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Biochemical and Physiological Responses of Two Wheat (*Triticum aestivum* L.) Cultivars to Drought Stress Applied at Seedling Stage

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ABSTRACT

Zagros (drought tolerant) and Pishtaz (drought susceptible) cultivars were classified on the basis of shoot dry weight and were used as plant material in this study. Total chlorophyll, carotenoids, antocyanins, proline, soluble sugar contents, lipid peroxidation, antioxidant enzyme activities and protein patterns were determined. Seedlings of wheat genotypes were grown in nutrient solution cultures under 16 h d^{-1} period at room temperature. With the decrease in osmotic potential, total chlorophyll initially increased but then decreased in the tolerant cultivar. The amount of total carotenoids and antocyanins in both cultivars increased in response to drought stress. However, the increase was only significant ($P< 0.05$) at some osmotic potentials. The increase was more pronounced in the tolerant cultivar. Soluble sugars and proline increased significantly in both cultivars, but were higher in the tolerant one. The sensitive cultivar showed higher rates of lipid peroxidation as compared to the tolerant cultivar. Antioxidant enzymes activities increased with the decrease in osmotic potential in both cultivars. The tolerant cultivar exhibited a higher antioxidant activity compared to the sensitive one. SDS-PAGE showed new protein bands under water stress. These results indicated that proline, soluble sugars contents and antioxidant enzyme activities are part of the defense mechanisms which confer water deficit tolerance to wheat cultivars. APX= Ascorbate peroxidase; CAT= Catalase; GR= Glutathione reductase; MDA= Malondialdehyde; ROS= Reactive oxygen species, SOD= Superoxide dismutase.

Keywords: Antioxidant, Compatible solute, Water stress, Wheat cultivars.

INTRODUCTION

Plants are constantly exposed to various biotic and abiotic stresses threatening their growth and development. Some of the abiotic stresses are drought, salinity, extreme temperatures and pollutants (Tas and Tas, 2007). Among the abiotic stresses, drought is the most common that plants are constantly exposed to, making up some 26% of all stresses (Tas and Tas, 2007). In fact, water deficiency due to drought is one of the most severe environmental stresses affecting plants growth and development (Rampino *et al.,* 2006). The IPCC Third Assessment (TAR) has reported that some weather events and extremes such as drought will become more frequent, more widespread and more intense during the 21st century and water shortage for settlements, industry and societies will reduce hydropower generation potentials and increase the potential for population migration. Drought is most common in regions of the world with either low rainfall or low water supply (Sankar *et al.,* 2007). The extent of damage to the plants depends on both the severity and the duration of water stress (Rampino *et al.,* 2006). In response to abiotic stresses, plants use different strategies to cope

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with which include changes in plants biochemical and physiological processes (Mohammadkhani and Heidari, 2008). Some of these changes include, decrease in water loss by increasing stomatal resistance, increase in the rate of water uptake by developing deep and extended root systems and accumulation of osmolytes (Rampino *et al.,* 2006). Adaptation to this stress is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline (Mohammadkhani and Heidari, 2008). Proline accumulation is one of the most widespread plant responses to environmental stresses especially to drought stress (Mohammadkhani and Heidari, 2008). Being a hydrophilic molecule, proline is known as a compatible solute which can be accumulated in cytosol without interfering with other metabolites or with cellular structures (Mohammadkhani and Heidari, 2008). Drought-induced overproduction of ROS increases the content of malondialdehyde (MDA). The content of MDA has been considered as an indicator of oxidative damage. MDA is considered as a suitable marker for membrane lipid peroxidation. A decrease in membrane stability reflects the extent of lipid peroxidation caused by reactive oxygen species (ROS) (Anjum *et al.,* 2011). Another strategy that plants adopt to cope with drought stress is to make use of antioxidants and expression of genes responsible for the synthesis of enzymes involved in the synthesis of such antioxidants (Dalmia and Sawhney, 2004). These antioxidants are effective in protecting plant cells from the harmful effects of ROS which are produced by oxidative stresses such as drought. Plant tissues contain several scavenging enzymes such as superoxide dismutase, catalase, peroxidase and glutathione peroxidase as ROS scavenging systems. Plants also contain a network of low molecular weight antioxidants such as ascorbate, phenolnic compounds and tocopherols (Blokhina *et al.,* 2003).

Moreover, drought stress affects genes expressions and protein synthesis and also causes quantitative changes in photosynthetic pigments such as chlorophylls, anthocyanins and carotenoids (Lobato *et al.,* 2008). Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers (Anjum *et al.,* 2011). Decreased or unchanged chlorophyll levels during drought stress have been reported in many species, depending on the duration and severity of drought (Anjum *et al.,* 2011).

