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Amelioration of the effects of polyethylene glycol-induced drought stress on six cultivars of Alfalfa by paclobutrazol treatment

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Abstract:

This investigation was carried out with the aim of determining the effect of polyethylene glycol 6000 (PEG; 0, 3%, 6% and 9% w/v) treatment on six cultivars of *Medicago sativa*. PEG treatment significantly decreased the germination index in cultivars of Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago, with the highest reduction in Ghare-Medicago and Hamedani cultivars by about 50% and 44%, respectively. PEG treatment significantly decreased germination percentage and germination rate in two cultivars of Ghare-Medicago (22% and 20%, respectively) and Hamedani (28% and 27%, respectively) while increased mean germination time. The seedlings height, fresh weight and water content significantly decreased in all PEG-treated cultivars. The highest reduction in the mentioned factors was observed in Ghare-Medicago (43%, 24% and 55%, respectively) and Hamedani (57%, 23% and 53%, respectively), while the lowest reduction was observed in Yazdi, by about 28%, 9% and 17%, respectively. Data showed that PEG treatment reduced seedling dry mass only in Ghare-Medicago and Hamedani cultivars by about 16% in both cultivars. PEG treatment significantly decreased the chlorophyll a, b and total, anthocyanins, phenols and α-tocopherol contents of six *Medicago* cultivars. The results of greenhouse experiments showed that PEG treatments resulted in a significant reduction in fresh weight, dry weight and water content of all studied cultivars. In conclusion, the findings of both petri dish and greenhouse experiments suggested that Ghare-Medicago and Hamedani cultivars can be regarded as drought sensitive cultivars while Yazdi is a drought tolerant cultivar. It should be noticed that Bami and Baghdadi cultivars were also relatively drought tolerant. Also, our study on paclobutrazol (PBZ) treatment showed that PBZ effectively decreased the negative effect of drought stress on growth of all cultivars of *Medicago*.

Keywords: Cultivar, Drought sensitive, Drought tolerant, *Medicago sativa*, Plant growth regulator, Water stress.

Introduction:

Drought is an important environmental stress that limits plant production. Drought impacts growth, yield, membrane integrity, pigments content, osmotic adjustment water relations, and photosynthetic activity (Anjum *et al.*, 2011). The International Water Management Institute predicts that by the year 2025, one-third of the world's population will live in regions that will experience severe water scarcity (www.iwmi.org). Therefore, it has become imperative for plant biologists to understand the mechanisms by which plants can adapt to water deficit while retaining their capacity to serve as sources of food and other raw materials. Iran has a complex climate with great differences in climate condition. Sixty-five percent of Iran is considered to be arid, twenty percent is semi-arid, and only fifteen percent of the country has a humid and semi-humid climate (Madani Larijani 2005). The negative effects of drought stress have been revealed on *Vigna radiate* (Zabeti *et al*., 2003), *Triticum aestivum* (Moaveni *et al.,* 2009), *Zea mays* (Alizade *et al.,* 2007) and etc. in Iran.

Aalfalfa (*Medicago sativa* L.) as a perennial forage crops is a leguminous plant species and has been grown for a variety of purposes such as soil improvement, forage, medicinal uses, and suitable foliage (Wang and Han, 2009). The establishment stage of the crop consists of three parts: germination, emergence and early seedling growth; that are particularly sensitive to drought stress. Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease (Welbaum *et al.*, 1990, Roundy *et al.*, 1985). Much information is available in literature about the effects of water quality, soil texture and soil salinity on germination and emergence (Jamil *et al.*, 2005). Retardation and negative impacts in seed germination have been reported under drought stress in the literatures. The decrease in germination rate

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particularly under drought stress condition may be due to the fact that seeds seemingly develop an osmotically enforced dormancy under water stress conditions. This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Gill *et al.*, 2003). The consequence of water stress could already be observed some days after emergence, the higher water stress, expressed by lower leaf water potential, stomatal conductance and evapotranspiration and lowering of the leaf area and dry matter production (Katerji *et al.*, 1994). For years, polyethylene glycols (PEG) with large molecular weights have been used to simulate drought stress in plants. They are non-penetrating osmotic agents and lower the water potential in a way similar to soil drying (Larher *et al.*, 1993).

Triazole compounds such as triadimefon, paclobutrazol and propiconazole are widely used as fungicides and they also possess varying degrees of plant growth regulating properties (Fletcher *et al.*, 2010). Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels. Triazoles have been called plant multi-protectants because of their ability to induce tolerance in plants towards environmental and chemical stresses. Paclobutrazol (PBZ) [(2*RS*–3 *RS*)-1-4(chlorophenhyl)-4, 4-dimethyl-2-1, 2, 4-triazol-1yl-penten-3-ol] is a member of the triazole family. PBZ has been reported to protect plants against drought stress (Marshall *et al.*, 2000, Zhu *et al.*, 2004).

Researchers are trying to produce the drought resistant crops. Thus, screening for drought-tolerant *Medicago* is important to determine whether there is a genetic basis for selection and breeding purposes and to whether there are useful genotypes or new genes for tolerance to drought stress. The present study was undertaken to study the responses of six *Medicago* cultivars to different levels of PEG and to determine the genotypic variability in their tolerance to drought both at the germination and seedling stages in petri dish and plant growth in greenhouse. For screening the different *Medicago* cultivars, we studied multiple physiological parameters such as seed germination, growth, photosynthetic pigments, anthocyanins, phenols and α-tocopherol under PEG treatment.

Materials and methods:

Plant material and growth conditions: Six cultivars of *Medicago sativa* L. were used in this study. All seeds (Hamedani, Isfahani, Bami, Baghdadi, Yazdi and Ghare-Medicago variety) were obtained from the seed company of Pakan Bazr in Isfahan, Iran. The experiment was conducted at Payame Noor University, Shahrekord, Iran in 2015. Similar seeds size and weight were selected. Seeds were surface-sterilized for 1–2 min in 95% ethanol, followed by treatment in 10% sodium hypochlorite (v/v) with 3 drops per liter of Tween-20 for 20 min. Then the seeds were washed three times with sterile distilled water. Twenty of sterile seeds were transferred to the 10 cm sterile petri dishes on sterile filter paper supplemented with PEG solutions under aseptic condition. Drought stress was applied by subjecting the seeds to 10 mL of control (no added PEG), 3%, 6% and 9% PEG solutions. Plates were sealed with parafilm and placed under controlled conditions (25±2°C by day; 16/8 h Light/ dark; irradiance 4500 Lux). Seeds were considered to have germinated when the radicle measured in excess of 2 mm (Montana *et al.*, 2014). The number of germinated seeds was recorded every day for one week. The whole experiment was repeated in three different times, every time with five replications per every treatment. The following parameters were analyzed at germination stage.

