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Full Length Research Paper

The studying effect of drought stress on germination, proline, sugar, lipid, protein and chlorophyll content in purslane (*Portulaca oleracea* L.) leaves

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Purslane (*Portulaca oleracea* L.) is one of the valuable medical plants. Drought also is one of the most important environment stresses that influences metabolism and growth of plant. The studying of physiological plant response rather than water stress can be influential equipment for understanding drought resistance mechanisms. In this study, the drought stress effects on germination and physiological characteristic of the medical plant (purslane) were studied. Drought imposed on polyethylene glycol (PEG) seedlings and experiments were done in completely random model framework, with 3 replications. Polyethylene glycol stress was in seven levels involving blank (distilled water), -0.1, -0.2, -0.4, -0.6, -0.8 and -1 MPa. In relation to germination, meaningful difference was not observed between blank and the different levels (P<0.01); and the least rate of germination in stress level was -0.4 MPa) and the highest rate of germination in stress level was -0.2 MPa). Meaningful increase of proline and sugar synchronized with PEG concentration was observed in statistical level (P<0.01) with meaningful difference. In this, blank had the least rates and stress level of -1 MPa and the highest proline and sugar rates. Lipid content in purslane leaves with increasing stress level was decreased. In the highest lipid rate in stress level (-0.2 MPa) and the least concentration in stress level (-1 MPa), it was observed that this difference in P<0.01 was meaningful. The concentration of leave proteins with meaningful decrease (P<0.01) had the highest protein concentrations in blank and the least concentration in stress level was -1 MPa. In stress effect on leaf chlorophyll content, meaningful increase of chlorophyll a and b synchronized imposed increase of stress level. It was observed that with chlorophyll a the highest concentration in stress level was -0.6 MPa and the least concentration exists in blank; and also with chlorophyll b the stress level, -1 MPa had the highest and blank had the least concentration (P<0.01).

Key words: Purslane (*Portulaca oleracea* L.), drought, polyethylene glycol, proline, sugar, lipid, protein, chlorophyll, germination.

INTRODUCTION

Plants resist drought stress by their morphological, physiological and metabolic changes reflected in all their organs (Cellier, 1998). Annual purslane resists drought and it is from the Portulacaceae family, its current name is purslane which has the highest rates of Omega-3 fatty acids and anti- oxidant vitamins. Hence, it is one of the valuable medical plants. Purslane, as a weed, has global distribution and taxonomy by 8 current plants groups in the world (Liu et al., 2000). The life cycle of this plant completes in 2 to 4 months (Chauhan and Johnson, 2007). Purslane can grow in salty and dry soil easily. As a result in Haloph data, it is known as Halophyte or salty plant (Aronson, 1989). This plant, being antiseptic, antispasmodic, diuretic, antipyretic; used for muscle loosening, anti-oxidant, reinforcing of safety system, blood filtering and removing of thirsty, has medical

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Abbreviations: PEG, Polyethylene glycol of molecular weight 6000; **MPa,** megapascal; **DW,** dry weight; **FW,** fresh weight.

Table 1. Contents of nutrient solution used for irrigation of Purslane seedlings.

g/lit (mother solution)	Source	Element name
59.025	$ca(no_3)_2.4H_2o$	Calcium nitrate
25.275	KNo_3	Potassium nitrate
61.6	$MgSo_4.7H_2o$	Magnesium sulfate
34.025	KH_2Po_4	Potassium de- hydrogen phosphate
0.715	Hbo_3	Boric acid
0.4525	$Mncl_2.4H_2o$	Manganese choloride
0.055	$Znso_4.5H_2o$	Zinc sulfate
0.02	$Cuso_4.5H_2O$	Copper sulfate
0.0225	$H_2M_0o_4.H_2O$	Molibedic acid

application (Miladi et al., 2007). Drought accounts for decrease in cultivated crops. With respect to uneasy magnitude of arid and semi-arid areas (90% of all country area), it is one the main problems in cultivating part in Iran (Rahimi and Kafi, 2008). Drought is relative term that occurs in mild regions in 15 days without any rain. In semi-arid region, there are two sessions: dry and damp. Dryness is a state where no remarkable rain occurs (Turner, 1979). Kramer (1983) defines drought stress as shortage of rain in plant environment, whose effect causes damage to plants. And the rate of this damage depends on the type of plant, maintaining of water capacity and atmosphere conditions in evaporation and condensation. In comparing the highest cultivated plants whose growth was decreasing and sensitive to drought, purslane has natural distribution in drought environments and is compatible with desert low- rain regions. It seems that photosynthesis mechanism of this plant is from c_4 mechanism and it can convert to CAM photosynthesis mechanism. The resistant reason and compatibility of this plant to stress environment is drought (Koch and Kennedy, 1981). With respect to Purslane as a medical and valuable plant and because of low information regarding its physiological reactions under stress environment condition, doing this project is necessary.