Worldwide, wheat (*Triticum aestivum* L.) is one the most important agricultural crops both economically and strategically. Because of its relatively good adaptation to different regions of the world with tropical climate and low water irrigation, it is usually considered as more resistant to abiotic stresses than the hexaploid wheat plants (Tas and Tas, 2007). About one-third of the world climates have been classified as arid and semi arid in which wheat is the major food source for their inhabitants. Consequently, finding the biochemical and physiological responses of some of the cultivated wheat plants to drought stress is helpful in selecting cultivars most adaptable to these climates. Accordingly, this study was carried out in order to examine the effects of drought stress on some wheat cultivars currently cultivated in Iran. The purpose of this investigation is to find the most tolerant cultivars to drought stress.

MATERIALS AND METHODS

Plant Materials

Seeds of the eight bread wheat cultivars (Sardari, Zagros, Pishtas, Falat, Shiraz, Marvdasht, Koohdasht and Azar-2) were provided by Zarghan Institute for Agricultural Research (Zargan, Fars Province, Iran). All chemicals used in this study were purchased from Sigma-Aldrich Company.

Seed Germination and Water Stress Induction

Experiments were conducted in two stages: (1) Effect of drought stress on seed germination, fresh and dry weight of roots and shoots to select the most and the least tolerant cultivar among eight cultivars. (2) Effect of drought stress at

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seedling stages on biochemical and physiological responses of two selected cultivars. In each stage of experiments, seeds of drought tolerant and susceptible wheat cultivars were first soaked in water for 2-3 hours followed by soaking in 10% sodium hypochlorite for 10-15 minutes. Seeds were then rinsed several times by tap water followed by a final rinse with distilled water. Seeds were germinated on a plastic mesh using tap water. Six-day-old seedlings were transferred to ½ strength Hoagland nutrient solution (Hoagland and Arnon, 1950). Experiments were designed in three replications for each treatment and three plants in each pot. The volume of nutrient solution in each pot was 370 ml. Using the Michel-Kaufmann equation, 18.53, 29.04, 65.98 and 82.75 g of polyethylene glycol-6000 were added to nutrient solutions to produce solutions of -0.05, -0.1, -0.4 and -0.6 MPa osmotic potential respectively (Michel and Kaufmann, 1973). Plants were allowed to grow in growth room with 16 h d^{-1} photoperiod provided by fluorescent lamps with 14000 lux (14000 lux= 184.21 µmol m^2 s (PAR)) luminescence. The solution was aerated at 15 minutes intervals and the ambient temperature was set at room temperature. Leaves and roots of 16-day-old seedlings were used for data analyses. The rate of seed germination, root and shoot length of germinated seeds and dry and fresh weights of the seedlings were determined for all treatments. Based on the roots and shoots fresh and dry weight of the eight wheat cultivars screened, two were selected as the most (Zagros) and the least (Pishtaz) drought tolerant cultivars, respectively for further investigation. The effects of drought stresses on the amount of chlorophyll, carotenoids, anthocyanins, proline, soluble sugars, lipid peroxidation and antioxidant enzyme activities including guaiacol peroxidase and catalase were determined in these two selected cultivars.

Anthocyanin, Total Chlorophyll and Total Carotenoids Contents

For the determination of photosynthetic pigments (total chlorophyll and total carotenoids), leaf samples (0.5 g) were homogenized with 80% acetone using quartz particles in a mortar. The homogenate was filtered and using 80% acetone, the final volume of filtrate was brought to 10 mL. The concentrations of chlorophyll and carotenoids pigments were determined by a spectrophotometer using Arnon (Arnon, 1956) and Lichtenthaler, 1987) methods, respectively. For anthocyanin determination, leaf samples (0.1 g) were added to 10 mL of methanol/hydrochloric acid (99:1) and kept in dark for 24 hourrs. Subsequently, the absorbance of the solutions was determined at 550 nm and the pigments concentrations were calculated according Wagner's method (Wagner, 1979).

Proline Contents

Proline was determined according to the method described by Bates and coworkers (Bates et al., 1973). Briefly, one-hundred milligram of frozen plant material was homogenized in 1.5 mL of 3% sulphosalicylic acid and the residues were removed by centrifugation. Then, 100 µL of the extract was reacted with 2 mL of glacial acetic acid (GAA) and 2 mL of ninhydrin (1.25g ninhydrin dissolved in 30 mL GAA and 20 mL of 6 M phosphoric acid) for 1 hour at 100ºC. To the reaction mixture 1mL toluene was added and the toluene containing chromophore was warmed up to the room temperature and its absorbance was determined by a spectrophotometer at 520 nm using standard curves.

