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Research Article

Effect of drought-induced stress by PEG6000 on physiological and morphological traits of chickpea (*Cicer arietinum* L.) seed germination in order to assortment of drought tolerant cultivars

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Abstract

In this study, the reaction of germination and plantlet growth different chickpea cultivars (Arman, Azad, Binalood and Hashem) in relation to osmotic stress due to polyethylene glycol solution (-2, -4, -6 and -8bar) and control (distilled water) using factorial design in completely randomized with four replications was evaluated. In this study, there was a significant difference between the different potentials of drought stress for all measured traits with 99% probability. With the reduction of water potential, the seed germination, germination rate, rootlet length, stemlet length, rootlet wet weight, rootlet dry weight, stemlet wet weight, stemlet dry weight, plantlet wet weight, as well as plantlet dry weight decreased. Also, proline and F_v/F_m (the efficiency quantum of the photosystem II) of cultivars at two levels of control and glycol polyethylene solution at the level of -2bar was different. So that the Hashem cultivar showed the highest amount of proline in the polyethylene glycol solution -2bar. However, the F_v/F_m had a reversed relationship with drought stress. That is, drought stress leads to a reduction in the F_v/F_m. The maximum amount of F_v/F_m was found in the Hashem cultivar under drought stress condition, and the lowest amount in Azad cultivar in stress conditions. The results of activity of antioxidant enzymes in this study showed that drought-resistant cultivars such as Hashem is to drought stress by increasing the capacity of the antioxidant system to remove reactive oxygen species and prevent membrane peroxidation Overall, the commercial cultivar Hashem showed drought tolerance at high stress levels, indicating their potential for production applications.

Keywords: Chickpea, Polyethylene Glycol, Drought Stress, Germination, Proline, Antioxidant enzyme activity

Introduction

Currently, there is food poverty in parts of the world called the developing world, which accounts for two-thirds of the world's productivity. Diet in these countries is mainly starch, and the lack of protein and malnutrition of millions of people living in these countries is one of the acute problems in these areas. Animal protein production is more difficult and expensive than plant protein. Dietary protein production from animals is a major source of greenhouse gas emissions, water use, and deforestation. Legumes are a rich source of protein but currently comprise a minor part of most human diets. Worldwide, the average consumption of legumes and meat is 21 and 112 g/person/day, respectively. Legumes are part of traditional diets in most cultures, have low greenhouse gas and water footprints, enrich the soil through nitrogen fixation (Bagheri, 1997; Abonus, 2001; Semba *et al.*, 2021). The protein in legumes is 2 to 3 times that of cereals and 10 to 20 times that of glandular plants (Bagheri, 1997). However, a deficiency in the amino acid lysine in cereals can also be compensated by consuming legumes (Bagheri, 1997; Adhikari *et al*., 2022). Meanwhile, due to the presence of nitrogen-fixing bacteria in the air, they also play an important role in soil fertility, and after harvesting, large amounts of nitrogen are added to the soil. Therefore, these plants play an important role in crop rotation (Abbasnejad, 2006; Marschner, 2011; Semba *et al.*, 2021).

These plants are also used as green manure to strengthen and improve soil physics (Bagheri, 1997; Semba *et al.*, 2021). On the other hand, drought stress is one of the most important stresses in reducing plant growth and production, so that it affects many aspects of plant metabolism and growth, because this stress reduces the speed and percentage of germination and finally Delays the establishment of the plant (Bradford 1995; Prisco *et al*., 1992; Ahmadloo *et al*., 2011; Hellal *et al.*, 2018). Water is one of the main factors activating germination and the ability of seed access to water is reduced by reducing the osmotic and matrix potential (Asghari, 1992; Chiang and Dandekar, 1995). Seed germination is extremely important in determining the final density of the plant per unit area so that sufficient

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plant density per unit area is obtained when the seeds are fully and rapidly germinated (Baalbaki *et al.*, 1990; Wu *et al.*, 2020).

Germination fluctuations that are affected by environmental factors are of ecological importance and also from the perspective of crop management (Syvertsen and ML, 1983; Ibrahim *et al.*, 2001; Turk *et al.*, 2004). The interaction between environmental factors and the internal mechanisms of a seed determines seed germination under certain conditions (Bradford and Haigh, 1994; Bradford, 1995). Since it is almost difficult to create and maintain a pure water potential in the soil environment, in this regard, establishing drought stress conditions using different osmotic materials to create osmotic potentials is one of the most important methods of studying the effect of stress on germination (Emmerich and Hardegree, 1990; De and Kar, 1995; Aniat-ul-Haq and Agnihotri, 2010).