MATERIALS AND METHODS

For studying the drought stress effect on germination and physiological factors of Purslane, seeds were prepared in Iran Jungle and Pasture Research Institute. The study was done under controlled condition and in Botany Laboratory located in Islamic Azad University of Tonekabon Branch since 2010. For antiseptic of seeds, 5% sodium hypochlorite was put them in this solution for 1 min and washed in distilled water (Falahati, 2006). After cultivating seeds in Petri dish and leaving them to grow to 3 leaves stage, seedlings were transported to washed sand pot. After transporting seedlings, for controlling of their growth, Hogland solution was added to the sand environment. This was performed every one week (Table 1). After multi-leave stage in seedlings, getting stress for 1 week was performed and after this period, the physiological factors of leaves were evaluated. In this research, drought by PEG was in seven levels, which included blank (distilled water), -0.1, -0.2, -0.4, -0.6, -0.8, -1 MPa; these resolving with 0, 8.1, 11.4, 15.8, 18.9, 21.2, 24.2 g of PEG resulted in 100 m/lit distilled water (Asadi-Kavan et al., 2009). After stress period, proline was evaluated by Bates method (1973), sugar by Nelson method (1943), protein by Lowry method (1951), chlorophyll by Lichtenthaler method (1987) and lipids by sokseleh set. Experiments in completely random framework, with 3 replications were performed. Data analysis, variance and comparison of means were done by Danken experiment and SPSS software; for drawing of diagrams, Excel software was used.

RESULTS

Results showed that drought stress effect on proline, sugar, leaf lipid, protein and chlorophyll contents was meaningful, while it had no meaningful effect on germination (P<0.01) (Table 2). In relation to germination percent synchronized with increasing stress level, its decrease was observed in statistical level 0.01, which was not meaningful. The greatest percent of germination in potential was observed in -0.2. It was more than blank; it was not expected to be so. It seems that the germination percent in potential -0.4 MPa exists when it is lower than -0.8, -1 MPa (Figure 1). The free proline rate in Purslane (*P. oleracea* L.) with increasing stress level had meaningful increase. The highest level of proline in stress was -1 MPa and the least rate of proline was in blank level (Figure 2). The concentration of leaf sugar synchronized with increasing stress level had meaningful increase; in proline, the highest sugar concentration in stress level was -1 MPa and the least concentration was in blank level (Figure 3). In leaf lipid content it was observed that with increasing drought,

Table 2. Effect of drought stress on germination, proline, carbohydrate, lipid, protein, chlorophyll a, and chlorophyll b in purslane leaves.

Treatment	Drought stress (MPa)						
	Control	-0.1	-0.2	-0.4	-0.6	-0.8	-1
Germination (percent)	11.33	10.33	11.66	9.33	10.66	10.33	9.66
Proline (mg/g FW)	0.091	0.170	0.171	0.181	0.287	0.273	0.432
Carbohydrate (mg/g DW)	0.071	0.158	0.120	0.140	0.155	0.220	0.234
Lipid (mg/g FW)	56.93	57.14	55.14	52.19	50.46	45.50	39.71
Protein (mg/g DW)	1.38	1.02	1.07	1.05	1.09	1.04	0.89
Chlorophyll a (mg/g FW)	0.399	0.511	0.506	0.549	0.558	0.521	0.451
Chlorophyll b (mg/g FW)	0.157	0.207	0.199	0.216	0.218	0.203	0.241

