

Antioxidant enzyme responses and crop yield of wheat under drought stress and re-watering at vegetative growth period

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Abstract

Drought induced stress is one of the most significant environmental challenges. This study was designed to evaluate the effects of drought stress on crop yield and antioxidant systems during the vegetative period of wheat. The study was carried out in a greenhouse using factorial experiment based on complete randomized block design (RCBD) in three replications in Razi University in Iran from 2011 to 2012. In the pot experiment, water stress was applied at vegetative growth stage (soil moisture 50 ± 5% of field capacity from the stemming to flowering periods) and various bread wheat genotypes ('Pishtaz', 'DN-11', 'Sivand', and 'Marvdasht') were examined as the second factor. Results showed that drought stress at vegetative growth stage considerably decreased plant height and crop yield. Under drought stress, the lowest and highest reductions in crop yield were noted seen in 'Marvdasht' and 'DN-11', respectively. The occurrence of drought stress at the vegetative growth stage in the experiment significantly reduced soluble protein content and membrane stability index. While drought stress considerably increased superoxide dismutase (SOD), it had no effect on the catalase (CAT) and peroxidase (POD). The studied parameters suggested that drought resistance of 'Pishtaz' and 'Marvdasht' might be due to the enhanced activity of antioxidant enzymes and low lipid peroxidation. Finally, antioxidant enzyme responses were found to provide a beneficial tool for depicting drought tolerance in different wheat genotypes in arid and semiarid regions.

Keywords: Wheat; oxidative stress; crop yield; reactive oxygen species; antioxidant defense system

Abbreviations: ROS: Reactive oxygen species; POD: Peroxidase; CAT: Catalase; SOD: Superoxide dismutase; APX: Ascorbate peroxidase; PPO: Polyphenol oxidase; GR: Glutathione reductase; MDA: Malondialdehyde; MP: Membrane permeability; O_2^- : Superoxide anion; HO⁻: Hydroxyl radical; 1O_2 : Singlet oxygen; NBT: Nitroblue tetrazolium; OC: Organic Carbon; MSI: Membrane stability index; YSI: Yield stability index

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in world, particularly

*Corresponding author *E-mail address: msaeidi667@gmail.com* Received: June, 2017 Accepted: September, 2017 Iran, which plays a special role in people's nutrition. It is also the world's most widely adapted crop, supplying one-third of the globe population with more than half of their calories and nearly half of their protein (Rajaram, 2001).

But, unfortunately abiotic stresses such as drought decrease wheat growth and development and limit plant production. The reactions of the plants to drought stress differ significantly at various organizational levels depending on intensity and duration of stress, as well as plant species and its stage of development (Chaves et al., 2003). Drought stress adversely affects a variety of vital biochemical and physiological processes in plant.

the One of biochemical changes encountered in plants subjected to drought stress is the production of reactive oxygen species (ROS). Drought leads to oxidative stress within the plant cell due to higher leakage of electrons towards O₂ during photosynthetic and respiratory processes leading to enhancement in ROS generation (Sánchez-Rodríguez et al., 2012). ROS such as superoxide anion (O_2^{-}) , hydrogen peroxide (H_2O_2) , hydroxyl radical (HO⁻) and singlet oxygen (¹O₂), are highly reactive and when the plant capacity for scavenging of these molecules is less than ROS production rate, they can seriously disrupt normal metabolism through oxidative damages on lipids, proteins, and nucleic acids (Cruz de Carvalho, 2008; Esfandiari et al., 2011; Kabiri and Naghizadeh, 2015). Also, according to Ahmad et al. (2011) who studied the role of oxygen radicals in different plants exposed to drought, water deficit or drought stress causes an overall inhibition of protein synthesis, inactivation of several photo-inhibition chloroplast enzymes, of photosynthetic apparatus, impairment of electron transport, increased membrane permeability, etc.

Plants produce a number of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), polyphenol oxidase (PPO), and glutathione reductase (GR) that protect their cells from the potential cytotoxic effects under stressful environments (Edreva, 2005; Liu et al., 2014). The enzymatic antioxidant system is one of the protective mechanisms against ROS. Under drought stress, SOD can catalyze the conversion of toxic O_2 to H_2O_2 , which is further decreased to O_2 and H₂O by POD and CAT (Scandalios, 1993). The relation between drought stress and enzymatic antioxidant systems has been studied in different plant species such as bread and durum wheat (Ahmad et al., 2011; Esfandiari et al., 2011). These reports provide substantial information about the