Among these materials, polyethylene glycol is widely used due to its environment-like conditions and is widely used in laboratory conditions (Kaufmann and Eckard, 1971; Gholamin and Khayatnezhad, 2010; Bradford, 1995; Prisco *et al*., 1992; Ahmadloo *et al*., 2011; Hellal *et al*., 2018), due to its high molecular weight, it cannot cross the cell wall and is therefore used to regulate water potential in germination tests. Polyethylene glycol with a high molecular weight (6000-PEG) is more suitable for creating drought stress compared to the smaller molecules such as (4000-PEG) because the percentage of seed germination in polyethylene glycol 6000 solution and in soil with the same water potential It is almost equal (Razzaq *et al.*, 2017).

The results of the study of the effects of drought stress due to polyethylene glycol solution at -2, -4, -6 and -8bar levels on different wheat cultivars, significant reduction in germination percentage, germination rate, root length, stem length and the dry matter showed what the root and the stem were (Gholamin and Khayatnezhad, 2010). In different conditions of drought stress, when plants seeds in different polyethylene glycol solutions with deferent osmotic potential were cultivated, different roots were observed. The results of the study of the effect of polyethylene glycol on cumin seeds showed that drought stress had important effects on plant physiological characteristics, so that by reducing the water absorption potential from zero to -8 bar, germination percentage, germination rate and growth index decreased, but the average germination rate increased and the highest recovery was observed at -8 bar (Bagheri, 1997).

Soleimani *et al*. (2011) reported that the interaction of salinity and drought due to dietary salt and polyethylene glycol on *Anabasis aphylla* was significant in reducing root size, stem and seed germination at different levels. Also, the most obvious and common effect of damage caused by drought stress to the plasma membrane is an increase in leakage of cellular sap compounds such as potassium, amino acids, carbohydrates and various electrolytes out of the cell. Reports indicate that cellular fluid leakage decreases during drought tolerance, indicating increased drought stress resistance (Levitt, 1980). The release of free amino acids as proline occurs most commonly in plants under environmental stress and is sometimes associated with increased resistance to drought stress in plants (Bates *et al.*, 1973). Probable physiological actions of proline can be considered as a factor in the regulation of osmotic pressure and the protective factor of cytoplasmic enzymes and membrane structure (Soleimani *et al.*, 2011). According to reports, osmotic stress caused stomatal closing, diminished intercellular CO_2 partial pressure, increased of active electrons, the generation of free radicals like hydrogen superoxide, hydroxide and oxygen active species, the turbulence of light-harvesting systems and damaged photosynthesis efficiency (Tavakol *et al*., 2018, 2022). Photosynthesis efficiency can be assayed by calculating the enhancing the number of electron carriers, finally, the safety of the photosynthetic electron transfer system using the chlorophyll fluorescence (Mc Kenzie and Hill, 1991; Maxwell and Johnson 2000; Zaiyou *et al*., 2020).

Various methods have been used by researchers to evaluate the response of crops to environmental stresses. In this regard, the present study to investigate the effect of polyethylene glycol on germination and other relevant traits of four cultivars of chickpeas in laboratory conditions in order to identify genotypes tolerant to adverse drought conditions and early screening of genotypes with the best characteristics of drought tolerance and to select the best suitable genotypes for aims in cultivation and breeding patterns was done.

Materials and methods

This study was conducted to evaluate the effect of different water potentials on some germination parameters and determine the best level of drought stress at the Karaj Seed and plant Improvement Institute (SPII) and the experiment was conducted as a factorial in a completely randomized design in four replications. Petri dishes were used for the experiment. Thus, the filter papers inside the petri dish were sterilized in an oven at 180 °C for two hours. Petri dishes were placed at 120 °C for 24 hours. Then, 50 chickpea seeds were placed inside each petri dish. The seeds were disinfected before being placed in petri dishes (disinfected in 3% sodium hypochlorite solution for 30 seconds, then rinsed with distilled water, and then reabsorbed with benomyl 2 per thousand fungicide solution for 30 seconds. (Disinfected and then rinsed 3 times with distilled water). After that, the seeds were treated with different levels of polyethylene glycol solution. To prepare a polyethylene glycol solution (with a molecular weight of 6000), the following equation was applied at five levels of drought stress of zero, 2-, 4-, 6- and 8-bar (Emmerich and Hardegree, 1990).

(1)

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

In which Ψ = Smooth load potential; C = concentration (gram per liter); T = temperature (C)

Distilled water was used to create zero water potential (control). 50 seeds were considered in each container and some polyethylene glycol solution with the corresponding osmotic potentials of 0.00, -2, -4, -6 and -8 Bar was added to each container so that the seeds were in contact with the solution. In order to avoid the negative effects of water evaporation, the amount of evaporated water was compensated by adding distilled water and solution. The containers were transferred to an incubator at a temperature of 24±1°C (according to ISTA data, a suitable temperature for germination was obtained). The seeds were inspected daily and the number of germinated seeds (with a root length of 1 to 2 mm) was recorded. On the last day, seed germination, germination rate, rootlet length, stemlet length, rootlet wet weight, rootlet dry weight, stemlet wet weight, stemlet dry weight, plantlet wet weight, and plantlet dry weight were calculated using the following formulas:

(2)

$$GR = \sum Ni.\ \sum Div \times 100$$

In which, GR = germination rate; ∑Ni= Total Number of germinated seeds up to the desired day; ∑Div = Total days elapsed from the start of the experiment

(3)

$$R = Ni.\ N \times 100$$

And in the Eq. (3) we have: R = germination percentage; Ni = the number of seeds sprouted up to the desired day; N = Total number of seeds

Meanwhile, proline levels were measured separately. To measure proline, the foliage of samples treated with polyethylene glycol with different negative and control loads were used. The separated foliage was placed in foil and transferred to the laboratory in a nitrogen tank. Samples were stored in the laboratory at -80°C until use. Proline was extracted from a sample of 0.5 g fresh foliage samples in 3% (w.v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of (Bates *et al.*, 1973). The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as µ mol proline g^{-1} FW:

(4)

$$\mu moles \frac{\text{proline}}{\text{g}} \text{of fresh weight material} = \frac{\left[\frac{\mu g \frac{proline}{ml} \times ml\ Toluene}{115.5\mu \frac{g}{\mu mole}}\right]}{\left(\text{g}\frac{sample}{5}\right)}$$

The fluorescence parameters were measured in a field utilizing a portable plant stress meter (OS5-FL modulated chlorophyll fluorometer, ADC Bio Scientific Ltd. Hoddesdon, Hert, EN11 0DB England). This way, foliage were dark-adapted for 30 minutes prior to the measurement of the minimal fluorescence (*F0*), maximum fluorescence (Fm), variable fluorescence (*Fv*) and maximum quantum efficiency of photosystem II (F_v/F_m). *Fv.Fm* was calculated through (F_m-F_0).F_m (Maxwell and Johnson, 2000).

Enzyme activity was determined as follows; First, 0.34 g KH_2PO_4, 0.7 g PVPP, and 0.3 g Na-EDTA were dissolved in 30 ml distilled water, and then the volume was dissolved in 50 ml (phosphate buffer pH = 6.8). Then, 0.44 grams of K_2HPO_4, 0.7 g of PVPP and Na-EDTA 0.3 in 30 mL distilled water were dissolved and the volume was diluted to 50 mL (pH = 7.2 with phosphorus). Finally, 39% of the first buffer was combined with 61% of the second buffer (phosphate buffer pH = 7). To prepare an oxidizing water buffer (H_2O_2) of 225 mM, 450 µl of H_2O_2 was mixed with 20 ml phosphate buffer. Furthermore, a 45 µM guaiacol buffer was provided with 112 µl guaiacol with 20 ml phosphate buffer. The extract extraction step was performed using Sun *et al.* (2015) method. These extracts were used to measure the activity of enzymes: Peroxidase activity was measured by method of Plewa *et al*. (1991). The assay mixture was consisted of 1.99 ml 50 mM sodium phosphate buffer (pH 7.0) supplemented with 0.1 μ M EDTA, 10 mM guaiacol and 15 mM H_2O_2 and 100 μ l of the enzyme extract in a total volume of 2 ml. Guaiacol oxidation and production of tetraguaiacol was monitored by increasing of absorbance of 470 nm. The activity of POD was calculated by extinction coefficient of tetraguaiacol (26.6 $mM^{-1}cm^{-1}$) and expressed as μ mol tetraguaiacol $min^{-1}mg^{-1}$protein. Activity of APX was determined by recording the decrease in absorbance of ascorbate at 290 nm as described by Nakano and Asada (1981). The reaction mixture (1.0 ml) contained 0.95 ml of 50 mM sodium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.2 mM EDTA, 0.2 mM H_2O_2 and 50 μ l of enzyme extract. APX activity was calculated by using the extinction coefficient 2.8 mM^{-1} cm^{-1} and expressed as μ mol decomposition of ascorbate min^{-1} mg^{-1} protein. SOD activity was measured by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) as described by Beauchamp and Fridovich (1971). The reaction mixture, consisted of 50 mM Na-phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine (Sigma-Aldrich Corporation), 75 μ M NBT (Sigma-Aldrich Corporation), 2 μ M riboflavin (Sigma-Aldrich Corporation), and 50 μ l of enzyme extract in a test tubes, were incubated for 15 min under fluorescent. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photochemical.

The data were analyzed by factorial design in completely randomized with three replications using SAS software (version 9.1 2002–2003, SAS Institute, Cary, NC, USA); the description was performed for traits with double interactions that were significant. The comparison of the meanings was done by the least significant difference (LSD) test ($P < 0.05$) with MSTAT-C software and the graphs were drawn also with exile software (version: 2019).