Germination index: Germination index (GI) was calculated as described by the Association of Official Seed Analysts (Basra *et al.*, 2005) as: $GI = \sum(\frac{GT}{Tt})$

GT: The number of the germinated seed on the nth day

Tt: The number of day from first day experiment

Germination percentage: Seven days after germination, the germination percentage (GP) was obtained by divided the number of germinated seeds in any petri dishes by the total number of seeds, multiplied by 100 (Cokkizgin and Cokkizgin, 2010).

$GP = (Gn/N) \times 100$

Gn: The number of germinated seeds in final count

N: The total number of seeds

Mean germination time: The mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981) as follows: $MGT = \sum Dn / \sum n$

D: The day of counting

N: The number of seeds germinated on each day

Germination rate: The germination rate (GR) was calculated to assess the rate of germination (Maguire, 1962) as follows:

$GR = X1/Y1 + (X2-X1)/Y2 + \ldots + (Xn-Xn-1)/Yn$

Xn: The germination percentage on the nth day

Yn: The number of day from first day experiment

Growth parameters: The height, fresh weight, dry weight and water content of the seedlings were measured immediately after the end experiment of stress treatment. The dry weight was measured by drying the samples at 75°C for 48 h, to give a constant weight. The water content was calculated as a difference between fresh and dry weight.

Photosynthetic pigments measurement: The leaves of the plants were extracted in 80 % (v/v) acetone. Chlorophylls and carotenoids contents were determined spectrophotometrically (Wellburn and Lichtenthaler, 1984).

Anthocyanins content: Anthocyanins were extracted from fresh leaves in acidified methanol (methanol: HCl, 99:1 v/v). Anthocyanins of samples were analyzed (Wagner, 1979) and calculated using an extinction coefficient of 33000 mol^{-1} cm^{-1}.

α-Tocopherol content: α-Tocopherol was extracted from fresh leaves in a mixture of petroleum ether and ethanol (2:1.6 v/v) and the supernatant was used for the assay. α-Tocopherol content was determined by spectrophotometer method (Baker *et al.*, 1980).

Phenols content: The amount of phenolic compounds in leaves was measured using Folin and Ciocalteu's reagent. The fresh leaves were homogenized in 95 % ethanol and kept for 24 hours in the dark. The supernatant was used for the assay (Singleton and Rossi, 1965). Samples were quantified using a standard curve based on a gallic acid.

PBZ treatment: According to the obtained results from primary analysis, we selected 5% of PEG to perform more experiments on *Medicago* plants in greenhouse. In the second stage of experiment, we studied the interaction of PEG and PBZ on cultivars of Isfahani, Hamedani, Baghdadi, Yazdi, Bami and Ghare-Medicago. The seeds were cultured in 1.5 l pots, filled with perlite in a greenhouse with supplementary light to extend the photoperiod to 16 hours per day. Air temperature ranged from 22 to 26 ºC during the day and 15 to 18 ºC during the night. Humidity ranged from 40 to 60%. Twenty seeds were sown in each pot. The plants were irrigated every other day and fertilized once a week by the standard Hoagland nutrient solution (pH 6.0). Ten days after sowing, the seedlings were thinned to ten similar plants per pot. The experiment was designed as a completely randomized block in a split plot with the PBZ treatment as main plot, cultivar as sub-plot and PEG treatment as sub-sub-plot. Each main plot consisted of 32 plots and there were four replications per PEG treatment. The plants were sprayed with 0 (distilled water) and 10 mg/l PBZ solution. PBZ treatment was carried out every other day for one week. Two days after the first PBZ treatment, the seedlings were irrigated with 0 (distilled water) and 5% PEG solutions. PEG treatment continued for three weeks. All growth measurements were performed two days after the last day of PEG treatment. The fresh weight, dry weight and water content were measured after harvesting plants.

Statistical analysis: All experiments were done by using a Randomized Complete Block Design with three replications. The data were analyzed by the Duncan test's SPSS (version 16) statistical package to assess significant differences (at the 5 % level) between means. The results of statistical analysis are shown by superscripted letters after the numbers in tables to reveal significant differences.

Results:

Cultivar variations in germination: Analysis of data revealed significant differences among the *Medicago* cultivars for germination index, germination percentage, mean germination time and germination rate. The results showed that the germination index decreased with increasing PEG concentration (Figure. 1a). Among the analyzed cultivars, Hamedani and Ghare-Medicago cultivars showed the highest reduction in germination index at 9% of PEG. The results of germination percentage are presented in Figure. 1b. The PEG treatment decreased germination percentage significantly in Hamedani and Ghare-Medicago, while PEG treatment had no significant effect on other cultivars. The lowest germination percentage in Hamedani and Ghare-Medicago was observed at 9% of PEG. The parameter of germination rate was negatively affected by PEG treatments in all cultivars (Figure. 2a). Germination rate of Hamedani and Ghare-Medicago revealed a significant decrease with increasing PEG level, with the highest reduction at 9% of PEG. With respect to mean germination time, there was a considerable increase in this character in Hamedani and Ghare-Medicago genotypes at 9% of PEG, while it was not significantly affected in other cultivars (Figure. 2b).

Cultivar variations in growth: Plant matter production is an important criterion to evaluate drought tolerance in plants since it permits direct estimations of economic returns under specified drought condition. Analysis of seedling height, fresh weight and water content showed that the levels of analyzed parameters decreased in all cultivars with increasing PEG concentration. With increasing PEG concentration, the highest and lowest reduction in plant height was observed in Ghare-Medicago and Yazdi cultivars, respectively (Figure. 3a). Under PEG treatment, the fresh mass production decreased significantly in Isfahani, Hamedani, Bami and Ghare-Medicago while no significant reduction was observed in Baghdadi and Yazdi, comparing to the control plants (Figure. 3b). The dry mass production was not affected by PEG treatment in Isfahani, Hamedani, Bami, Yazdi cultivars (Figure. 4a). By contrast, dry mass production showed a significant reduction in Hamedani and Ghare-Medicago cultivars at 9% of PEG treatment. Interestingly, the water content of six analyzed cultivars significantly decreased with increasing PEG level from 3% to 9% (Figure. 4b). There were obvious differences among the cultivars in water content value. Comparing different cultivars under PEG treatments, the highest and lowest water content were observed in Yazdi and Hamedani cultivars, respectively.