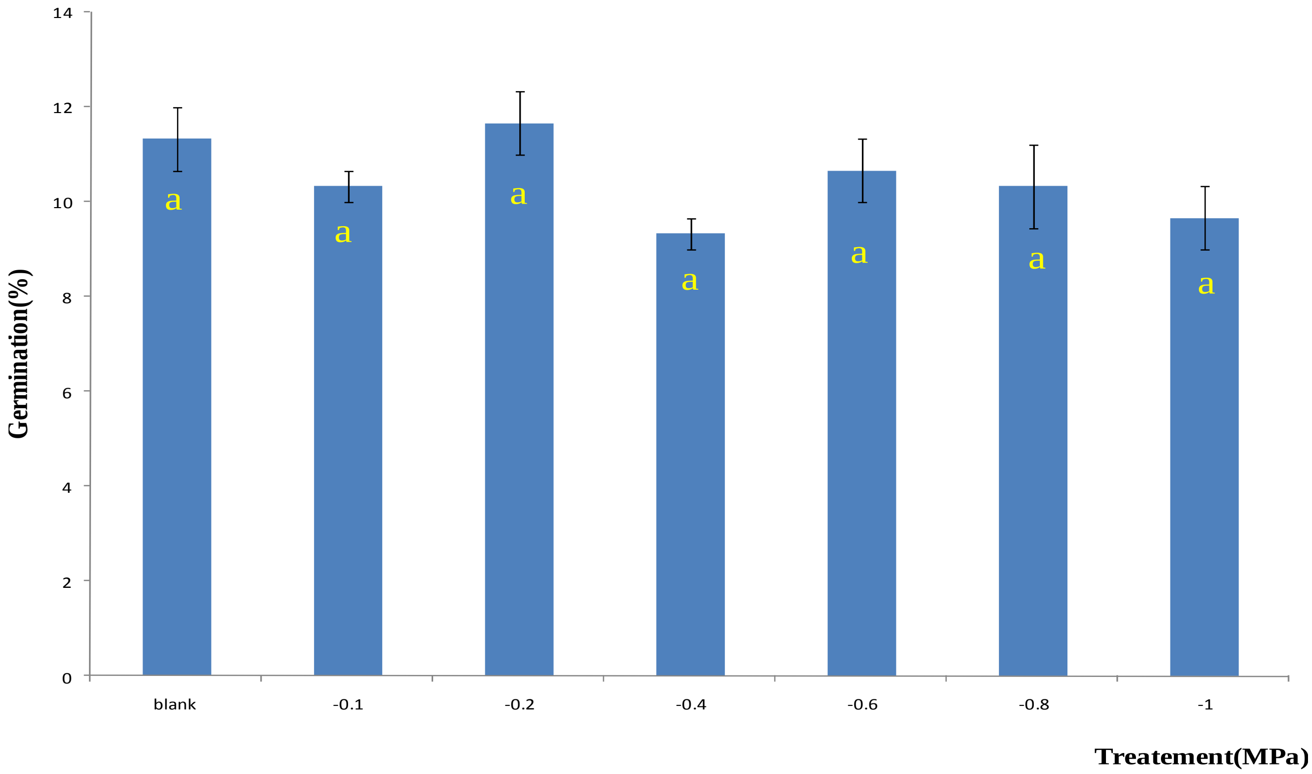

Figure 1. Effect of drought stress on seed germination.

meaningful decrease occurred in lipid concentration, in which the least rate of lipids in stress level was -1 MPa and the highest rate in stress level was -0.2 MPa were. These were more than blank (Figure 4). The decreasing of leaf protein concentrations synchronized with increasing stress level was meaningful, while the highest proteins concentrations were in blank level and the least concentrations in stress level were observed in -1 MPa (Figure 5). Drought was imposed due to increase in chlorophyll a and b content in purslane (*P. oleracea* L.) leaves and this increase in statistical level 0.01 was meaningful. In chlorophyll a content, the highest concentration in drought level was -0.6 MPa and the least concentration was in blank level (Figure 6). Also the highest concentration of chlorophyll b in drought level was -1 and the least concentration was in blank level (Figure 7).

