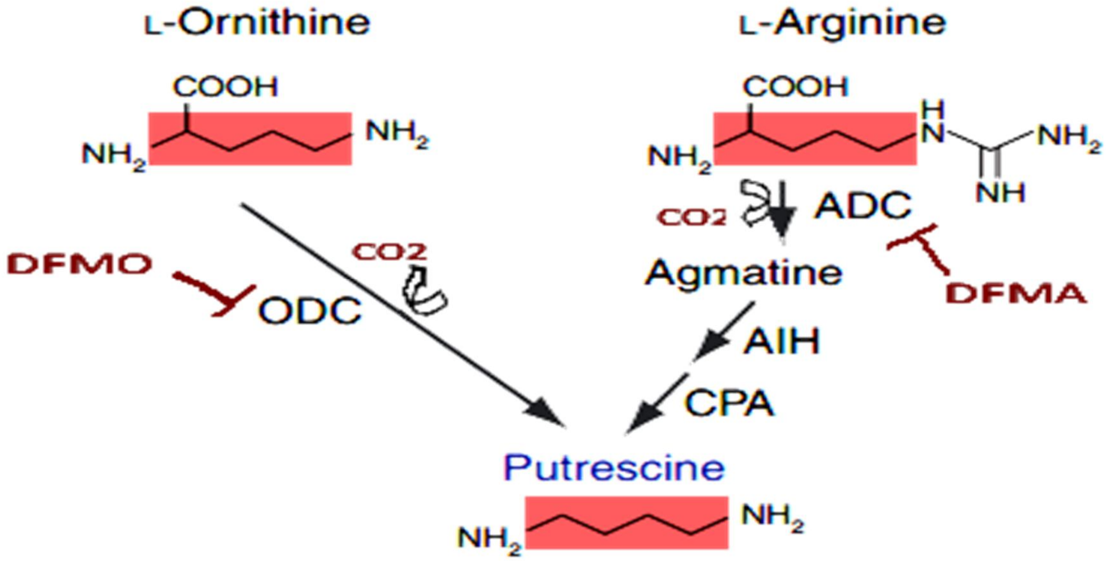


Figure 2: Biosynthesis pathway of putrescine

Soybean is considered as one of the oldest agricultural plants. It contains abundant amounts of protein, carbohydrate, oil, phosphorus, calcium, iron, magnesium, zinc, fiber, and vitamins (thiamin, riboflavin, and niacin) [4]. Water is very important of growth and development of soybean. It is sensitive to drought in germination and great loss of germination occurs during drought stress. Also, lack of sufficient moisture in germination of soybean brings about slower growth [3].

Polyamines can have variety of applications for tolerating stress in agricultural plants. They are small organic polycations found in all living creatures and have considerable role in cell cycles, gene expression, signaling, growth and development of plants, and tolerance to stress. Accumulation of polyamines in plants has been proven during abiotic stress leading to higher tolerance to stress. Authors have introduced polyamines as a way in order to increase tolerance to various stresses in crop plants [4].

Considering high application and importance of soybean as well as adverse effect of drought stress on its characteristics, the present study aimed at evaluation of putrescine treatment on growth factors of soybean (*Glycine max* L.) under drought stress induced by polyethylene glycol (PEG).

2- MATERIALS AND METHODS

Polyamine putrescine was purchased from Pajohan-Sanaat-Homehr. The required amount of PEG with molecular mass of 6000 was derived by the following relation in order to provide osmotic potential of 0.3 MPa:

$$S = -(1.18\times10^{-2})\ C - (1.18\times10^{-4})\ C^2 + (2.67\times10^{-4})\ CT + (8.39\times10^{-7})\ C^2T$$

where C, T, and S stand for concentration of PEG 6000 ($g.l^{-1}$), temperature (°C), and osmotic potential (MPa), respectively. PEG concentration was found to be 35.42 g.

Solutions were made on Aug 2013 in Research Laboratory of Islamic Azad University of Gorgan – Iran.