Reducing Sugars

The shoot tissues (200 mg) were extracted twice in 10 ml ethanol (80%) at 90ºC followed by 4 times extraction in 70% ethanol. The extracts were used for total reducing sugars determination as described by Nelson (Nelson, 1944). Briefly, one-hundred milliliters of extracted solution was added to 0.9 mL distilled water plus 1 mL alkaline copper reagent solution and warmed in a water bath (78˚C) for 20 seconds. After cooling, 1 mL arsenomolybdate and 7 mL

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distilled water were added to the solution. The solution absorbance was determined by a spectrophotometer at 520 nm. For preparing the alkaline copper solution, 12.5 g $NaCO_3$, 12.5 g sodium potassium tartrate, 10 g $NaHCO_3$ and 10 g Na_2SO_4 were solved in 500 ml distilled water. Then 3 g $CuSO_4$, which was solved in 20ml distilled water, was added to the final solution. Then 0.2ml sulfuric acid was added. The final solution was blue in color. Also, the arsenomolybdate solution was prepared as follows: 12.5 g ammonium molybdate was solved in 225 ml distilled water. Then 10ml sulfuric acid was added. 1.5 g sodium arsenate solved in 12.5 ml distilled water was added to the solution. The solutions were kept in dark at 37˚C for 24-48 hours.

Lipid Peroxidation

Lipid peroxidation was analyzed according to the procedure described by Heath and Packer (Heath and Packer, 1968). Briefly, 0.4 g of the shoot tissue was homogenized in liquid nitrogen with a mortar and pestle. The homogenized tissue was suspended in 5 mL ice-cooled 0.1% trichloroacetic acid (TCA), and then was transferred into centrifuge tube with a further addition of 1 mL TCA. After centrifugation at 22,000×*g* for 10 minutes, 1 mL of the supernatant was mixed with 4 mL TCA (20%) containing 0.5% Thiobarbituric acid (TBA), kept for 30 minutes in a 95°C water bath and then rapidly cooled down on ice and centrifuged at 22,000×*g* for 10 minutes. The absorbance of the supernatant containing malondialdehyde was determined at 532 nm, subtracting from its absorbance at 600 nm.

Antioxidant Enzymes Activities

For the extraction of catalase (CAT) enzyme, leaf samples (1 g) were homogenized with 10 mL 200 mM phosphate buffer, pH 0.7 in a mortar. 10µL triton X-100 0.1% was added to the homogenized sample, mixed and after centrifuging at 1,000×g at 4°C for 10 minutes, the supernatant was used for the catalase activity determination according to Aebi's method (Aebi, 1984).

Briefly, catalase activity was assayed in a reaction mixture containing 1 mL 30 mM H_2O_2, 2 mL 50 mM phosphate buffer, pH 7.0, plus 0.1 mL extraction mixture. Catalase activity was estimated by the decrease in solution absorbance at 240 nm.

To extract guaiacol peroxidase (GPX), plant samples (1 g) were homogenized in 10 mL 0.1M phosphate buffer, pH 6.0 containing 0.6g KCl. After centrifuging at 1,000×g at 4°C for 10 minutes, the supernatant was used for the determination of soluble guaiacol peroxidase activity (Mac-Adam et al., 1992).

The reaction mixture contained 50 µL H_2O_2 (0.03 M), 3 mL extraction buffer pH 7.0, 50 µL 0.2M guaiacol and 0.1 mL extraction mixture. After adding 0.03M H_2O_2, the change in the reaction mixture absorbance at 436 nm was recorded every 30 seconds for 5 minutes.

Gel Electrophoresis

The leaf proteins were analyzed using Sodium Dodesyl Sulfate Poly Acryl amide Gel Electrophoresis (SDS-PAGE), containing 15% gel according to the standard protocol described by Laemmli *et al.* (1970). The protein bands were visualized using Coomassie brilliant Blue R-250 staining and were destained in the solution of 15% methanol and 10% glacial acetic acid (Merck).

Experimental Designs and Statistical Analysis

Experiments were arranged in a complete factorial design with three replications for each treatment. The statistical analysis was performed using SPSS 14 by Duncan

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multiple range test (α= 0.05). Excel 2003 program was used to produce standard curves and graphs.

RESULTS AND DISCUSSION

Based on the roots and shoots fresh and dry weight, the two wheat cultivars, Zagros and Pishtaz, were selected as the most and the least drought tolerant among the eight cultivars used, respectively (Tables 1 and 2). Accordingly, in our subsequent studies these two cultivars were used to investigate their physiological and biochemical responses to drought stress.