DISCUSSION

Reduction of water entrance to seed increases drought

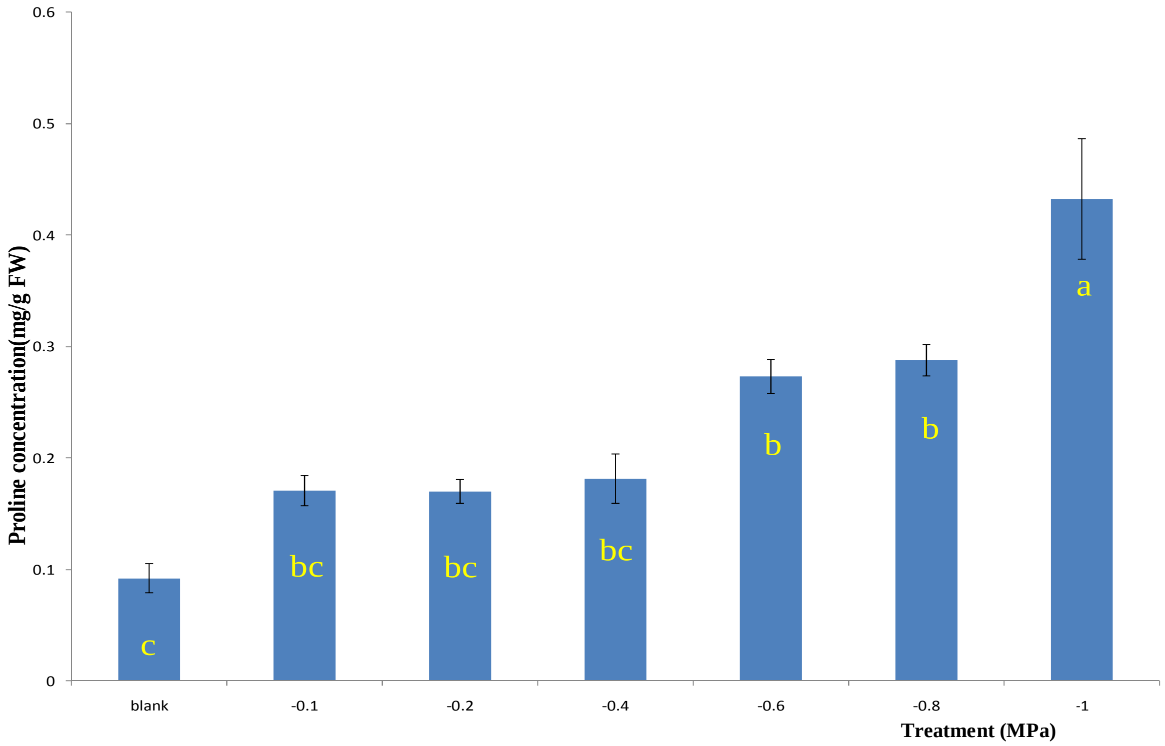

Figure 2. Effect of drought stress on proline content.

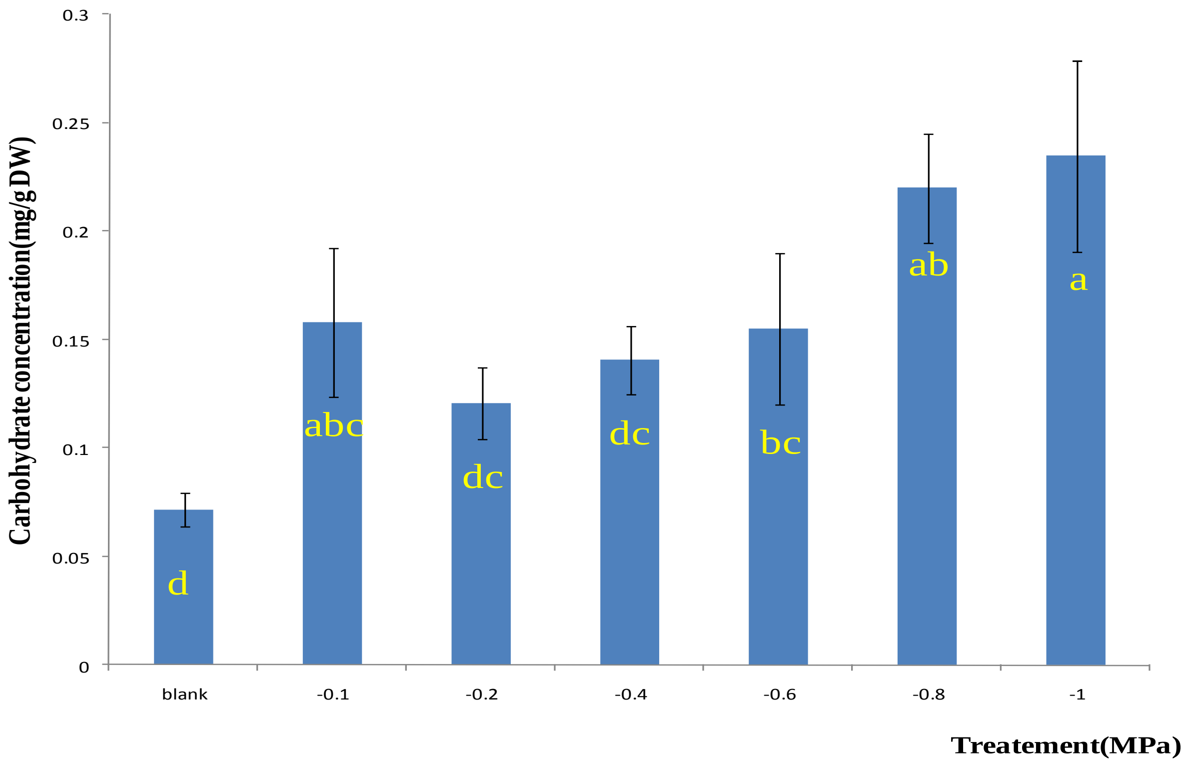

Figure 3. Effect of drought stress on carbohydrate content.