Table 1. Variation analysis results of the effect of polyethylene glycol (PEG) and cultivar on different morphological traits of chickpeas

Sources of variations	Degrees of freedom	MS									
		Seed germination	Germination rate	Rootlet length	Stemlet length	Plantlet wet weight	Rootlet wet weight	Stemlet wet weight	Plantlet dry weight	Rootlet dry weight	Stemlet dry weight
PEG	4	**	**	**	**	**	**	**	**	**	**
Cultivar	3	**	**	*	**	**	**	**	**	**	*
PEG×Cultivar	12	**	*	**	**	**	**	**	**	**	**
Error	38	2.2130	2.3016	1.0010	0.1680	1.0310	0.4037	1.0180	0.9213	5.1160	0.9875
Total	59										

****: Significant at 1% probability level; *: Significant at 5% probability level**

Results and discussion

The results of the analysis of variance of the data collected from seed germination, germination rate, rootlet length, stemlet length, rootlet wet weight, rootlet dry weight, stemlet wet weight, stemlet dry weight, plantlet wet weight, plantlet dry weight in Table 1 showed that the traits have significant differences.

Also, the results of comparing the average of the studied traits are presented below:

Germination percentage. The results of comparing the means (Table 2-1) show that the highest percentage of germination is related to -2 bar treatment with 98.54%, while the lowest percentage of germination is related to -6 bar with zero percent, if we compare these parameters with the control (with 99.13% germination) and it is clear that the ratio was higher than all treatments. Also, considering Hashem, it had a higher germination percentage (5.47%) and the lowest germination percentage was observed in the Azad genotype (0.00%) at -6 bar. Regarding the decrease in germination percentage with increasing load, according to the studies of De and Kar (1994), increasing the load, of polyethylene glycol reduces the germination percentage and survival of plants (Muscolo *et al.*, 2014).

Germination rate: The results of analysis of variance in this study not only showed a significant difference at the level of 1% on the rate of germination between different treatments, but also different chickpea genotypes had a significant difference in terms of germination rate at the level of 1% (Table 1). On the other hand, according to the comparison of averages (Table 2-2), the highest germination rate was related to the treatment of -2 bar (the number of germinated seeds compared to the days passed) in Hashem (17.50 day), while the lowest germination rate is related to Azadi in -4 bar (7 87 day), if we compare these parameters with control, it is clear that the ratio is higher than all of treatments. One of the important indicators in evaluating tolerance to drought stress of cultivars is their germination rate, so that cultivars with high germination rate in drought stress conditions have the possibility of faster germination than other cultivars. The higher the germination rate in some genotypes, the higher the rate of water uptake and seed germination (Levitt, 1980). Also, The RGR and WC of shoots and roots all decreased with increasing PEG concentration, with the greatest reductions occurring under the highest water stresses (Partheeban *et al*., 2017).

Rootlet length: The results of the study showed that the maximum root length was observed in both treatment (control) and treatment -2 bar, 28.99 and 18.660 mm respectively, while no root length was observed in -6 and -8 bar. Hashem genotype had a higher root length (18.67 mm) than Arman genotype (14.67 mm) and the lowest root length was observed in Binalood genotype (4.98 mm) (Table 2-3). Reducing the length of the root, based on the results of Asghari's research (1992), to reduce the absorption of water by seeds, which reduces the secretion of hormones and enzyme activity, and thus disrupts root growth and its length.

Stemlet length: The results of the experiment showed differences between different treatments. So that the maximum length of the stem was related to the treatment of -2 bar (2.01 mm), while no the stemlet was detected in -4, -6- and -8-bar. Also, there were the significant differences between chickpea genotypes in terms of stemlet length (Table 2-4). The higher stemlet length was observed in Hashem genotype that higher than other genotypes. In a report of research by Robin *et al*. (2021), PEG-induced osmotic stress alters root morphology and root hair traits in wheat genotypes. It cited the causes of reduced stemlet length under stress, reduced or no transfer of nutrients from the cotyledons to the stemlet.

Root wet weight: Different chickpea cultivars have a significant differences in terms of root wet weight, so that the highest wet weight of the root is related to the treatment of -2 bars with 15.98 mg, while wet weight of the root is corresponding to the control with 24.56 mg (Table 2-5). Munir *et al.* (2004) in their study of the effect of osmotic stress on a number of lentil cultivars stated that the root and stem weight of the lentil cultivars studied ramped down with decreasing osmotic potential.