Effects of Osmotic Potential on the Amount of Leaves Chlorophylls and Carotenoids

The results presented in Figure 1-A show that the amount of total chlorophyll in leaves of Pishtaz (drought sensitive) started to decrease at -0.05 MPa and continued to decrease at more negative nutrient solutions osmotic potentials. However, in Zagros (drought tolerant cultivar), the amount of total chlorophyll increased significantly ($P< 0.05$) at -0.05 and -0.1 MPa osmotic potentials. Further decrease (more negative) in solutions osmotic potentials (-0.4 and -0.6 MPa) resulted in a decrease in total chlorophyll content. Figure 1-B shows that the total cartenoids contents of the two wheat cultivars increased with the decrease in solutions osmotic potential. Except for Pishtaz, at osmotic potentials of -0.4 and -0.6 MPa, the increase in carotenoids remained unchanged. The initial increase in total chlorophyll content in response to drought stress indicates that cell growth is more sensitive to water shortage than chlorophyll synthesis (Gimenez *et al.,* 1992), thus smaller leaf cells will result in higher chlorophyll content per unit leaf fresh weight. However, the amount of chlorophyll in both wheat cultivars decreased with the increase in the duration of drought stress probably due to the destruction of chloroplast envelope by drought stress (Figure 1-A). The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of photo-oxidation of photosynthetic pigments (Anjum *et al.,* 2011).

The increase in total carotenoids in response to drought stress in both cultivars can help in quenching the reactive oxygen species (ROS) produced by drought stress and to some extent reduce their effects on cell destruction (Howltt and Pogson, 2006).

Effects on Anthocyanins Synthesis

The amount of anthocyanins in both cultivars increased in response to drought stress (Figure1C). However, increases in anthocyanins in plants grown in solutions with osmotic potentials of -0.05, -0.1 and -0.6 MPa were only significant ($P< 0.05$) for the tolerant cultivar (Zagros). The increase in leaves anthocyanins content in response to drought stress has also been reported by Spyropuolos and Mavrommatis (2005) for three oak species (*Quercus sp.*). One of the functions of anthocyanins is the protection of plants against photoinhibition and photodamage to chloroplasts caused by high light intensity (Merzlyak *et al.,* 2008). Anthocyanins also protect plants against environmental stresses such as UV radiation, high temperatures, drought and nutrient deficiencies (Hughes and Pogson, 2007). In our study, the increase in anthocyanins in Zagros cultivar was higher than that in Pishtaz which is in agreement with the role of this pigment as being more effective in protecting Zagros cultivar against drought stress.

Effects of Osmotic Potential on Proline Accumulation

The effects of drought stress on proline accumulation in the leaves and roots of both

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Table 1. Effects of osmotic potential produced by PEG-6000 on shoot and root fresh weight (g).

		Osmotic potential (MPa)				
Organ	Cultivars	0	0.05	0.1	0.5	0.75
Shoot	Zagros	3.5Ad [a]	3.05Bd	2.60Ca	1.85Dc	1.60Ee
	Pishtaz	2.60Ab	2.01Bc	1.80Cb	1.20Dd	0.90Eb
	Sardary	3.65Ad	3.55Bb	2.95Cc	2.20De	1.40Ed
	Falat	2.60Bb	2.40Aa	2.35Ad	1.60Cb	1.10Dac
	Azar2	3.20ABa	3.10Bb	2.90Ae	1.50Ca	1.05Da
	Koohdasht	3.15Aac	2.50Ba	2.65Cf	2Df	1.40Ed
	Marvdasht	3Bc	2.45Aa	2.45 Ag	1.35Cg	1Dab
	Shiraz	3.20Aa	2.65Ba	2.40Ch	1.55Dab	1.20Ec
Root	Zagros	2Ba	1.70Ac	1.70Ac	1.65Abc	1.25Cc
	Pishtaz	1.65Dd	1.35Cc	1.20Ba	1.05Aa	1.05Ad
	Sardary	1.90Aab	1.75Ba	1.50Cd	1.40Dd	1.20Ebc
	Falat	1.70Ddc	1.40Cbc	1.30Ba	1Aa	1.30Ba
	Azar2	2.20Be	2.0Ae	2Ab	1.60Cb	1.80Be
	Koohdasht	1.95Aa	1.90ABd	2ACb	2.10Ce	0.95Ea
	Marvdasht	1.90CAab	1.45Bb	1.30Ca	1.05Da	1.35Df
	Shiraz	1.80Bcbc	1.75ABa	1.85C	1.70Ac	1.25Cc

[a] Capital and small letters that are different in rows and columns, respectively, have significant difference at α= 0.05 level based on Duncan's analysis. Each number is average of three replications. For example, for shoot fresh weight: Zagros cultivar on the first line has significant difference in all osmotic potentials, so we showed different letters, capital A, B, C, D, E for them. On the first column, at 0 MPa osmotic potential, Zagros cultivar has significant difference with Pishtaz, Falat, Azar2, Koohdasht, Marvdasht and Shiraz cultivars, therefore it was showed this with different small letters, but there is no difference with Sardary, therefore we showed the same small letter for them, b.