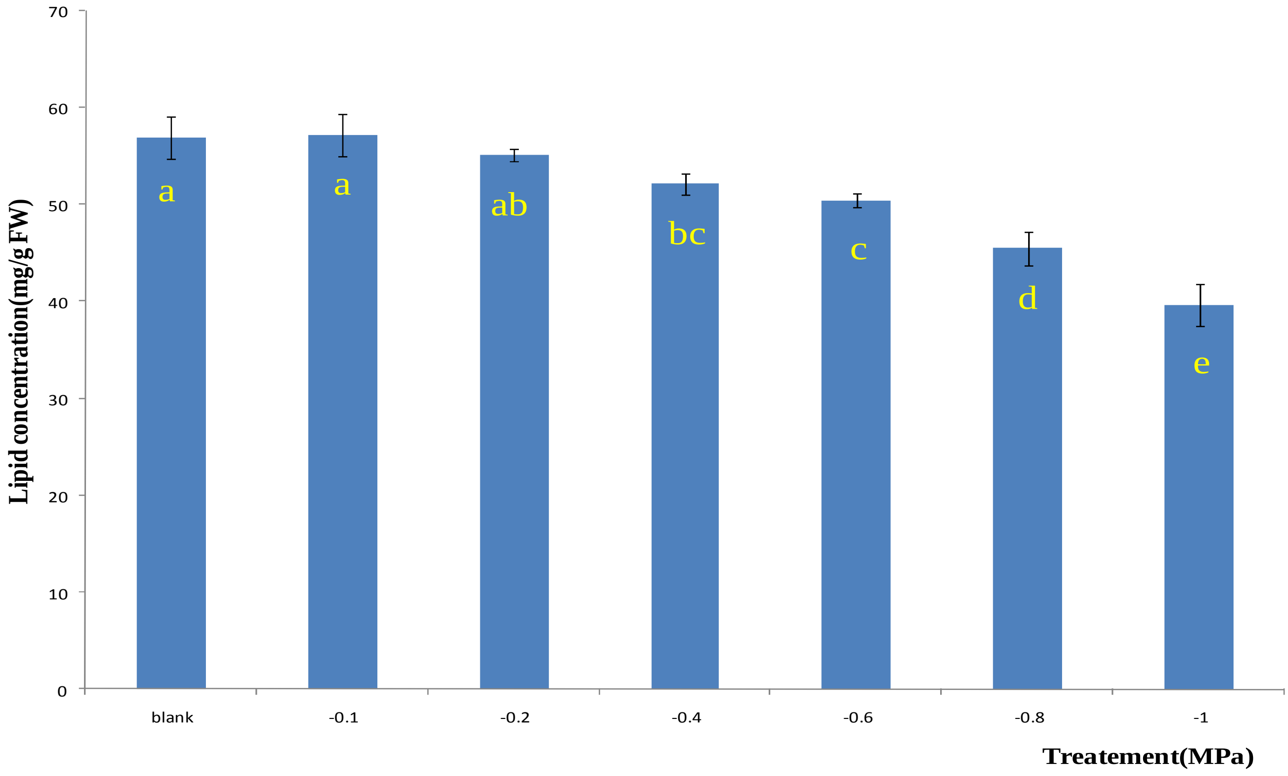

Figure 4. Effect of drought stress on lipid content.