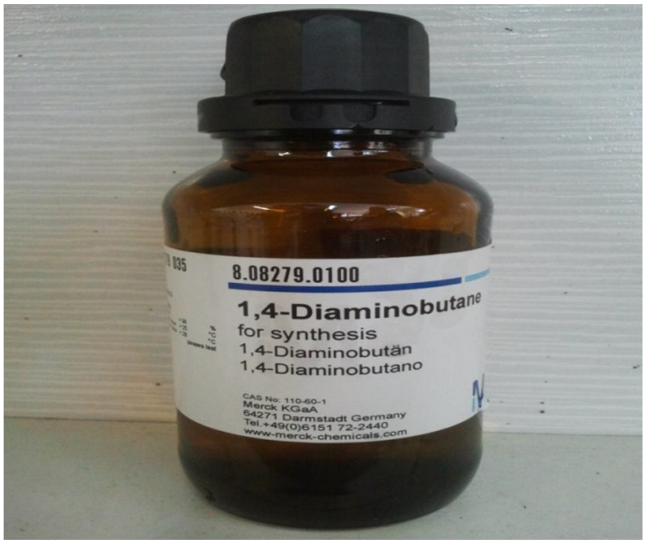


Figure 3: The purchased putrescine

Putrescine solution with concentration of 0.5 mM was made as follows:

$0.1 \quad mm = \frac{1}{10000}$ M

$$C_M = \frac{C}{M} \quad => \frac{1}{10000} = \frac{C}{88.15} \quad => \quad C = \frac{88.15}{10000} = 0.008815\ {}^{g}/_{lit}$$

0.0088 × 5 = 0.044

For preparation of 2 lit of putrescine solution, 2×0.044 was added into 2 lit of distilled water.

In order to provide PEG+Put solution, 70.82 g PEG (2×35.42 for 2 lit) were weighed and the putrescine required was achieved as follows:

$$Put \quad \frac{0.088}{2000} = \frac{x}{2058} \quad => \quad x = 0.09$$

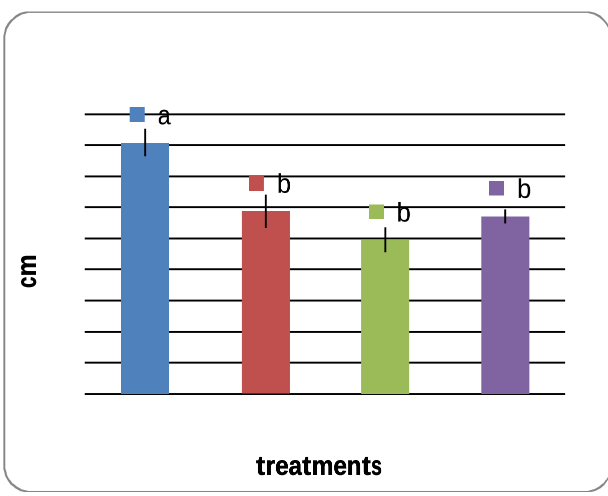
Soybean (*Glycine max* L.) seeds were purchased from Araghi-Mahalleh Station – Gorgan – Iran and 600 seeds were placed in hypochloride sodium 10% for 10 min after rinsing with distilled water. Petri dishes were rinsed with boiling water and then disinfected with hypochloride sodium 10%. Petri dishes and seeds were rinsed immediately after disinfection. Cleansing fabrics were disinfected with distilled water and hypochloride sodium and finally they were washed with distilled water. The seeds were sorted in 5 petri dishes between two cleansing fabrics. In each petri dish, seeds were sorted in 5 rows each with 20 seeds. Then, the petri dishes were placed at 25°C at darkness and were irrigated with Put, PEG, PEG+Put, and control solutions every 8-hour intervals. After 7 days, length, fresh weight, and dry weight of seedlings were measured for each treatment. Then, the seedlings were planted in 1-kg pots. Three seedlings were planted at the depth of 1 cm in each pot. Irrigation was performed with five treatments: control (irrigation in alternate days), drought (irrigation once in five days), PEG (irrigation with PEG solution), Put (irrigation with putrescine solution), and PEG-Put (irrigation with PEG and putrescine solution). Then, growth factors (length and weight) were measured.