Cultivar variations in physiological parameters: Among control plants, the highest and lowest Chl a, Chl b, total Chl and carotenoids contents were observed in Bami and Ghare-Medicago cultivars, respectively (Figure. 5 a and b; 6 a and b). Applied PEG treatment significantly decreased photosynthetic pigments in all cultivars. The highest negative affect of PEG treatment on Chl a, Chl b, total Chl and carotenoids were observed in Ghare-Medicago cultivar. The negative effect of PEG treatments on photosynthetic parameters of Bami and Yazdi cultivars was less than cultivars of Isfahani, Hamedani, Baghdadi and Ghare-Medicago.

The anthocyanin contents of plant significantly decreased with increasing PEG level from 0 to 9% in all cultivars (Figure. 7a). Interestingly, Bami and Hamedani cultivars, respectively, showed the highest

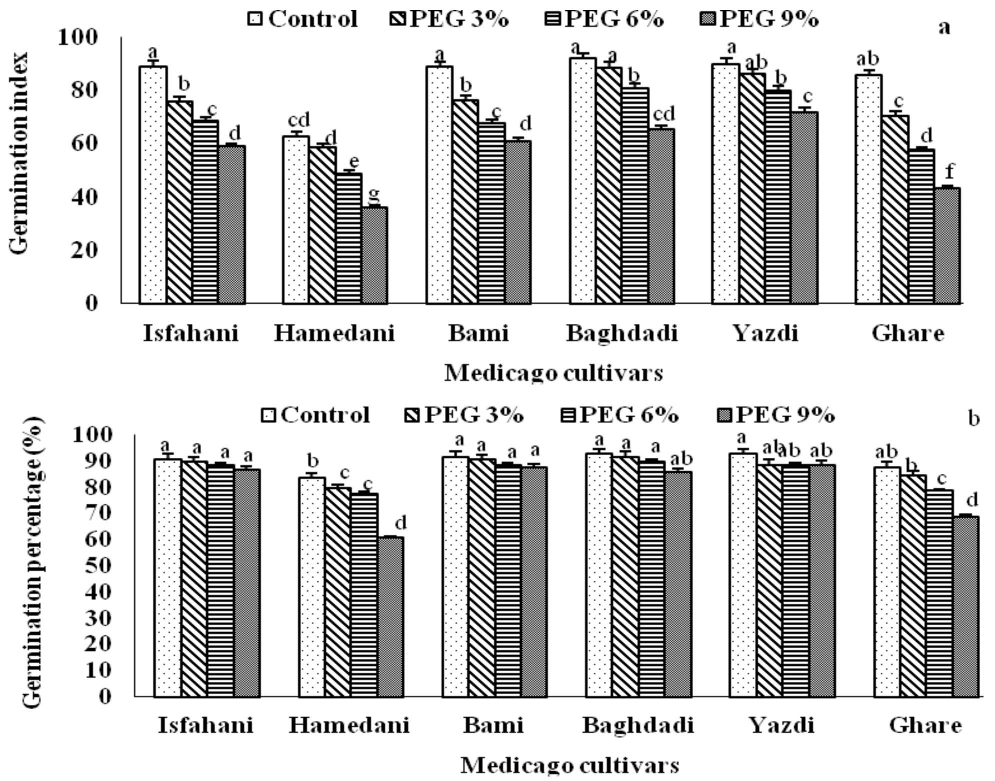


Figure 1. The effect of drought stress (0, 3, 6 and 9 % PEG) on germination index (a) and germination percentage (b) in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago). Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