Root dry weight: In this study, different chickpea

Table 2-1. Effect of drought-induced stress by PEG6000 on seed germination of chickpeas

PEG (Bar)	Seed germination (%)			
	Binalood	Hashem	Azad	Arman
-2	97.45[a]	98.54[a]	27.56[b]	84.00[b]
-4	39.67[b]	97.34[a]	24.98[b]	67.00[c]
-6	0 00[d]	5.47[b]	0.00[c]	2.00[d]
-8	0 00[d]	0.34[bc]	0.00[c]	0.00[d]
0	99.03[a]	99.13[a]	98.08[a]	98.45[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table 2-2. Effect of drought-induced stress by PEG6000 on seed germination rate **of chickpeas**

PEG (Bar)	Germination rate (day)			
	Binalood	Hashem	Azad	Arman
-2	14.89[b]	15.50[b]	7 87[b]	17.58[a]
-4	9 67[c]	15 78[b]	3.58[c]	9 56[b]
-6	0 00[d]	0 00[c]	0 00[d]	0 00[c]
-8	0 00[d]	0 00[c]	0 00[d]	0 00[c]
0	18 67[a]	18 31[a]	17 98[a]	17 95[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table 2-3. Effect of drought-induced stress by PEG6000 on rootlet length of chickpeas

PEG (Bar)	Rootlet length (mm)			
	Binalood	Hashem	Azad	Arman
-2	9.34[b]	18.67[b]	4.98[b]	14.67[b]
-4	4.45[c]	14.78[c]	2.89[c]	4.98[c]
-6	0 00[d]	0 00[d]	0 00[c]	0 00[d]
-8	0 00[d]	0 00[d]	0 00[c]	0 00[d]
0	31.56[a]	34.99[a]	21.69[a]	24.87[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table 2-4. Effect of drought-induced stress by PEG6000 on stemlet length of chickpeas

PEG (Bar)	Stemlet length (mm)			
	Binalood	Hashem	Azad	Arman
-2	1.10[b]	2.01[b]	0.99[b]	1.11[b]
-4	0 00[c]	0 00[c]	0 00[c]	0 00[c]
-6	0 00[c]	0 00[c]	0 00[c]	0 00[c]
-8	0 00[c]	0 00[c]	0 00[c]	0 00[c]
0	1.82[a]	2.12[a]	1.62	1.92[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table2-5. Effect of drought-induced stress by PEG6000 on rootlet wet weight of chickpeas

PEG (Bar)	Rootlet wet weight (mg)			
	Binalood	Hashem	Azad	Arman
-2	12.43[b]	15.98[b]	10.67[b]	12.67[b]
-4	5.99[c]	6.34[c]	4.04[c]	5.98[c]
-6	0 00[d]	0 00[d]	0 00[d]	0 00[d]
-8	0 00[d]	0 00[d]	0 00[d]	0 00[d]
0	23.31[a]	24.56[a]	14.78[a]	18.67[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

genotypes were different in terms of dry root weight (Table 2-6). So that the highest dry weight of the roots is related to the treatment of -2 bar with 0.38 mg, while in the control the highest dry root weight was observed in comparison with all treatments. (Goicoechea *et al.*, 1997) stated that the difference in root dry weight can be due to differences in the content of nutrients in the seeds, so that larger seeds provide more nutrients to the roots.

Stemlet wet weight: Comparison of means (Table 2-7) shows that the highest wet weight of the Stemlet related to the treatment of -2 bar with 3.34 mg, while if these parameters are associated with control such as Hashem genotype (with a higher stem wet weight of 14.40 mg) Our comparison shows that the ratio is higher than all of treatments. Munir *et al.* (2004) stated in their studies on the effect of osmotic stress on a number of lentil cultivars that, the root weight and stem of the lentil cultivars studied ramped down with decreasing osmotic potential.

Stemlet dry weight: The results of the study of dry weight of the stem or between different treatments showed that the highest dry weight of the stem is related to the treatment of 2 times (0.13 mg), while in the treatment control, Binalood genotype has dry stem weight. What was higher (0.34 mg) than free genotypes

Table 2-6. Effect of drought-induced stress by PEG6000 on rootlet dry weight of chickpeas

PEG (Bar)	Rootlet dry weight (mg)			
	Binalood	Hashem	Azad	Arman
-2	0.20[b]	0.38[b]	0.10[b]	0.29[b]
-4	0.09[c]	0.24[c]	0.04[c]	0.15[c]
-6	0 00[d]	0 00[d]	0 00[d]	0 00[d]
-8	0 00[d]	0 00[d]	0 00[d]	0 00[d]
0	0.31[a]	0.50[a]	0.21[a]	0.47[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table 2-7. Effect of drought-induced stress by PEG6000 on stemlet wet weight of chickpeas

PEG (Bar)	Stemlet wet weight (mg)			
	Binalood	Hashem	Azad	Arman
-2	3[b]	2[b]	2[b]	3[b]
-4	0[c]	0[c]	0[c]	0[c]
-6	0[c]	0[c]	0[c]	0[c]
-8	0[c]	0[c]	0[c]	0[c]
0	13[a]	14[a]	0	9[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table 2-8. Effect of drought-induced stress by PEG6000 on stemlet dry weight of chickpeas