Table 2. Effects of osmotic potential produced by PEG-6000 on shoot and root dry weight (g).

		Osmotic potential (MPa)				
Organ	Cultivars	-0	-0.05	-0.1	-0.5	- 0.75
Shoot	Zagros	0.37Cc [a]	0.31Ba	0.28Aac	0.27Ab	0.27Ac
	Pishtaz	0.30Ab	0.24Bb	0.22Cd	0.20Dd	0.18Ee
	Sardary	0.39Cd	0.38BCc	0.361Be	0.27Ab	0.26Ac
	Falat	0.29Cb	0.24Bb	0.22Ad	0.21ACd	0.21Aab
	Azar2	0.33Aa	0.36Bc	0.30Cb	0.23Da	0.21Eab
	Koohdasht	0.34Da	0.30Ca	0.27Bc	0.23Aa	0.22Ab
	Marvdasht	0.33Ba	0.30Aa	0.29Aab	0.22Cac	0.20Da
	Shiraz	0.36Ac	0.32Ba	0.29Cab	0.27Db	0.24Ed
Root	Zagros	0.11Aa	0.11Ab	0.12ABcd	0.11Aa	0.13Ba
	Pishtaz	0.09Aa	0.10Aab	0.1Aab	0.07Bc	0.06Be
	Sardary	0.1ABa	0.09Aa	0.1ABab	0.11Ba	0.13Ba13Ca
	Falat 1.70Ddc	0.09Bb	0.10Aab	0.1Aab	0.09Ab	0.09Ad
	Azar2	0.11Aa	0.13Bc	0.11Abc	0.11Aa	0.12ABabc
	Koohdasht	0.15Aa	0.14ABc	0.13BCd	0.12CDa	0.11Dbc
	Marvdasht	0.1Aa	0.09Aa	0.09Aa	0.09Ab	0.1Acd
	Shiraz	0.12ABa	0.11Ab	0.12ABcd	0.12ABa	0.13Ba

[a] Capital and small letters that are different in rows and columns, respectively have significant difference at α= 0.05 level based on Duncan's analysis. Each number is average of three replications.

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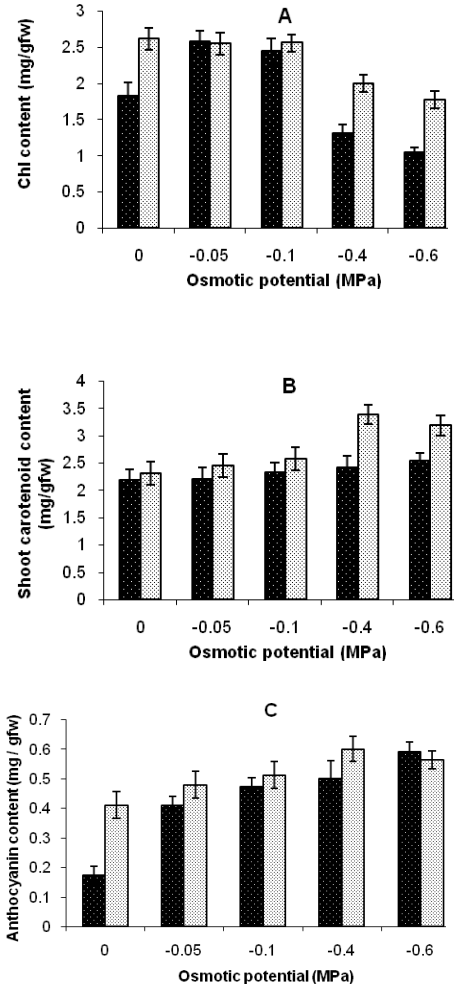

Figure 1. Effects of osmotic potential produced by PEG-6000 on the amount of leaves pigments (A: Total chlorophyll; B: Total carotenoids, C: Anthocyanins). In each graph, the black column shows Zagros cultivar and the white column is Pishtaz cultivar. Each number is the average of three replications.

cultivars are shown in Figure 2. The amounts of leaves and roots proline in both cultivars increased with the decrease in osmotic potential. The increase in proline content in plants grown in solutions with osmotic potentials of -0.1, -0.4 and -0.6 MPa for shoot and -0.4 and -0.6 MPa for root were significant in Zagros cultivar. The increase in proline was significantly higher in Zagros cultivar as compared with Pishtaz