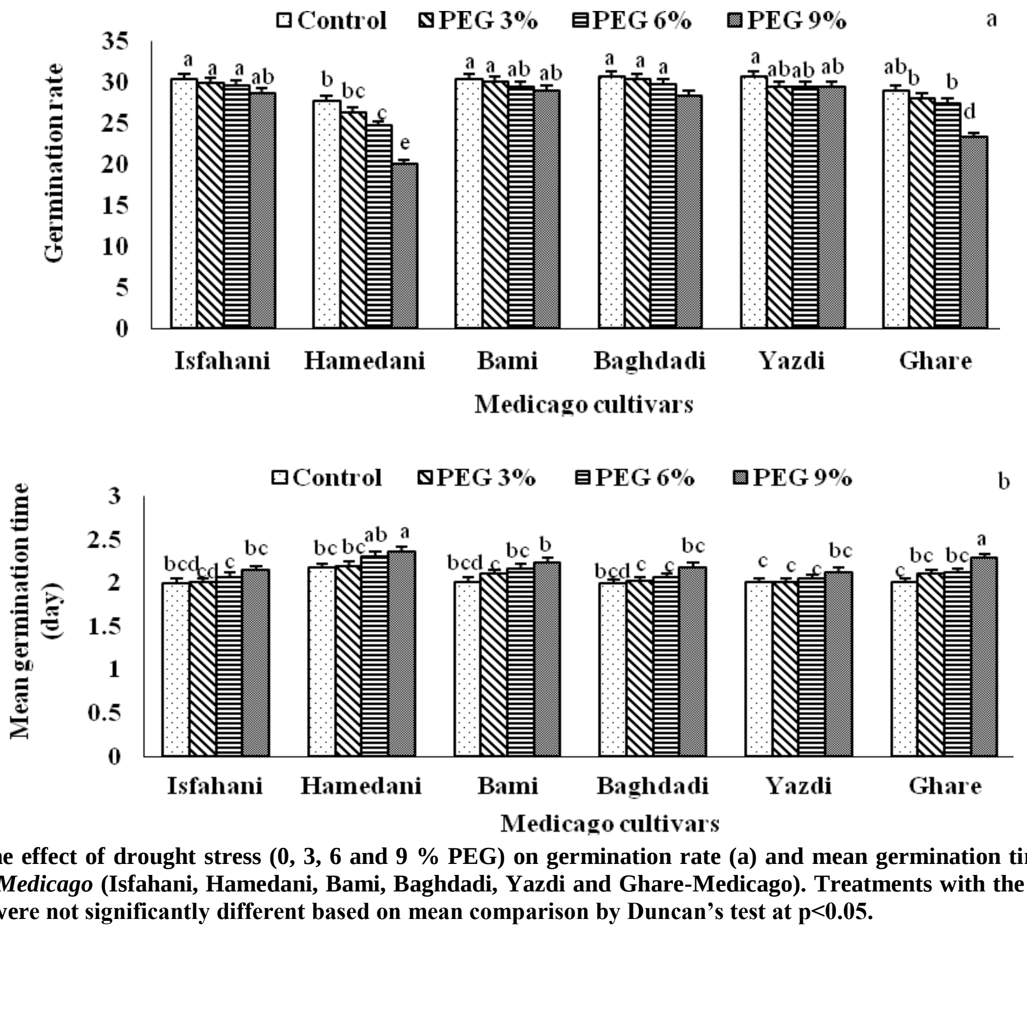


Figure 2. The effect of drought stress (0, 3, 6 and 9 % PEG) on germination rate (a) and mean germination time (b) in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago). Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

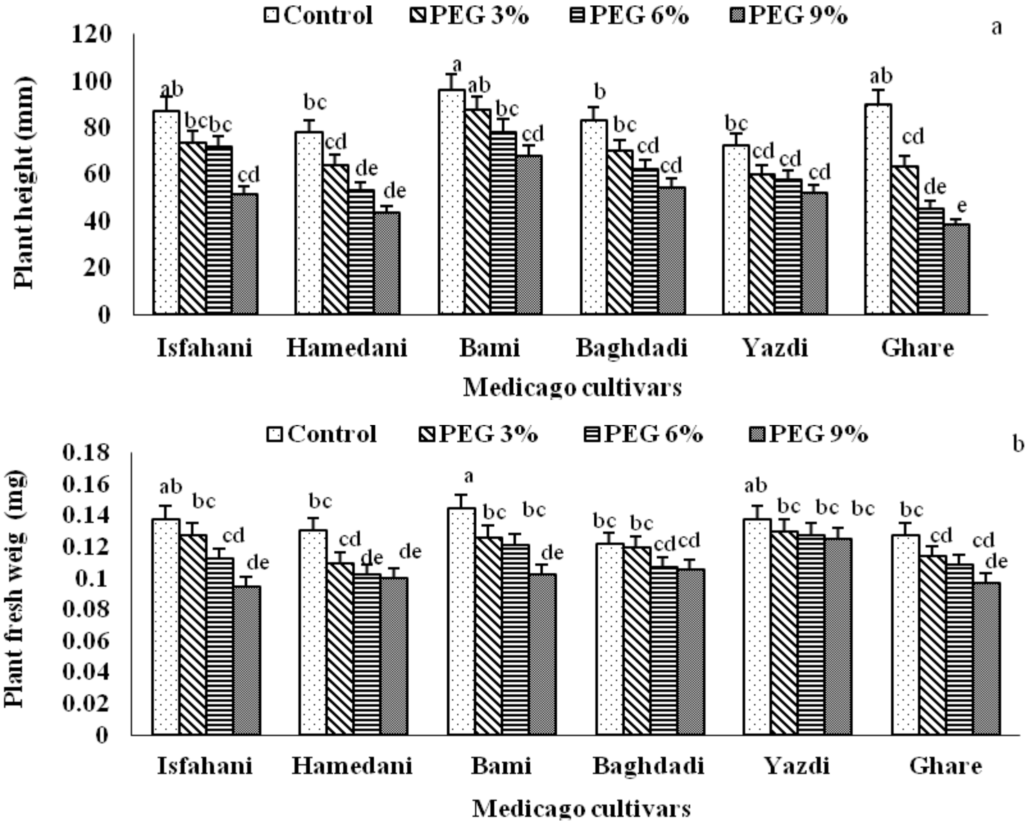


Figure 3. The effect of drought stress (0, 3, 6 and 9 % PEG) on plant height (a) and fresh weight (b) in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at seedling stage. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

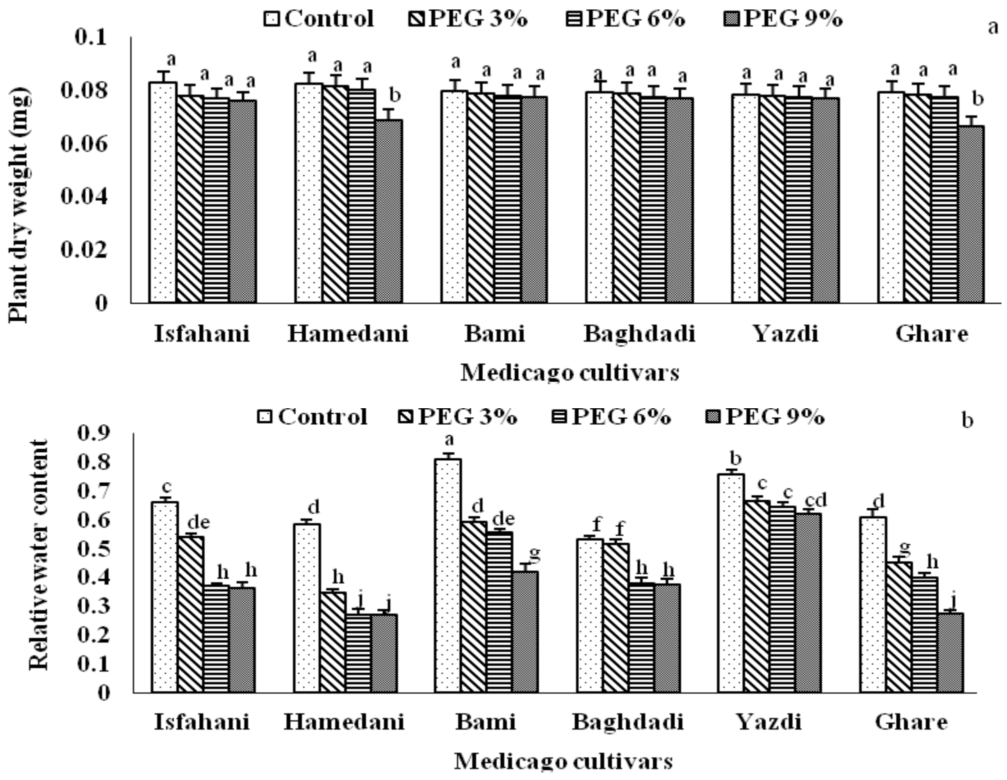


Figure 4. The effect of drought stress (0, 3, 6 and 9 % PEG) on plant dry weight (a) and plant water content (b) in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at seedling stage. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