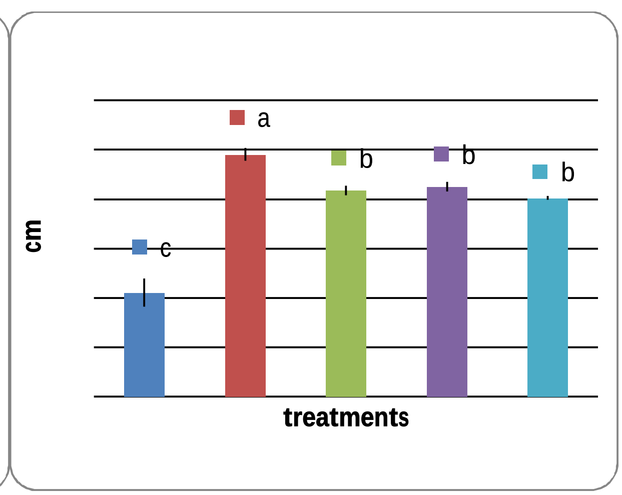
Figure 4: Treatments

Statistical analyses were performed by one-way ANOVA through Duncan Test at p≤0.05 in SPSS (Version 21) in three iterations. Graphs were drawn in Excel Software (Microsoft Office, 2010).

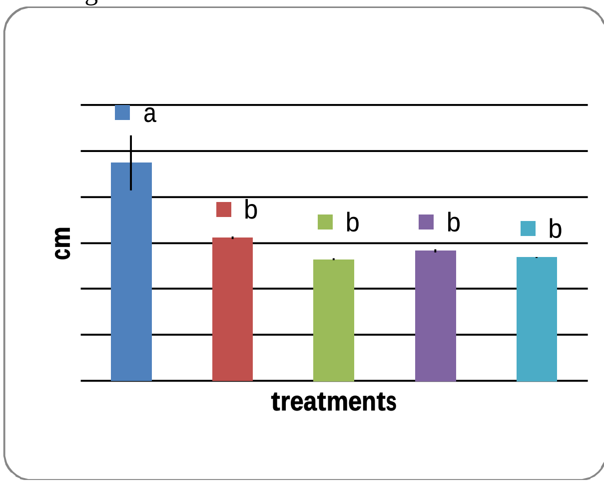
3- RESULTS

Figure 5 shows the results achieved from measurement of length of root, shoot, seedling, stem, plant, hypocotyl, and first internode. As it can be seen, the highest and lowest seedling lengths were seen in control and Put treatments, respectively. There was a significant difference between control and other treatments (p<0.05). The lowest root length was detected in control treatment which had a significant difference with other treatments (p<0.05). The highest root length was seen in drought treatment with significant difference with other treatments (p<0.05). Also, the highest shoot length was found in control treatment which had significant difference with other treatments (p<0.05) while the lowest was in PEG which had no significant difference with drought, Put, and PEG-Put treatments (p>0.05).Moreover, the highest stem length was detected in control treatment with significant difference with other treatments (p<0.05) whereas the lowest was in PEG (22.325 cm) which had no significant difference with drought, Put, and PEG-Put treatments (p>0.05). The highest and lowest hypocotyl lengths were seen in drought and PEG treatments, respectively. There was a significant difference between the treatments (p<0.05). Furthermore, the highest and lowest first internode lengths were found in control (13.50 cm) and PEG (11.175 cm) treatments, respectively. There was a significant difference between control treatment and other treatments (p<0.05). In addition, the highest and lowest plant lengths were seen in control and PEG treatments (56.25 and 47.375 cm), respectively. No significant difference was detected between PEG and PEG-Put treatments (p>0.05).