protective mechanisms in the crops that prevent oxidative injury under drought stress conditions. High activities of antioxidant enzymes were also shown to improve drought tolerance of genotypes of tea (Upadhyaya et al., 2008), olive (Ben Ahmed et al., 2009), and woody plant species (Liu et al., 2011). However, the responses of the antioxidant defense system in plant species to drought stress are still relatively unknown. Sairam et al. (2000) found differences in the level of activities of various antioxidants among tolerant genotypes of wheat so that one tolerant genotype had very high levels of ascorbic acid and APX, whereas another tolerant genotype exhibited higher SOD and CAT intermediate ascorbic acid and activities. Moreover, Shao et al. (2005) observed variation in POD activity in wheat genotypes under water deficit at maturation period and suggested that water stress tolerance was closely associated with POD activities. In addition, Naderi et al. (2014) reported that among the antioxidant enzymes, APX activity increased most drastically in severe stress condition. These findings indicate the importance of research about drought stress tolerance mechanisms especially ROS scavenging systems in wheat.

The aims of this study were (a) to investigate the recovery capacities of wheat after a drought stress, (b) to select the ideal genotype based on higher crop yield and better growth under stress conditions, and (c) to study the impact of drought and re-watering stress on wheat antioxidant systems and understand the role of antioxidant systems in drought tolerance.

Materials and Methods

Experimental procedure and design

The pot experiment was conducted during the growing season from 2011 to 2012 in the greenhouse of Campus of Agricultural and Natural Resource, Razi University at Kermanshah in the west of Iran (47°, 9'/E; 34°, 21'/ N), with 1319 m elevation from sea level. The climate based on Domarten classification is semi-arid cold in the test area. The experiment was laid out in a randomized complete block design (RCBD) in a factorial arrangement with three replications. It comprised of four bread wheat genotypes i.e.,

'Pishtaz', 'Sivand', 'Marvdasht', and 'DN-11' and two drought stress treatments i.e., control or well water (irrigation at 95 ± 5% of field capacity in all stages of plant growth normally), and drought stress at vegetative growth stage (soil moisture around 50 ± 5% of field capacity from the stemming to flowering periods - 31 to 59 of the Zadok's scale, 1974). These four wheat genotypes were chosen because they have the highest area under cultivation in Kermanshah province and genotypes with unknown they are new biochemical and physiological characteristics. Some growing characteristics of bread wheat genotypes used in the experiment are shown in Table 1. The seeds of wheat genotypes were obtained from Agricultural and Natural Resources Research Center of Kermanshah, Iran. Seeds were sown in plastic pots (PVC) with a diameter of 20 cm and height of 30 cm, filled with 2.5 kg fertilized peat and soil (1:4). Some of the physical and chemical properties of the soil are shown in Table 2. Ten seeds per pot were sown at distances and depth and one week after their emergence, the number of the seedlings was reduced to five per pot. It was collected from the top 0-30 cm layer.

Sampling

Twenty-five uppermost leaves of 5 plants per pot (to means 5 leaves per plant) were harvested at 10, 20 and 40 days after drought

Table 1

Characteristics o	f genotypes use	ed in the	experiment
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enzymes assay, as well as other fresh leaves for measurement of membrane stability index.

Enzyme extraction

For SOD, CAT, and POD extraction, leaf samples (0.5 g) were homogenized in 10 mL ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4 °C in Beckman refrigerated centrifuge for 15 min at 15000 g. The supernatant was used for enzyme activity assay.

Antioxidant enzymes assay

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by recording the decrease in absorbance of superoxide nitro-blue tetrazolium complex by the enzyme (Sen Gupta et al., 1993). About 3 mL of reaction mixture containing 0.1 mL of 200 mM methionine, 0.01 mL of 2.25 mM nitroblue tetrazolium (NBT), 0.1 mL of 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer, 1 mL distilled water, and 0.05 mL of enzyme extraction were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 μ M) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped

Genotypes Grain yield		Physiological maturity	Plant height	Grain weight		
Pishtaz	High	Late maturing	Tall	High		
Sivand	High	Late maturing	Tall	High		
Marvdasht	Medium	Late maturing	Tall	Low		
DN-11	High	Early maturing	Medium	Medium	Medium	
Adapted from Abdo	li and Saeidi (2012)					

. Table 2

Physical and chemical properties of the soil used in the experiment

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Physical Property		Chemical Property (Saturation extract)		
Sand (%)	17	K (mg kg ⁻¹)	329	
Silt (%)	39	Available P for plant (mg kg ⁻¹)	8.0	
Clay (%)	44	N (%)	0.098	
Field capacity (0.033 MPa, cm ³ cm ⁻³)	0.35	рН	7.41	
Bulk density (g cm ⁻³)	1.23	OC (g kg ⁻¹)	1.14	

K: Potassium, P: Phosphorus, N: Nitrogen, pH: level of acidity, OC: Organic Carbon

stress at vegetative growth stage. Samples were frozen in liquid nitrogen for 10 min and stored at - 80 °C for soluble protein content and antioxidant

by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal color. A non-irradiated complete reaction