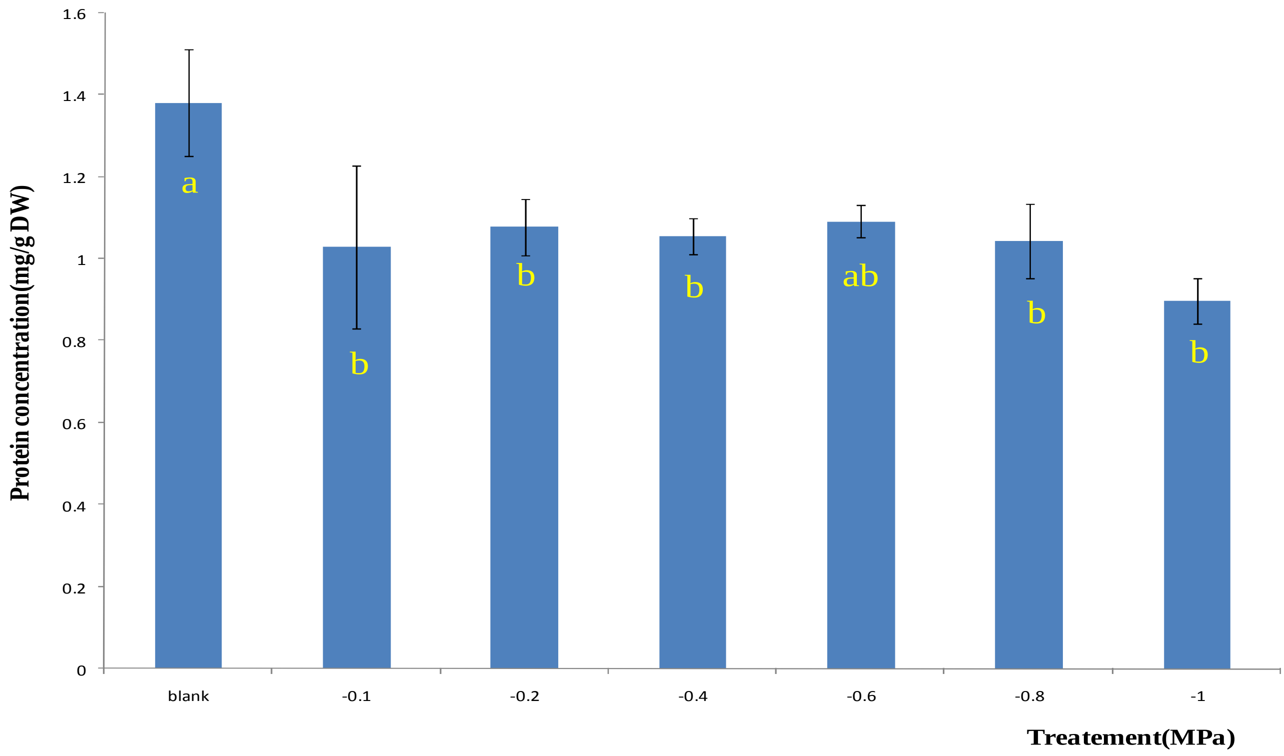

Figure 5. Effect of drought stress on protein content.

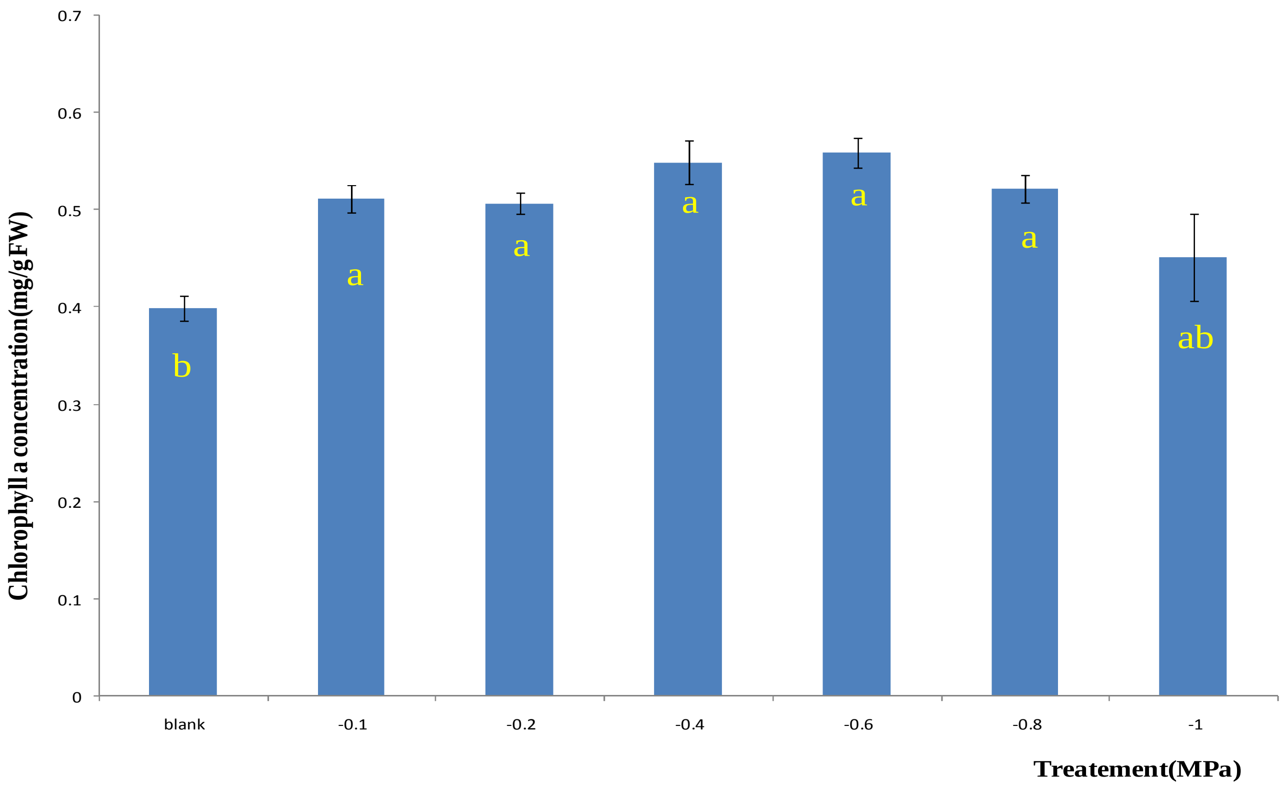

Figure 6. Effect of drought stress on chlorophyll a content.