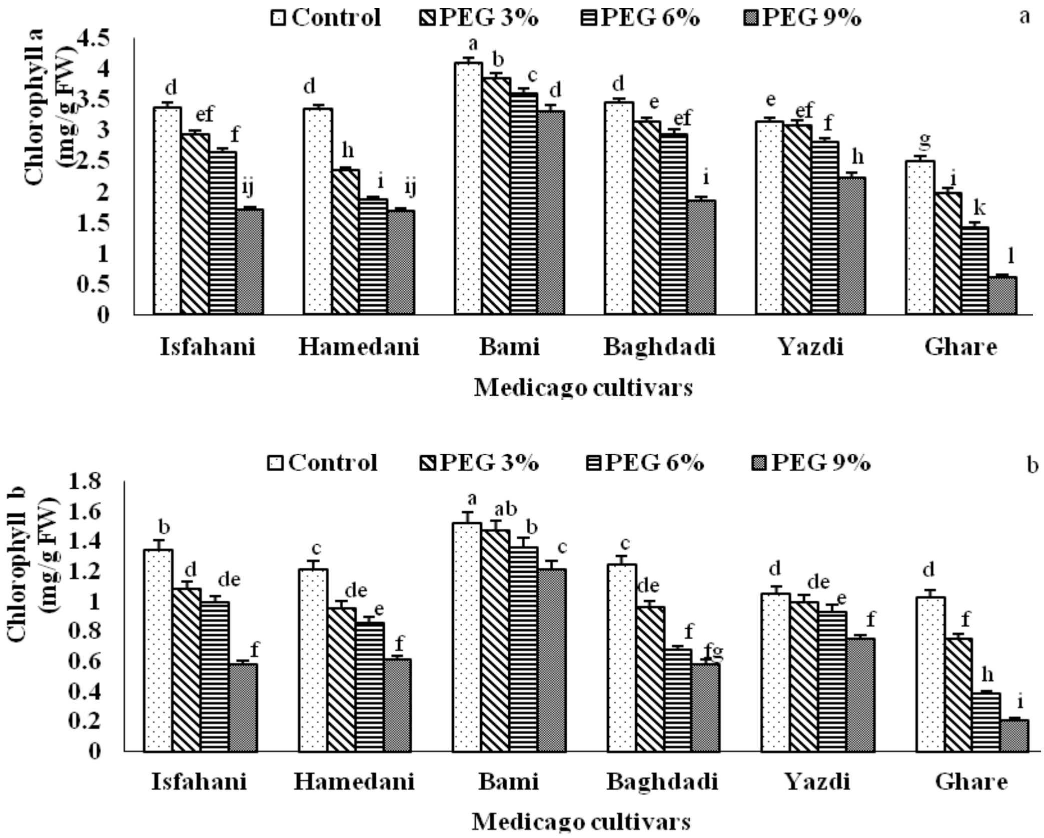


Figure 5. The effect of drought stress (0, 3, 6 and 9 % PEG) on Chlorophyll a (a) and Chlorophyll b (b) contents in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at seedling stage. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

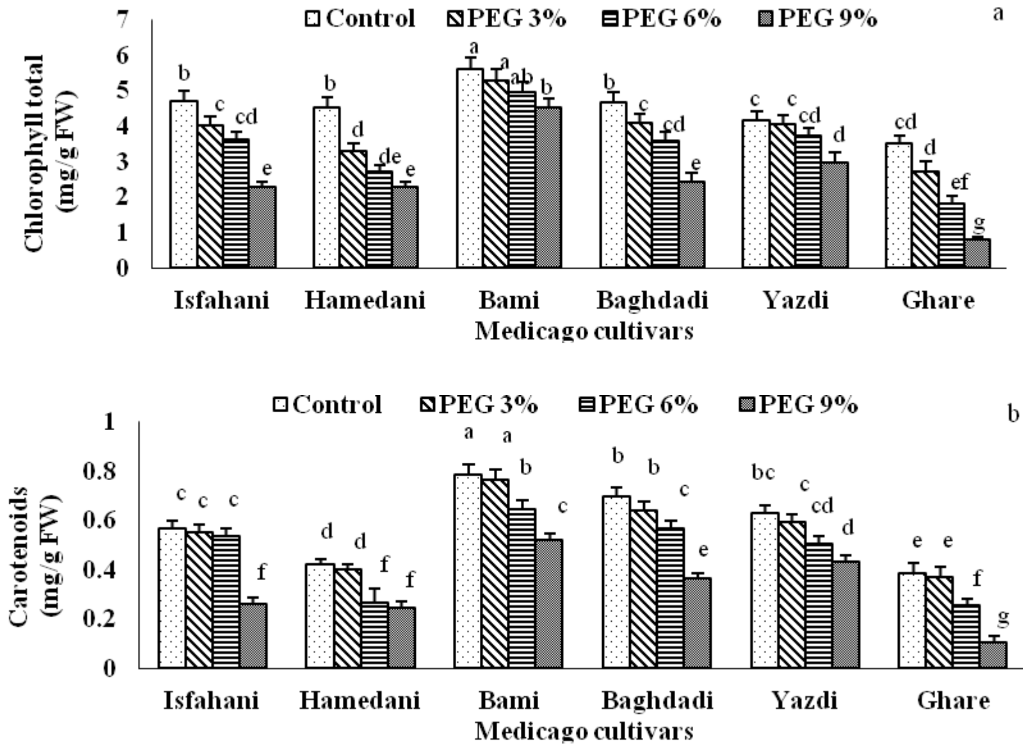
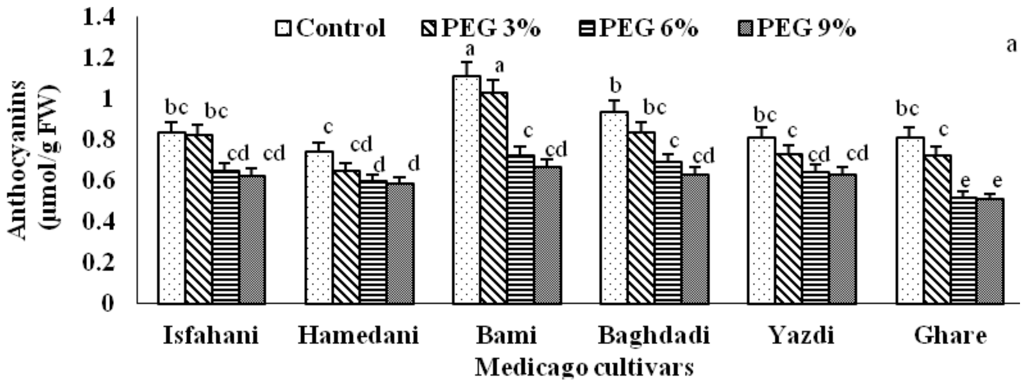