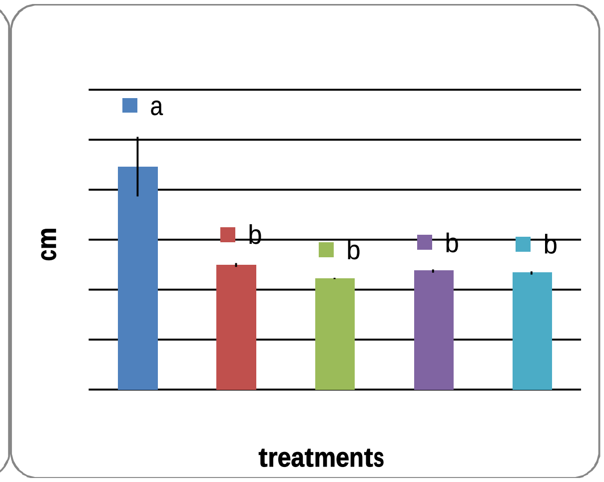




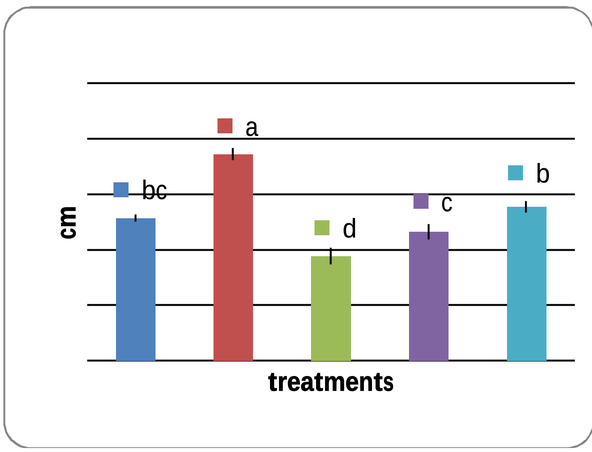
Seedling



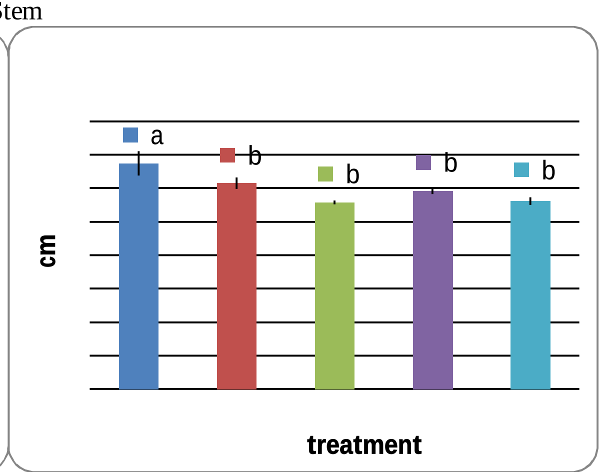
Root



Shoot

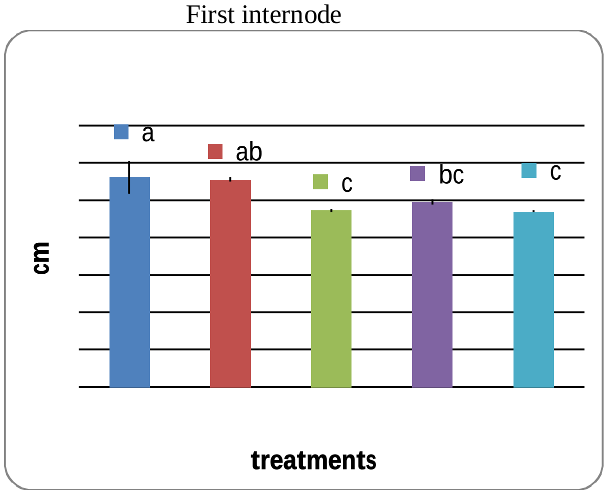


Stem



Hypocotyl

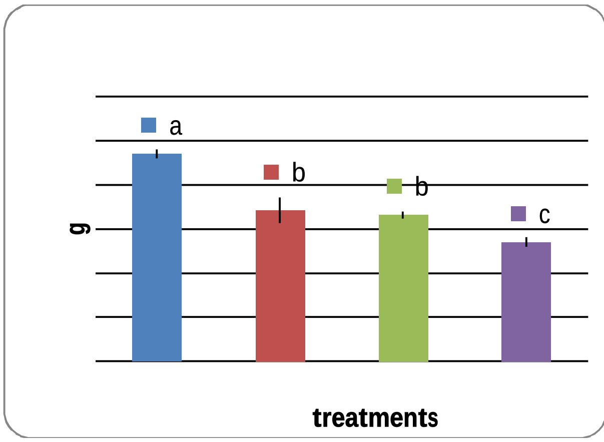
First internode



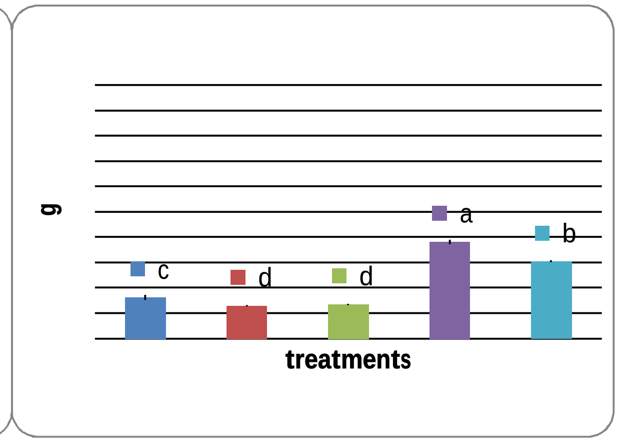
Plant

Figure 5: Lengths of seedling, root, stem, shoot, hypocotyl, first internode, and plant.

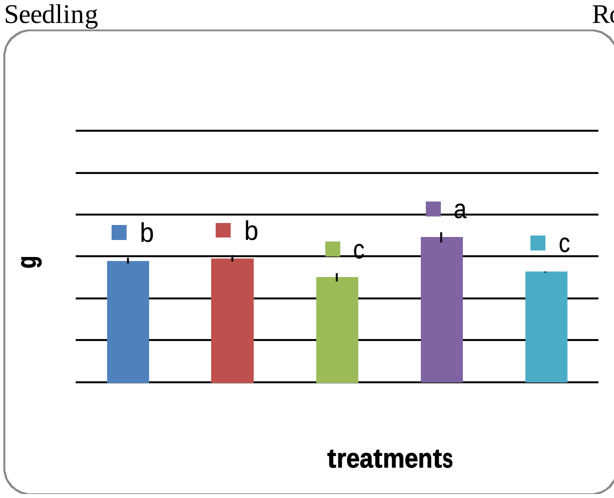
Figure 6 depicts the results obtained from measurement of fresh weight of seedling, root, shoot, and plant. As it can be seen, the highest fresh weight of seedling was in control treatment (0.9425 g) which was significantly higher than other three treatments ($p<0.05$). The lowest seedling fresh weight was in PEG-Put treatment (0.6187 g) which had significant difference with Put and PEG treatments ($p<0.05$). The highest root fresh weights were seen in Put and PEG-Put treatments which had significant difference with other treatments ($p<0.05$). Also, the lowest root fresh weight was in drought treatment (0.13075 g) which had no significant difference with PEG treatment ($p>0.05$). Moreover, the highest and lowest shoot fresh weights were seen in Put and PEG treatments, respectively and significant differences were seen between them ($p<0.05$). In addition, the highest and lowest plant fresh weights were in Put and PEG treatments, respectively. There was a significant difference between them ($p<0.05$).