PEG (Bar)	Stemlet dry weight (mg)			
	Binalood	Hashem	Azad	Arman
-2	0.11[b]	0.10[b]	0.13[b]	0.04[b]
-4	0[c]	0[c]	0[c]	0.04[b]
-6	0[c]	0[c]	0[c]	0[bc]
-8	0[c]	0[c]	0[c]	0[bc]
0	0.33[a]	0.18[a]	0.30[a]	0.12[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

and Hashem (0.32 and 0.17 mg) and the lowest dry stem weight was observed in Arman genotype (0.10 mg) (Table 2-8). It seems that one of the reasons for the reduced dry weight of the stem, whether in low water potentials, is the low motility of nutrients and their lower transfer from the cotyledons to the embryonic axis. It is noteworthy that factors that affect the growth rate of the embryonic axis can affect the motility of food and their transfer from the cotyledons to the embryonic axis. This has been confirmed by Daffalla *et al*. (2011).

Plantlet wet weight: The highest weight of seedlings was related to -2 bar treatment (87.00 mg) among of polyethylene glycol treatments, while the Binalood genotype had a higher seedling weight (116.54 mg) than others in normal (control) condition (Table 2-9). The results of different research on plants seeds in drought stress conditions showed that the reduction of water uptake by seeds reduces the secretion of hormones and enzyme activity and thus disrupts plant growth and weight (Yang *et al*., 2021).

Plantlet dry weight: The dry weight of seedlings was different between different treatments, so that the highest dry weight of seedlings was related to the treatment of -2 bar (0.38 mg) related to Binalood cultivar, while the same genotype had a dry seedling weight of 0.69 in normal conditions (Table 2-10). The results of this experiment are similar to the results of Abonus (2001) that stated as the osmotic pressure increases due to drought stress, the growth of the plant decreases. In this regard, the reduction of dry weight of Plantlet due to reduced transfer of metabolic products due to enzymatic hydrolysis of seed storage materials.

Proline content: The results of analysis of variance in Table 3 showed a significant difference at the level of 1% on the amount of proline between different treatments. So that the highest amount of proline is related to the treatment of -2 bar (0.41 µmol.g) in Hashem cultivar, while this amount of proline seen at same cultivar (0.14 µ mol.g) under control condition. Chickpea genotypes also had a significant difference in terms of proline level of 1% (Table 3). Hashem genotype had a higher proline level (0.41µ mol.g) than Arman, Binalood and Azadi genotypes (0.35, 0.29 and 0.19 respectively) (Fig. 1). It has been shown that with increasing drought stress, proline content in the plant ramped up and the amount of protein decreased due to their higher fracture to produce proline so that the plant could better withstand stress (Chiang and Dandekar, 1995). Many researchers have investigated the role of proline as osmolite in plant response to water stress. Their reports have indicated that increased proline levels protect the plant from stress (Hayat *et al.*, 2012;

Table 2-9. Effect of drought-induced stress by PEG6000 on plantlet wet weight of chickpeas

PEG (Bar)	Plantlet wet weight (mg)			
	Binalood	Hashem	Azad	Arman
-2	78[b]	77[b]	78[b]	68[b]
-4	78[b]	69[b]	85[b]	71[b]
-6	0[c]	0[c]	0[c]	0[c]
-8	0[c]	0[c]	0[c]	0[c]
0	116[a]	98[a]	101[a]	84[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table 2-10. Effect of drought-induced stress by PEG6000 on plantlet dry weight of chickpeas

PEG (Bar)	Plantlet dry weight (mg)			
	Binalood	Hashem	Azad	Arman
-2	0.28[b]	0.19[b]	0.28[b]	0.18[b]
-4	0[d]	0[d]	0.16[c]	0.17[b]
-6	0[d]	0[d]	0[d]	0[d]
-8	0[d]	0[d]	0[d]	0[d]
0	0.69[a]	0.30[a]	0.56[a]	0.27[a]

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test

Table 3. Results of analysis of variance of the effect of polyethylene glycol and cultivar on chickpea proline and F_v/F_m in laboratory conditions

Sources of variations	Degrees of freedom	MS					
		Proline (µ mol.g)	F_v/F_m	APX	CAT	POD	SOD
				(µmol/gfw min^{-1})			
PEG	1	*	**	**	**	**	**
Cultivar	3	**	**	*	**	**	*
Cultivar ×PEG	3	**	**	**	*	**	**
Error	13	0.000161	0.000970	0.00012	0.00002	0.000019	0.00001
Total	22						