Figure 6. The effect of drought stress (0, 3, 6 and 9 % PEG) on total Chlorophyll (a) and carotenoids contents (b) in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at seedling stage. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.



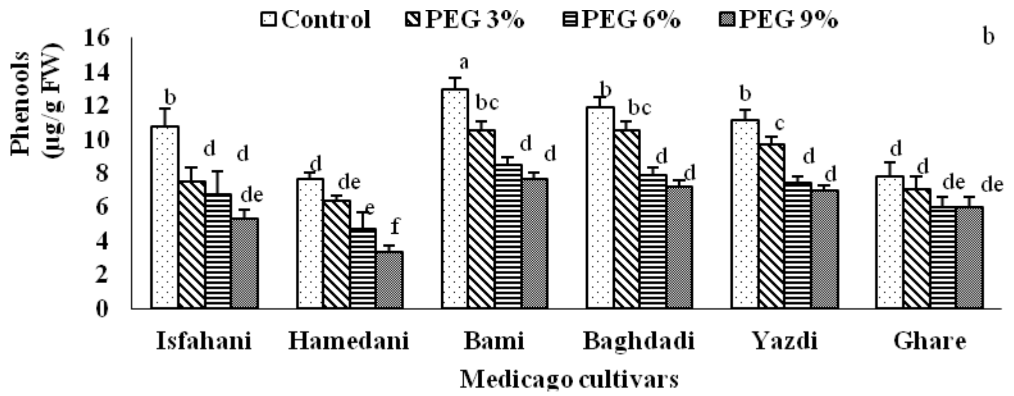


Figure 7. The effect of drought stress (0, 3, 6 and 9 % PEG) on anthocyanin (a) and phenol (b) contents in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at seedling stage. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

and lowest reduction of anthocyanin contents at 9% of PEG. The phenol contents were analyzed in treated plants (Figure. 7b). Among the control cultivars, Bami accumulated the highest amount of phenols while Ghare-Medicago and Hamedani accumulated lowest amount of phenols. PEG treatment decreased significantly the phenol contents in all cultivars with the highest reduction in Bami, comparing to control plant. α-Tocopherol content was negatively affected by PEG treatment in all cultivars (Figure. 8). The most negative effect of PEG treatment was observed in Ghare-Medicago and Hamedani cultivars while the lowest negative effect of PEG was revealed in Bami cultivar, comparing to the control plant.

Cultivar variations in growth under PEG and PBZ treatments: Plant growth was measured in *Medicago* cultivars under interaction of PEG and PBZ treatments in greenhouse condition. Concerning plant fresh weight, there was a highly significant reduction in all cultivars under both PBZ and PEG treatments (Figure. 9a). Under PBZ treatment, all cultivars showed the same fresh weight and no significant differences were observed in their fresh weight. Comparing the PEG and PBZ treatments, the observed reduction in PEG treatment was significantly higher than PBZ treatment. It should be mentioned that the interaction of PBZ and PEG treatments significantly reduced the negative effect of PEG in Hamedani and Ghare-Medicago cultivars.

Plant dry weight was measured in *Medicago* cultivars and then was compared to PBZ and PEG treatments (Figure. 9b). PBZ treatment had no significant effect on plant dry matter production in analyzed cultivars of Isfahani, Hamedani, Bami, Ghare Medicago, Yazdi and Baghdadi. In comparison with PBZ treatment, PEG treatment significantly decreased the plant dry weight in all cultivars, with the most reduction in Hamedani and Ghare-Medicago cultivars, compared to the control plants. PBZ treatment significantly reduced the negative effect of PEG treatment on plant dry mass under PBZ+PEG treatment.

The classical explanation of water stress in plants growing in a dry environment is the reduced access of soil water due to its osmotic potential. The results in Figure 10 show that there was a remarkably decrease in water content of all cultivars under PBZ treatment. Similar to plant fresh weight parameter, the water content of all cultivars of Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago were almost the same and no significant variations were observed among them. PEG treatment significantly decreased water content in all cultivars with the highest reduction in Ghare-Medicago and Hamedani cultivars, compared to the control plants. The interaction of PBZ and PEG treatment significantly reduced the negative effect of PEG treatment in Hamedani and Ghare-Medicago cultivars.