stress due to hydraulic reduction; and as a result, physiological and metabolic germination process is being influenced and rate of their speed decreases (Masoumi et al., 2007). Rahimi and Kafi (2008) reported that meaningful reduction in germination is under the influence of imposed drought stress by poly ethylene glycol. They expressed that germination percent up to -0.75 MPa had no meaningful difference and after that it had meaningful reduction ($P<0.05$). As a result, plants in response to environmental stresses had synthesis or accumulation materials such as enzymes, proteins, mineral material and amino acid (Barsa et al., 1997).One of the physiological responses that plants use against drought is proline accumulation (Girousse et al., 1996). It is reported that one of the most important reasons why free proline level increases is because of abscises acid hormone effect on light processes during proline metabolism. Existing of energetic components resulted from photosynthesis due to proline synthesis simulation. Drought occurs from two ways due to increase of proline, which includes increasing synthesis of proline enzymes levels and decreasing of destructive proline enzymes actions (Rontein et al., 2002). Bandurska and Jozwiak, (2010), in studying the effect of imposing drought in *Lolium Perenne*, *Festuca rubral* species synchronized with decreasing relative water content, observed more accumulation from proline, while in blank, proline content in stress duration had no changes. Also, intensive accumulation of proline in phloem sap of Medicago (Farahani et al., 2009), wheat (Ahmadi and Sio-se, 2004) and Colza (Mossavi et al., 2006) is reported. Decreasing of turgor pressure is the first reason for proline accumulation under drought stress (Girousse et al., 1996). Amino acid proline resulted from proteins degradation whose response to drought stress is due to its compatibility with osmosis. Many researchers reported that its accumulation is during drought experience. Under condition of stress, plant cells have the ability to prohibit decreasing of water. Usually, plants resist these stresses by accumulation of compatible solution such as proline reaction, which can bear environmental stress. These solutions can aggregate in concentrations without damaging the metabolism. In many plant species, free proline accumulates in response to vast range of stresses such as drought and salt.

There exist a lot of relationships between proline accumulation and resistance to stress. In researching various plants, it is observed that proline has important role in osmosis balance (Fedina et al., 2002). Also, plants are faced with osmotic stress because they accumulate non-important molecules such as sugar and store many percent of them in their cytoplast. The first role of sugar