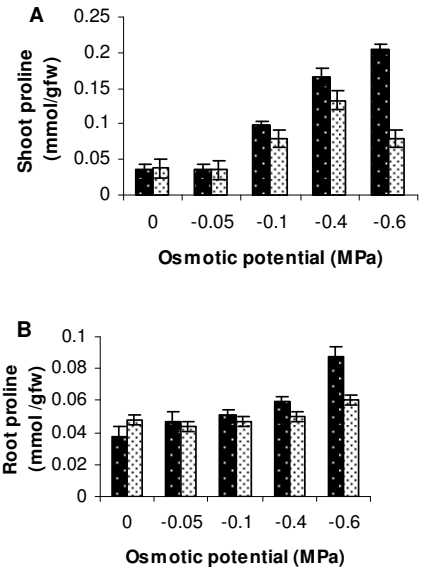

Figure 2. Effects of osmotic potential produced by PEG-6000 on proline content (A: Shoot proline content, B: Root proline content). In each graph, the black column shows Zagros cultivar and the white column is Pishtaz cultivar. Each number is the average of three replications.

cultivar. Also, the increase in proline content was higher in shoots than in roots. Tolerant plants accumulate more proline in their shoots and roots in response to drought stress. Mohammadkhani and Heidari (2008) reported that under drought condition, increase in proline content occurred in tolerant varieties of maize and the increase was higher in shoots than in roots (Mohammadkhani and Heidari, 2008). The multiple roles of proline in protecting plants against abiotic stresses are very well documented. As an osmoticum it is used for osmotic adjustment under salt and drought stresses, protects cell structure and acts as a scavenger of damaging free radicals (Nanjo *et al.,* 1999).

Changes in Soluble Sugars

In both wheat cultivars, the amount of soluble sugars increased with the increase in

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drought stress (Figure 3). In all treatments, the increase in soluble sugars was higher in the tolerant cultivar (Zagros). Osmotic adjustment is considered to be an adaptive characteristic by which an increase in the solute content of cells can lead to the maintenance of turgor and turgor related processes at low water potentials (PasbanEslam, 2011). In most plant species, soluble carbohydrates play a major role as osmoprotectants protecting plants against dehydration stress (Li and Li, 2005).

Effects on Membrane Lipid Peroxidation

Drought stress increased the rate of membrane lipid peroxidation and malondialdehyde (MDA) production in both cultivars (Figure 4). However, the peroxidation rate was higher in Pishtaz (sensitive) cultivar at -0.05, -0.4 and -0.6 MPa. Our results are similar to those reported by Fazeli *et al.* (2005) for two sensitive and tolerant sesame (*Sesamusindicum*) cultivars. Protecting membrane integrity under stress conditions is of prime importance for cells and plants survival. The increase in the rate of reactive oxygen species production under abiotic stress conditions is mainly responsible for membrane lipid peroxidation rendering the cell incapable of carrying out its metabolic activities (Ismail *et al.,* 2005).

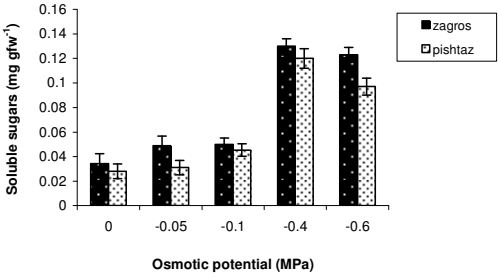

Figure 3. Effects of osmotic potential produced by PEG-6000 on reducing sugars. In each graph, the black column shows Zagros cultivar and the white column is Pishtaz cultivar. Each number is the average of three replications.

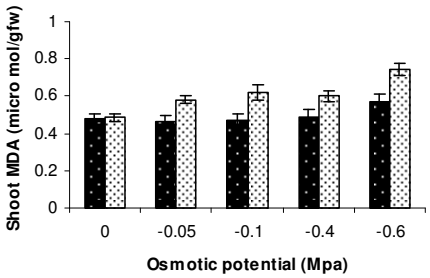

Figure 4. Effects of osmotic potential produced by PEG-6000 on membrane lipid peroxidation. In each graph, the black column shows Zagros cultivars and the white column is Pishtaz cultivar. Each number is the average of three replications.

Effects on the Guaiacol Peroxidase Activity

The activity of guaiacol peroxidase was higher both in the roots and shoots in Zagros cultivar than Pishtaz under drought conditions (Figure 5). Oxidative damages in plants caused by biotic and abiotic stresses are usually reduced by enzymatic antioxidants (superoxid dismutase (SOD), ascorbate peroxidase (APX), glutathionreductase (GR), and catalase (CAT)) and by non-enzymatic antioxidants (β-carotene, tocopherol, ascorbate and glutathione) (Ismail *et al.,* 2005). The ability of antioxidant enzymes to scavenge ROS and reduce their damaging effects may confer drought resistance to plants. Yang *et al*. (2009) exhibited that as compared with 100% field capacity, at 25% field capacity the increased activities of CAT, SOD, POD, APX and GR were 4.3, 103, 172, 208 and 56% in *P. cathayana*, respectively, whereas these increases were 8.1, 125, 326, 276 and 78% in *P. kangdingensis,* respectively.