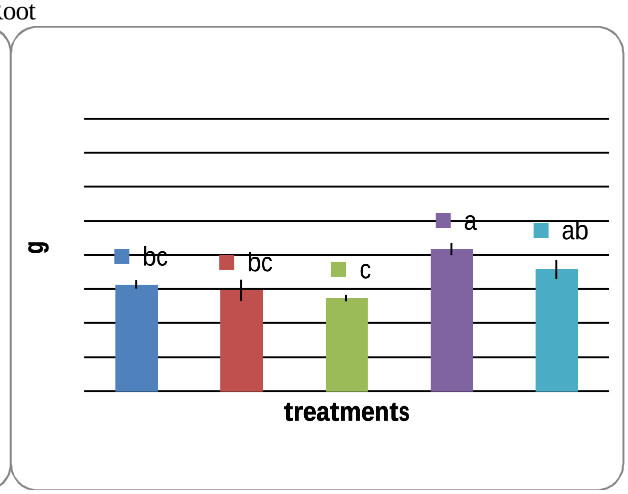
Seedling



Root



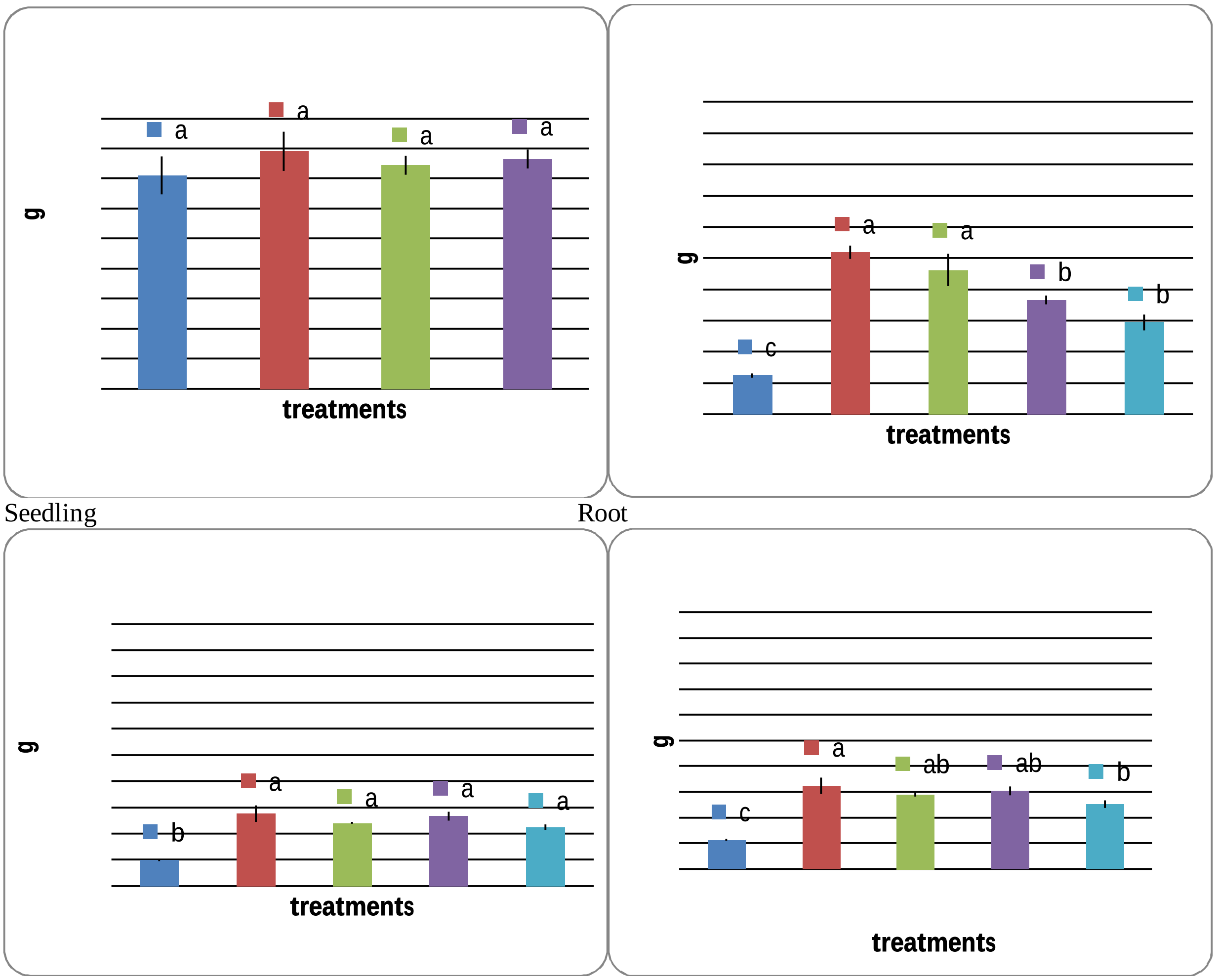
Shoot



Plant

Figure 6: Fresh weights of seedling, root, shoot, and plant.

Figure 7 depicts the results acquired from measurement of dry weights seedling, root, shoot, and plant. As it is evident, the highest and lowest seedling dry weights were in PEG and control treatments, respectively. No significant difference were seen between the treatments ($p>0.05$).The lowest root dry weight was seen in control treatment (0.0125 g) which had significant difference with other treatments ($p<0.05$). Also, the highest root dry weight was detected in drought treatment (0.52 g) which had significant difference with PEG treatment ($p<0.05$).Additionally, the highest and lowest shoot dry weights were detected in drought and control treatments, respectively. A significant difference was seen between control and other treatments ($p<0.05$). No significant differences wereseen between other treatments ($p>0.05$). Finally, the highest and lowest plant dry weights were in drought and control treatments, there was a significant difference between control and other treatments ($p<0.05$).



Seedling Root

ShootPlant

Figure 7: Dry weights of seedling, root, shoot, and plant.

4- DISCUSSION AND CONCLUSION

Over 80% of plant tissues is water and water loss may have evident adverse effects and can be considered as of the most important limiting factors for growth and development of plants. In order for a plant to absorb water, water potential around root cells should be lower than its surroundings. As a matter of fact, during drought stress, water potential surrounding the plant is lower than natural status and the plant will encounter difficulty in water absorption. Several studies have been performed about the effects of water loss on growth and development of plants. The results obtained from these studies indicate that reduction of growth occurs for many reasons. When plantsare not provided with sufficient water, growth-inhibitors increase in plants [5].Plant abilities in tolerance to different stress conditions are different. The tolerance can be classified into stress avoidance and stress tolerance. By providing