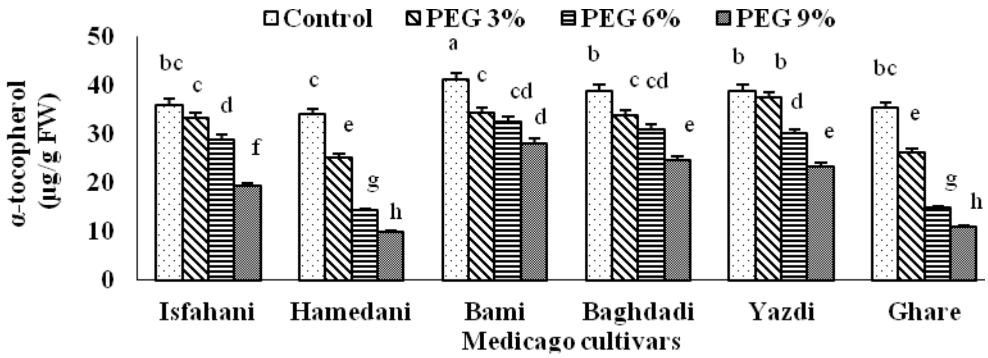


Figure 8. The effect of drought stress (0, 3, 6 and 9 % PEG) on α-Tocopherol content in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at seedling stage. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

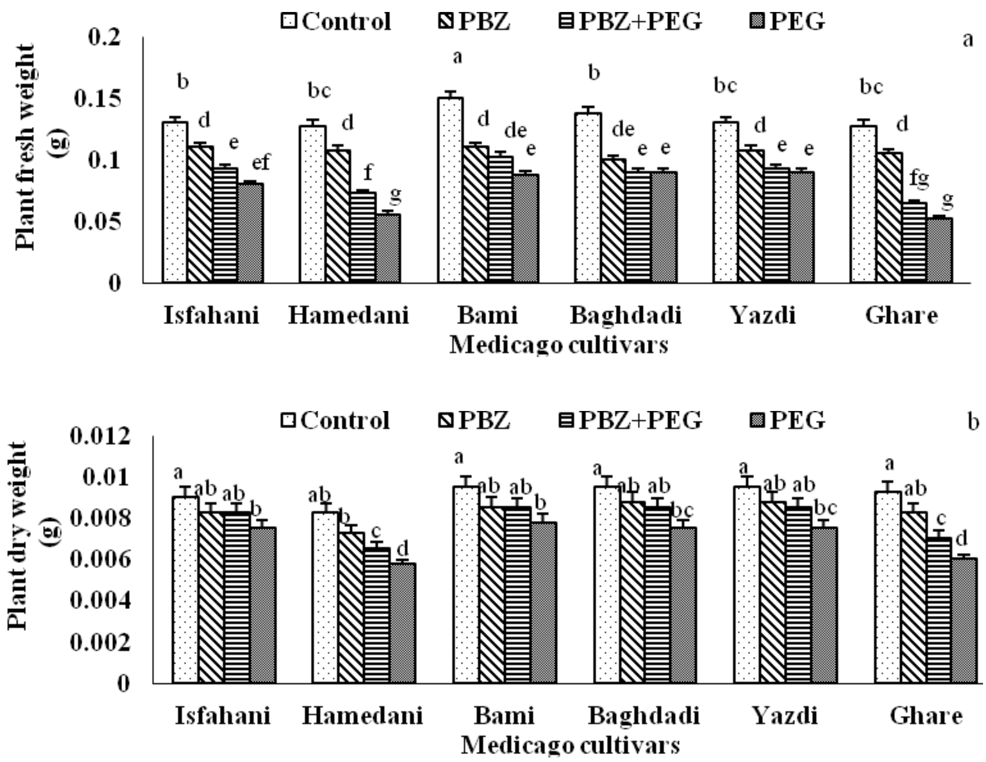


Figure 9. The effect of drought stress (0, 3, 6 and 9 % PEG) on plant fresh weight (a) and plant dry weight (b) in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at greenhouse experiment. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

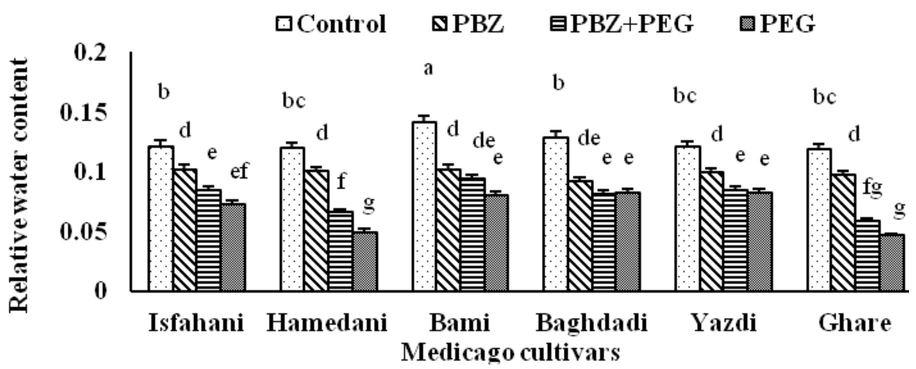


Figure 10. The effect of drought stress (0, 3, 6 and 9 % PEG) on plant water content in six cultivars of *Medicago* (Isfahani, Hamedani, Bami, Baghdadi, Yazdi and Ghare-Medicago) at greenhouse experiment. Treatments with the same lower-case letters were not significantly different based on mean comparison by Duncan's test at p<0.05.

Discussion:

Drought is a multifaceted stress condition that causes serious crop yield limitations depending on plant growth stage, stress duration, and severity. Germination is the most critical and sensitive stage in the life cycles of plants (Ahmad *et al.*, 2010). The seeds exposed to unfavorable environmental conditions such as drought may compromise the subsequent seedling establishment (Albuquerque and De Carvalho, 2003). Genetic variability within a species offers a valuable tool for studying mechanisms of drought tolerance. Our results highlighted significant differences among the cultivars exposed to drought stress with a remarkably decreased and delayed germination in Hamedani and Ghare-Medicago cultivars.

Germination index is a valuable parameter to compare the negative effect of PEG treatment among different cultivars of *Medicago*. Germination index was negatively affected by increasing PEG level. It decreased by about 50%, 44%, 35%, 32%, 29% and 21%, respectively in Ghare-Medicago, Hamedani, Isfahani, Bami, Bghdadi and Yazdi cultivars, at 9% of PEG. All studies conducted thus for have proved that the increasing water stress decreased the germination proportion in plants (Falusi *et al.*, 1983, Boydak *et al.*, 2003). It has been indicated that water stress decreased the germination percentage of Pinus contorta and Picea engelmanii seeds with a proportion of 50% (Kaufmann and Eckard, 1977). At highest level of PEG, the germination percentage of Ghare-Medicago and Hamedani decreased by about 22% and 28% less than the control plants while the cultivars of Isfahani, Bami, Yazdi and Baghdadi revealed no significant reduction.