Effects on the Catalase Activity

Drought stress caused an increase in catalase activity in leaf tissues of both wheat

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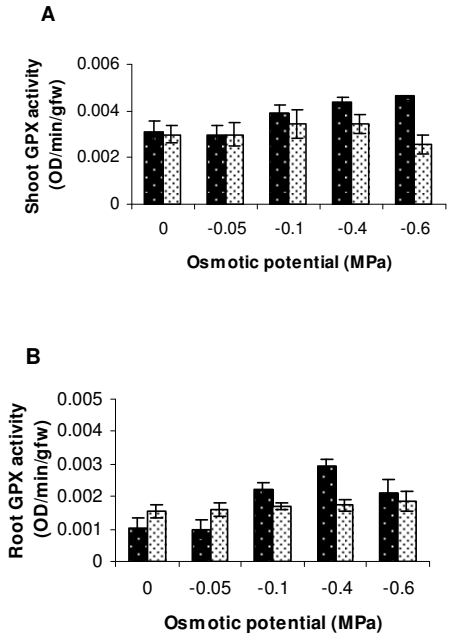

Figure 5. Effects of osmotic potential produced by PEG-6000 on guaiacol peroxidase activity (A: Shoot guaiacol peroxidase activity, B: Root guaiacol peroxidase activity). In each graph, the black column shows Zagros cultivars and the white column is Pishtaz cultivar. Each number is the average of three replications.

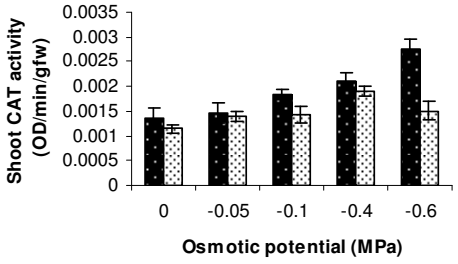

Figure 6. Effects of osmotic potential produced by PEG-6000 on shoot catalase activity. In each graph, the black column shows Zagros cultivar and the white column is Pishtaz cultivar. Each number is the average of three replications.

cultivars (Figure 6). However, the increase was higher in Zagros which is a tolerant cultivar. In fact, at external osmotic potential of -0.6 MPa, catalase activity in Pishtaz started to decrease (Figure 6). Catalase plays a key role in detoxification of H_2O_2 produced specially in chloroplasts under oxidative stresses (Blokhina et al. 2003). Our results agree with those reported by Ismail *et al.* (2005) who found higher catalase activity in drought tolerant bean plants (*Phaseolusacutifolus*) as campared with *Phaseolus vulgaris* which is drought sensitive.

Effects on Protein Profile

The examination of proteins prepared from the leaves of the two cultivars by SDS-PAGE revealed a 175 KD band for the tolerant cultivar exposed to -0.4 MPa external osmotic potential which was absent in control plants. Two other bands (33 and 48 KD) were also present both in the tolerant cultivar at -0.4 MPa and in control plants and were absent in the sensitive cultivar (Figure 7).

Zulkarnain *et al.* (2009) reported that under drought stress, all varieties of Malaysia rice showed a high level of protein expression, as compared to well-watered plants.

CONCLUSIONS

The present study was carried out to demonstrate that genetic variation can cause drought resistance in plants growing in dry and semi-dry regions of Iran and similar countries in the world. Our results revealed that the Zagros wheat cultivar can cope with drought stress by employing strategies such as the production of compatible solutes (reducing sugars, proline) and antioxidant enzymes. This cultivar might be considered as a suitable candidate for cultivation and semi arid regions of this country.

[DOR: 20.1001.1.16807073.2012.14.7.13.5]

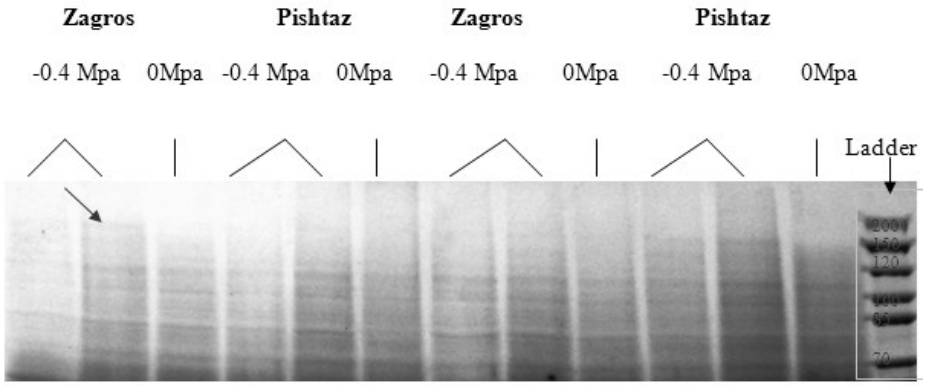

Figure 7-A. Effects of osmotic potential produced by PEG-6000 on protein profile of two wheat cultivars (Zagros and Pishtaz). The red arrow shows a 175KD new band in Zagros cultivar. The protein markers are shown on the right (Ladder) with different molecular weights.