some physical and/or metabolic barriers, a plant can avoid stress conditions. In tolerance condition, plants tolerate the damages and losses caused by stresses and try to minimize such effects. In this condition, the plants are subjected to stresses but the damages are expected to be alleviated [6]. Drought stress was induced by use of polyethylene glycol. PEG is very popular because of its ability to make more realistic drought conditions [7]. PEG results in reduction of hydrolysis of seeds' stored materials and consequently, lower growth by making drought condition [8]. In the present study, use of putrescine after drought induced by PEG results in improved root length although it was not significant ($p>0.05$). This is consistent with the results obtained by Nayyar et al. (2005). They claimed that external putrescine (0.1 mM) reduced injuries caused by drought by 38% and 43% in pea and soybean, respectively [9]. Despite the fact that accurate reason of these variations in polyamines between two species is not clear, some authors attributed it to the difference in expression of polyamines' biosynthetic enzymes such as ADC and ODC. Niakan et al. (2011) reported that use of spermidine improves shoot length significantly [10] although putrescine in the present study could not have such effect. Various studies have shown that exposure of plants to different stresses result in putrescine accumulation [11,12,13]. Several investigations have indicated that external polyamines can somewhat improves growth during stresses which is indicative of effect of polyamines in reduction of cellular damage [13,14,15,16]. In addition, the results obtained by the present study are not in agreement with those of Zapata et al. (2004) who found that putrescine treatment led to improvement of physical properties of seedling [17]. Also, the results obtained from the present study are not in agreement with those of Farrokhi e al (2004). They reported that drought stress in germination of soybean results in significant reduction of length and dry weight of root and stem [18].

Acknowledgment

The authors declare that they have no conflicts of interest in the research.

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