The ability to early germinate contributes to differences among cultivars for drought tolerance and is considered an important trait in selection of drought resistant cultivars. The factor of mean germination time is of prime importance which can be negatively increased in drought sensitive plants. Data indicated that 9% of PEG negatively increased germination time in Ghare-Medicago and Hamedani while no increase was observed in other cultivars. Also, the studies have shown that the factor of germination rate is also important to determine the tolerance for seeds to drought stress. In arid environment, less water will be available for seeds which negatively affect water absorption by seeds and consequently reduce germination rate (Guan *et al.*, 2013). In this study, germination rate of Ghare-Medicago and Hamedani was negatively affected by PEG, by about 21% and 26% less than the control plants at highest level of PEG. The results of our study on seed germination showed that Yazdi cultivar performed better seed germination properties under PEG treatment compared to five other cultivars. We also found that the cultivars of Ghare Medicago and Hamedani are not compatible for arid environment.

Early seedling growth is a critical stage for plant establishment (Guan *et al.*, 2013). Accordingly, we analyzed growth and some physiological parameters in early seedling growth stage in six cultivars of *Medicago* under drought stress. PEG treatment revealed a significant negative effect on plant height. At 9% of PEG, the plant height of plants reduced by about 58%, 45%, 42%, 35%, 31% and 28% respectively in Ghare-Medicago, Isfahani, Hamedani, Bami, Baghdadi and Yazdi cultivars, less than the control plants. Obtained results showed that drought stress negatively affected growth of plant and more sensitive plants showed lower tolerance to drought. A common adverse effect of drought stress on plants is the reduction in fresh and dry biomass production due to the decrease in the number of leaves per plant and leaf size (Anjum *et al.*, 2011). Obtained results revealed that the fresh weight of seedlings significantly decreased under PEG treatment. The cultivars of Isfahani, Hamedani, Bami and Ghare-Medicago showed significant reduction of plant fresh weight under PEG treatment while Yazdi and Baghdadi did not show significant reduction in treated plants. Regarding plant dry mass, PEG treatment only adversely affected Ghare-Medicago and Hamedani cultivars. Also, PEG treatment decreased water content in the studied cultivars, as well documented also for other species under similar experimental conditions (Bajji *et al.*, 2000, Abrams, 1990, Wu *et al.*, 2013). According to our results, cultivars of Yazdi stored higher amount of water under PEG treatment compared to other cultivars and it is important for seedling to tolerate drought stress.

The decrease in chlorophyll contents under drought stress has been considered as a typical symptom of oxidative stress and might result from pigment photo-oxidation and chlorophyll degradation (Farooq *et al.*, 2009). Total chlorophyll of Ghare-Medicago, Isfahani, Hamedani, Baghdaddi, Yazi and Bami respectively decreased by about 77%, 54%, 52%, 48%, 30% and 20%, at highest level of PEG. The decrease in carotenoids under stress condition is due to the degradation of ß-carotene and formation of zeaxanthins, which are apparently involved in protection against photo-inhibition (Sultana *et al.*, 1999). The carotenoid contents significantly decreased in all cultivars under PEG treatment which was highly announced in Ghare-Medicago cultivar. Anthocyanins are water-soluble pigments derived from flavonoids via the shikimic acid pathway (Herrmann, 1995). The negative effect of PEG treatment on anthocyanins of all studied *medicago* cultivars was significant. Anthocyanins are responsible for the antioxidant capacity in plant tissues (Larson, 1988).

α-Tocopherol is a lipophilic antioxidant. It plays an important role in adsorbing and neutralizing free radicals which results in antioxidant protection (Wang and Quinn, 2000). PEG treatment negatively affected α-tocopherol content in six analyzed cultivars with the highest negative effect in two cultivars of Ghare-Medicago and Hamedani more than other cultivars. Phenols are very important plant constituents because of their scavenging ability, which is mainly due to their hydroxyl groups (Hatano *et al.*, 1990). Results showed that PEG treatment significantly decreased phenols content. It is reported that drought stress resulted in an increase of some soluble proteins and a decrease of others. Interestingly, Ghare-Medicago and Hamedani cultivars accumulate less phenol contents in both normal condition and PEG treatment, compared to other cultivars, which is propably correlated to low resistance to drought stress.

On the what, these findings suggest that Ghare-Medicago and Hamedani cultivars can be considered as drought sensitive cultivars while making a firm decision on drought tolerant cultivar is critical in this step. Our data highlighted an opposite response to drought tolerance because the three cultivars of Yazdi, Baghdadi and Bami were found drought tolerant. We will further study in greenhouse to make a better overview as well as using PBZ treatment to open a new gate for further study. PBZ, as a gibberellin biosynthesis inhibitor, reduces plant height, leaf size and subsequently plant weight (Fletcher *et al*., 2010). PBZ treatment could partly restore plant weight under drought stress. Reduction of plant water content under drought stress could partly be quenched by PBZ treatment. Navarra *et al.,* (2007) reported that PBZ-treated plants showed better water status which supports our results. Drought associated oxidative stress slows down metabolism and modifies membranes which causes lipid peroxidation. Fletcher *et al.,* (2010) reported that the stress protective effect of triazole against stress is mediated by an increase in antioxidant contents. Sankar *et al.,* (2007) reported that PBZ increased drought tolerance in *Arachis hypogaea* which supports our results.

Conclusion:

The results of plant fresh weight, plant dry weight and water content measurements from greenhouse experiment confirmed that PEG treatment revealed the highest negative effect on two cultivars of Hamedani and Ghare-Medicago. According to greenhouse experiments, Yazdi cultivar was more tolerant to PEG treatment with comparison to the other cultivars. Thus, it can be concluded that Yazdi cultivar establishment in drought condition is better than other studied cultivars. Also, we should mention that PBZ was an effective treatment to reduce negative effects of PEG treatment on plants but it needs more studies on biochemical and physiological parameters. In conclusion, comparing the responses of plant growth, photosynthetic pigments, anthocyanins, phenols and α-tocopherol to PEG treatment suggested Ghare-Medicago and Hamedani as drought sensitive cultivars, Isfahani as semi drought tolerant cultivar, Baghdadi and Bami as relatively drought tolerant cultivars, and Yazdi as a drought tolerant cultivar. To be performed we suggest more experiments with PBZ for better understanding and comparison of the drought tolerant mechanism among different cultivars of *Medicago*.

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