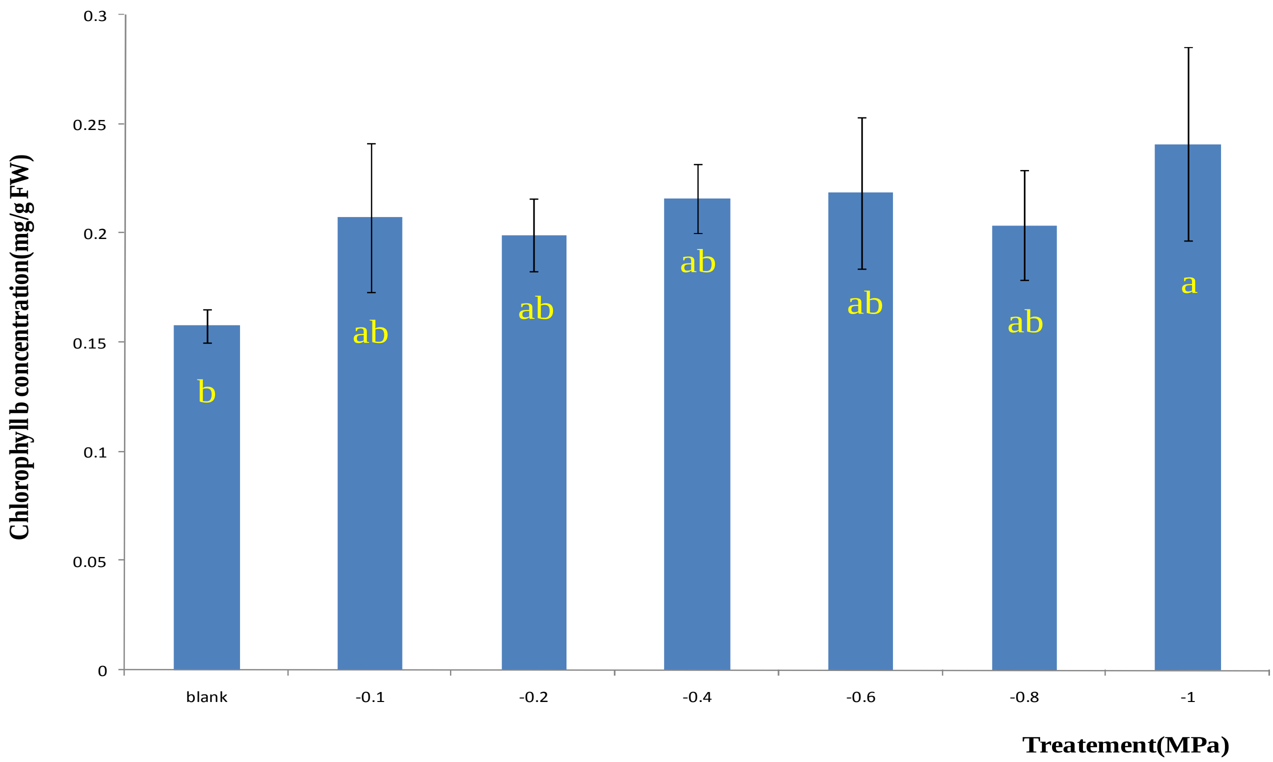

Figure 7. Effect of drought stress on chlorophyll b content.

solution is maintaining of turgor pressure and increasing of sugar solution to hydrolyze increase of starch because of hydrolytic enzymes actions during environmental stress (Bartels and Ramanjulu, 2005). Of course, other reasons for stress include sugar synthesis by non-photosynthesis pathways, non-conversion of these components to other crops and decreasing of leaf transporting and increase of sugar solution in leaf during drought stress (Premachandre et al., 1991). Khavarinejad et al. (2002) expressed that in studying *Onobrychis radiate*, *Onobrychis Viciifoli*, sugar solution concentrations in shoot organs in every 2 species of plants increased drought intensity because of prohibiting plasmolysis and osmosis adjustment .There are similar results for studying medical plants such as Rosemary by Munne and Alegre, (1999), bean by Subbaro et al. (2000), Soybean by Niakan and Ghorbanli (2006) and barely by Heidari-Rikan et al. (2006).

In relation to drought effect on leaf proteins concentration, it is expressed that drought is due to hydrolysis and destruction of leaf proteins and as a result of accumulation of free amino acids for maintaining and adjusting osmosis pressure in cells. This case is reported in soybean, corn and sunflower (Izzo et al., 1990). Of course, there are many groups of proteins that increase under condition of stress; they are called dehydrin proteins. They have similar role with proline in resisting drought and adjusting osmosis (Mohammadkhani and Heidari, 2008).It is reported that decrease of the content of protein solution in shoot organs synchronized with increasing stress level in some plants such as Legume (Ashraf and Foolad, 2005), rice (Sikuku et al., 2010) and onion (Arvin and kazemipour, 2001). It is expressed that drought is due to disorder in synthesis of proteins. Studies showed that drought can be due to decreasing of polysomic and proteins in cells such as weed plants (Creelman et al., 1990). In studying of soybean leaves, Niakan and Ghorbanali (2006) observed that with increasing drought level, the amount of protein solution in shoot organs decreased. That is this process accompanied by increase in proline concentration is due to protein destruction and its synthesis reduction is proportional.

Along with proteins, lipids are the most abundant component of membranes and they play a role in the resistance of plant cells to environmental stresses. Strong water deficit leads to a disturbance of the association between membrane lipids and proteins as well as to a decrease in the enzyme activity and transport capacity of the bilayer (Yordanov et al., 2003). Drought stress provoked considerable changes in lipid metabolism (Kesri,G et al, 2002). MGDG is major leaf glycolipid that can decrease in strong water deficit which shows the chloroplast membrane destruction (Yodanov et al., 2003). In relation to drought effect on chlorophyll a and b in leaf, we can express that drought is due to chloroplastic proteins hydrolysis, decreasing of leaf pigments and chlorophyll destruction as a primary stage in degradation of proteins (Synerri et al., 1993). As a result, chlorophyll concentration decreases under condition of stress by chlorophylase, peroxidase enzymes and phenolic components production (Abaaszadeh et al., 2007). Decreasing of chlorophyll content in plants such as *Paulownia imperialis* (Astorga,G and Melendez, 2010), bean(Beinsan et al., 2003) and also *Carthamus tinctorius* (Siddiqi et al., 2009) is reported under drought stress. But non -reduction of chlorophyll under condition of environmental stress expresses bearing of plant proportional to light damage to chloroplast, while anti-oxidant enzymes action and anti-oxidant component that conserve chlorophyll have direct relationship (Yang, 2006). In fact, the reason for increase in chlorophyll level under condition of environmental stress can be proportional to increase in leaf chloroplast in stress leaf where photosynthesis occurs. This is one of the resistant symbols in plants that are proportional to stress. As a result, the results express that purslane (*P. oleracea* L.) has high resistance to environmental stress such as drought and we can use it as a medical plant in low-raining regions.

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