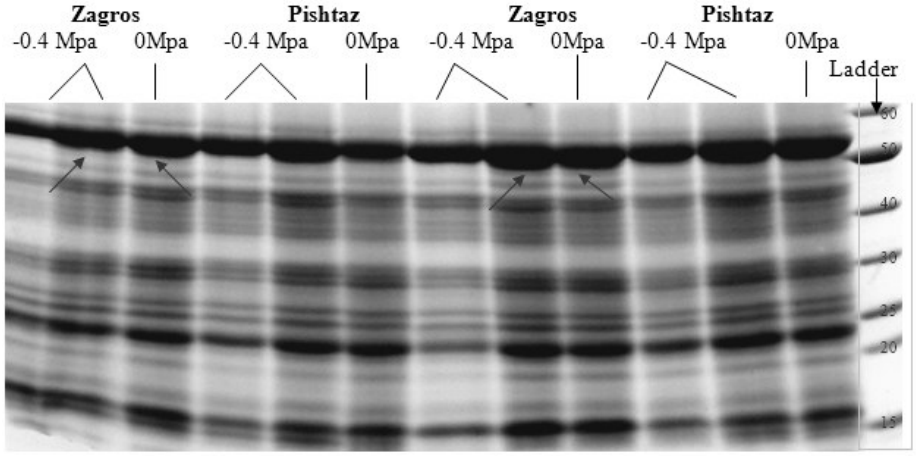

Figure 7-B. Effects of osmotic potential produced by PEG-6000 on protein profile of two wheat cultivars (Zagros and Pishtaz). The red arrow shows a new band in Zagros cultivar.

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پاسخ‌های بیوشیمیایی و فیزیولوژیک دو رقم گندم (*Triticum aestivum* L.) نسبت به تنش خشکی در مرحله گیاهچه‌ای

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چکیده

بر اساس میزان کاهش وزن تر و خشک ساقه‌چه، از میان هشت رقم گندم یک رقم حساس (پیشتاز) و یک رقم مقاوم (زاگرس) انتخاب گردیدند. سپس اثر تنش کم آبی بر میزان کلروفیل، کاروتنوئید، آنتوسیانین، پرولین، قندهای محلول، جذب یون پتاسیم، اکسایش لیپیدهای غشایی، فعالیت آنزیم های گایاکول پراکسیداز و کاتالاز و الگوی پروتئینی در ارقام حساس و مقاوم مورد بررسی قرار گرفت. گیاهچه‌های گندم در محلول غذایی، تحت رژیم نوری مناسب و در دمای آزمایشگاه رشد داده شدند.

با کاهش پتانسیل اسمزی میزان کلروفیل در رقم مقاوم ابتدا افزایش نشان داد ولی با کاهش بیشتر پتانسیل اسمزی میزان کلروفیل در هر دو رقم کاهش یافت که میزان کاهش در رقم مقاوم بیشتر بود. با کاهش پتانسیل اسمزی مقادیر کاروتنوئید و آنتوسیانین تنها در بعضی از ارقام و پتانسیل‌های اسمزی افزایش معنی‌دار نشان دادند. قندهای محلول، پرولین و فعالیت آنزیم‌های آنتی اکسیدان افزایش معنی-داری نسبت به گروه شاهد نشان دادند که میزان افزایش در تمام موارد فوق در رقم مقاوم بیشتر از رقم حساس بوده است. میزان اکسایش لیپیدهای غشایی نیز با افزایش تنش کم آبی در هر دو رقم افزایش نشان داد که این افزایش در رقم پیشتاز بیشتر از زاگرس بوده است. با کاهش در پتانسیل اسمزی فعالیت آنزیم‌های آنتی اکسیدان در هر دو رقم افزایش یافت. الگوی پروتئینی دو رقم زاگرس و پیشتاز مشخص می‌کند که در رقم زاگرس تحت شرایط تنش باندهای پروتئینی جدیدی ایجاد می شود. مطالعات فوق نشان می دهند که میزان پرولین و قندهای محلول و فعالیت برخی از آنزیم‌های آنتی اکسیدان نظیر کاتالاز می‌توانند جزئی از سیستم ایجاد مقاومت به تنش خشکی در گیاه گندم باشند. بنابراین با تغییر در فعالیت ژن‌های مسئول در ایجاد پرولین و آنزیم‌ها و مواد آنتی اکسیدان می‌توان به افزایش مقاومت به خشکی در گیاه گندم اقدام نمود.

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