****: Significant at 1% probability level; *: Significant at 5% probability level**

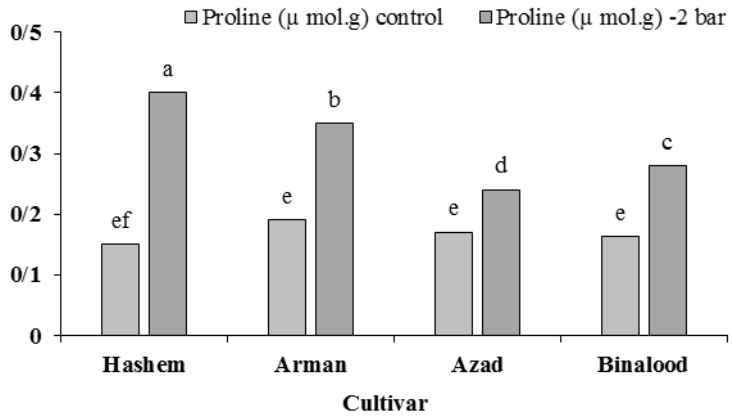

Fig. 1. Chickpea proline content in different treatments

Arteaga *et al*., 2020). A stressful condition has been reported to lead to overproduction of proline in plants, which in turn creates stress tolerance by maintaining cellular turgor or osmotic balance. Stabilization of the membranes thus prevents electrolyte leakage. Putting the concentration of reactive oxygen species (ROS) in the normal range, thus protecting plants from oxidative explosion (Hayat *et al*., 2012; Desoky *et al*., 2021). Interestingly, when the drought condition was extended to 10 days, the free proline contents in Zarin and Sardari cultivars of wheat, respectively, increased to 5 and 4 times that of the control (Jahanbakhsh *et al*., 2017). Also, previous reports that has been showed water stress induces higher proline contents in drought condition and

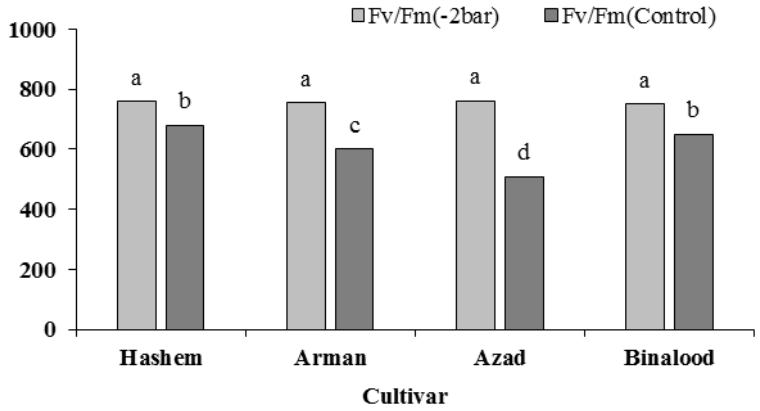

Fig. 2. Chickpea *Fv/Fm* in different treatments

the greatest amounts of free proline accumulate in the leaf tissues of crops under stress, (Vendruscolo *et al.*, 2007; Shamsi, 2010; Hussain *et al.,* 2011).

Quantum efficiency of the photosystem II (*Fv.Fm*). The results related to quantum efficiency of the photosystem II (Table 2) showed that individual effects of cultivar and drought stress, as well as dual interactions of cultivar × drought stress were significant ($P < 0.01$). In this line of inquiry, the results of interactions of cultivar × drought stress (Table 3), indicated that drought-induced stress by PEG6000 leads to a reduction in the *Fv/Fm*. The maximum amount of quantum efficiency of photosystem II (*Fv/Fm*) was found in the Hashem cultivar (0.669) under -2 bar condition, and the lowest amount in Azad cultivar 0.500 (Fig. 2). The results related to quantum efficiency of the photosystem II in all cultivars were different. This difference might be due to reducing the consumption of electron transport chain products (NADPH and ATP), which leads to both a reduction in ferredoxin and, consequently, results in thylakoid membrane proteins destruction. Furthermore, the above mentioned changes, first, disturb the electron transferring process from the receptive site of photosystem II and the maximum productivity of this photosystem II. Then, they cause an increase in chlorophyll fluorescence as well (Piper *et al.*, 2007).

As a matter of fact, under drought stress condition, the amount of received solar energy by the leaf, which is generally consumed for electron transferring and phosphorylation in normal cases, is mostly transformed into heat. Moreover, the produced heat is recognized by chlorophyll fluorescence. Additionally, in tolerant cultivars exposed to drought stress, less amount of received solar energy was wasted. In other words, the highest photosystem II quantum efficiency (Fv/Fm) occured in the tolerant variety.

The activity of antioxidant enzymes: In order to evaluate the response of antioxidant system of four chickpea cultivars to drought stress induced by PEG6000, enzyme activity APX, CAT, POD and SOD were examined in the foliage (Table 4). Both cultivars and drought stress factors had a significant effect on POD, CAT, APX and SOD enzymes activity in chickpea foliages. chickpea cultivars were significantly different in this regard. The highest activity of these enzymes (POD, CAT, APX and SOD) were observed in Hashem (0.442 µmol/GFW min^{-1}, 1.536 µmol/GFW min^{-1}, 1.792 and 2.303 µmol/GFW min^{-1}) and (0.183 µmol/GFW min^{-1}) and the lowest level of activity in cultivar of Azad (0.183 µmol/GFW min^{-1}, 1.025 µmol/GFW min^{-1}, 1.411 and 1.743 µmol/GFW min^{-1}) under stress conditions respectively. Comparison of stress with the control showed that drought stress increased enzymes activity compared to the control (Table 4).

In the present study, chickpea cultivar and drought stress had a significant effect on the activity of leaf antioxidant enzymes including SOD, POD, CAT and APX. In all of the cultivars, the activity of these enzymes increased under the influence of drought stress treatment. Differences between control and treated plants in terms of SOD activity in all chickpea cultivar and drought stress were significant. According to reports, when plants are exposed to stress, including drought stress, the activity of antioxidant enzymes in the photosynthetic apparatus protects the plant against oxidative damage (Nasr Esfahani, 2013). Superoxide dismutase (SOD) removes the superoxide anion (O^{2-}) produced by the electron transfer chain in chloroplasts and mitochondria, and then, the H_2O_2 produced by SOD activity is eliminated by APX and POD in different parts of the cell. In addition, CAT also eliminates the H_2O_2 produced in the light-breathing pathway inside the peroxisomes (Foyer and Noctor, 2003). The sweeping capacity of reactive oxygen species (ROS) and the reduction of their harmful effects may be associated with the drought tolerance of plants (Mafakheri *et al.*, 2011).

In scientific reports, the close relationship between the high activity of antioxidant enzymes and increased resistance to drought stress has been repeatedly pointed out and emphasized. For example, the activity of SOD, CAT, and APX increased significantly compared to a drought-sensitive plants, leading to lower lipid peroxidation levels and electrolyte leakage (Alscher *et al.*, 2002; Turkan *et al*., 2005). Meanwhile, a drought-

Table 4. Comparison of mean the activity of leaf antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) of selected Chickpea cultivars in response to drought stress

Genotype	POD	POD	CAT	CAT	APX	APX	SOD	SOD
	(μmol/gfw min^{-1})							
	stress(-2 bar)	Control	stress(-2 bar)	Control	stress(-2 bar)	Control	stress(-2 bar)	Control
Arman	0.314^{b}	0.173^{a}	1.246^{b}	0.556 ^{a}	1.731^{b}	0.541^{a}	2.183^{bc}	1.3140^{a}
Azad	0.183^{d}	0.169^{a}	1.025^{c}	0.544^{b}	1.411^{d}	0.502^{ab}	1.743^{d}	1.097^{ab}
Hashem	0.442^{a}	0.179^{a}	1.536^{a}	0.567^{a}	1.792^{a}	0.601^{a}	2.303^{b}	1.401^{a}
Binalood	0.376^{c}	0.178^{a}	1.087^{c}	0.541 ^{a}	1.554^{c}	0.534 ^{a}	2.061^{d}	1.163^{ab}

Means in each column with the same letters are not s significantly different at the 5% level

resistant bean species showed higher SOD, CAT, POD, and APX activity, as well as lower levels of lipid peroxidation, compared to its drought-sensitive species (Foyer and Noctor, 2003; Turkan *et al*., 2005).

Conclusion

In the present study, all conditions were the same for all chickpea genotypes (Arman, Azad, Binalood and Hashem) and the genotypes were evaluated based on different drought levels. Genotypes in all traits were significantly different from the treatment at the level of 1%, which to evaluate the tolerance of chickpea genotypes to drought stress in the germination stage, the best trait percentage of germination. The Azad cultivar had the lowest level of drought tolerance of -2 bar. Also, studies on the measurement of *Fv.Fm* and proline at two levels of control and glycol polyethylene solution at the level of 2-bar showed that there was a significant difference between the treatments at the level of 1%. The results related to *Fv/Fm* in all cultivars were different. The maximum amount of F_v/F_m was found in the Hashem cultivar (0.669) under -2 bar condition, and the lowest amount in Azad cultivar 0.500. Also, examination of chickpea cultivars revealed that there was a difference between them in terms of proline content under normal conditions and stress. So that the hashem cultivar showed the highest amount of proline in the polyethylene glycol solution -2 bar. The best level of drought stress for germination and plant establishment was -2 bar, which decreased with decreasing water potential.

The results of activity of antioxidant enzymes in this study showed that drought tolerate cultivars were tolerant to drought stress by increasing the capacity of the antioxidant system to remove reactive oxygen species and prevent membrane peroxidation. Therefore, the reasons of the observed differences can be attributed to genetics, so that in this study, Hashem, Arman, Binalood and Azad genotypes were ranked in the next ranks in terms of tolerance of drought stress caused by polyethylene glycol, respectively. Overall, the commercial cultivar Hashem showed drought tolerance at high stress levels, indicating their potential for production applications.

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