	Specific activity of enzymes		Soluble				
Treatments	CAT	POD	SOD	protein	Membrane stability index	Plant	Crop yield
	(Units / m	(Units / mg Protein . min)		content (mg/g fw)	(%)	height (cm)	(g/plant)
Irrigation levels							
Well water	182 a	667 a	27.0 b	55.0 a	55.5 a	57.7 a	3.76 a
Drought stress	184 a	639 a	34.0 a	44.0 b	34.7 b	44.7 b	1.72 b
Changes (%)	+1	-6	+26	-20	-38	-22	-54
Genotypes							
Pishtaz	183 ab	625 a	41.2 a	47.6 a	44.5 a	52.7 a	2.86 a
DN-11	192 a	704 a	25.1 b	51.6 a	42.5 a	50.0 a	2.52 ab
Sivand	195 a	692 a	27.1 b	50.6 a	47.0 a	43.4 b	2.36 b
Marvdasht	164 b	547 b	29.7 b	48.4 a	45.2 a	51.2 a	2.56 ab
Times sampling after dr	rought stress						
10	209 a	908 a	45.6 a	50.3 a	-	-	-
20	214 a	725 b	20.7 c	41.1 b	-	-	-
40	127 b	341 c	26.0 b	57.2 a	-	-	-
CV (%)	17.0	20.5	28.6	24.9	19.6	7.76	12.6

Table 3

Mean comparison of the effect of irrigation levels, genotypes, and time of sampling after drought stress on some biochemical and physiological traits and crop yield

Means followed by the same letters in each column are not significantly different at 5% level, according to Duncan's Multiple Range test.

mixture which did not develop color served as blank. Absorbance was recorded spectrophotometrically at 560 nm by Elisa (PowerWave XS, BioTek, USA) and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

Catalase (CAT, EC 1.11.1.6) activity in the leaves was measured according to Sinha (1972). This method uses H2O2 as the substrate. The reaction mixture of 1.5 mL consisted of 1 mL phosphate buffer (0.01 M, pH 7.0), 0.4 mL distilled water, and 0.1 mL of centrifugation supernatant. Reaction was started by adding 0.5 mL H2O2 (320 mM), incubated at 25 °C for different time intervals and the reaction was stopped by the addition of 2 mL of dichromate: acetic acid reagent (1:3 ratio). The tubes were immediately placed and kept in a boiling water bath for 20 min and were then centrifuged for 15 min (1500 g). The green color developed during the reaction was read at 570 nm in a spectrophotometer set. Control tubes, devoid of enzyme, were also processed in parallel. The enzyme activity is expressed as nmol H2O2 consumed min-1 mg-1 protein.

Peroxidase (POD, EC 1.11.1.7) activity was based on the method described by Chance and Maehly (1955). The reaction mixture contained 3,3'-diaminobenzidine-tetra hydrochloride dehydrate solution containing 0.1% (w/v) gelatin, 150 mM Na-phosphate-citrate buffer (pH 4.4), and 0.6% H2O2. The increase of absorbance was followed for 5 min at 465 nm by a spectrophotometer Elisa (PowerWave XS, BioTek, USA). A unit of POD activity was defined as μ M H₂O₂ decomposed ml⁻¹ min⁻¹ mg⁻¹ protein.

Soluble protein content measurement

The contents of soluble protein were assayed as described by Bradford (1976); bovine serum albumin (BSA, Sigma chemical) was used as the standard. To determine total soluble proteins, 50 mg of leaf fresh matter was incubated in 5 mL of extraction buffer including Tris-HCL at 25 mM (pH 7.6). The mixture was centrifuged at 2000 g for 15 min. Finally, total soluble proteins were read spectrophotometrically at 595 nm by Elisa (PowerWave XS, BioTek, USA).

Membrane stability index measurement

At flowering stage, membrane stability index (MSI) was determined by recording the

electrical conductivity of leaf ions leaching in double distilled water (Sairam et al., 2002). Leaf samples (0.1 g) were taken in test tubes containing 10 mL of double distilled water in two sets. One set was kept at 40 °C for 30 min and another set at 100 °C in boiling water bath for 15 min and their respective electrical conductivities, EC₁, and EC₂, were measured by a pH-EC meter (Hanna, HI 2004, Hanna Instruments, Inc. Padova, Italy). Then, leaf MSI was calculated by the following formula:

MSI (%) = $[1 - (EC_1 / EC_2)] \times 100$

Crop yield and yield stability index measurements

For measuring crop yield and plant height, 10 plants were harvested at physiological maturity stage from each treatment (2 pots). Yield stability index (YSI) was used to differentiate the resistant and susceptible genotypes and was calculated using the formula suggested by Bouslama and Schapaugh (1984) as:

 $YSI = (Y_s / Y_p) \times 100$

where Y_s and Y_p representd yield under drought stress and non-stress conditions, respectively.

Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) using Statistical Analysis System Software (version 9.1, SAS Institute Inc.). Differences were considered statistically significant when P<0.05. The figures were drawn using Excel software (version 10.0).

Results

Antioxidant enzymes activities

Results showed that genotypes significantly differed for antioxidant enzymes activity (Table 3). So that 'DN-11' and 'Sivand' possessed the highest and 'Marvdasht' had the lowest catalase (CAT) and peroxidase (POD) enzymes activities under both conditions. However, 'Pishtaz' possessed the highest and the other genotypes had the lowest superoxide



Fig. 1. Influence of drought stress at vegetative growth stage on POD: Peroxidase (A), SOD: Superoxide dismutase (B) enzymes activity and crop yield (C) of different wheat genotypes; means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's Multiple Range test. Vertical bars represent \pm SE.

dismutase (SOD) enzyme activities under both conditions (Table 3).

Our findings indicated significantly higher activity levels of SOD in water-stressed plants than in well-watered plants (26% increased to control) whereas the CAT and POD did not change with drought stress (Table 3). This suggests that drought stress activated an antioxidant enzyme defense system in wheat leaves in order to alleviate oxidative damage under drought conditions.

In the current study, genotype and its interaction with drought stress significantly affected POD and SOD enzymes activity (Figs. 1A, B). In this case, various genotypes had different reactions so that under drought stress, the highest increase in POD enzyme activity was seen in 'Pishtaz'. But, decrease of POD enzyme activity in the other genotypes under drought stress condition (Fig. I, A) Showed, as well as, under drought stress, the lowest and highest increases in SOD enzyme activities were seen in 'DN-11' and 'Pishtaz', 23.1 and 67.2%, respectively. However, decrease of SOD enzyme activity in 'Marvdasht' genotype under drought stresswas shown (Fig. 1B). Generally, on the basis of antioxidant activity and crop yield, 'Pishtaz' genotype appears to be better adapted to drought stress (Figs. I, A, B, and C).

With increasing exposure time to drought stress, activities of almost all antioxidant enzymes including POD and CAT declined (Figs. II, A and B) so that the re-watering did not increase antioxidant enzyme activity (Fig. II). But, on day 10, CAT and POD activities under drought stress were significantly higher by approximately 24.9% and 18.0% more than under well-irrigation, respectively (Figs. II, A, B). Furthermore, the activity of SOD in the leaves of wheat remained almost unchanged with increasing drought time (Fig. II, C).

In this study, the interaction between wheat genotypes and time of sampling after drought stress significantly affected CAT, POD, and SOD enzymes activity (Figs. 3). In different bread wheat genotypes antioxidant enzyme activities declined significantly (P<0.01) in later stages after drought stress e.g., for SOD 20 days after stress, for POD 40 days after stress, and for CAT during the 40 days after stress (Figs. II, A, B, C).



Fig. II. Changes in CAT: Catalase (A), POD: Peroxidase (B) and SOD: Superoxide dismutase (C) enzymes activity in well-watered and drought stress at the vegetative growth stage; Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's Multiple Range test. Arrows indicate the rewatering in the drought stress.

Furthermore, the highest CAT and POD enzymes activities were seen 10 and 20 days after drought stress of 'Sivand' and 'DN-11' genotypes, respectively while the lowest activities of these enzymes were recorded 40 days after drought stress (re-watering) in 'Pishtaz' genotype (Figs. III, A, B). Generally, re-watering after 40 days caused an increase in POD enzyme activities in all



Fig. III. Influence of times sampling after drought stress at vegetative growth stage on CAT: Catalase (A), POD: Peroxidase (B) and SOD: Superoxide dismutase (C) enzymes activity of different wheat genotypes. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's Multiple Range test. Arrows indicate the re-watering in the drought stress.

genotypes except for 'Pishtaz' genotype (Fig. III, C). This suggests the genetic diversity among wheat genotypes.



Fig. IV. Yield stability index in wheat genotypes at the physiological maturity stage; Vertical bars represent \pm SE.

Soluble protein content and membrane stability index

According to Table 3, water stress caused a significant increase soluble protein content and membrane stability index (MSI) in leaves. A reduction in the soluble protein content and MSI occurred during drought stress so that drought stress at the vegetative growth stage caused 20% and 38% reduction in soluble protein contents and MSI on the average, respectively (Table 3). In the current study, genotype and its interaction with drought stress did not have any significant effect on the soluble protein content and MSI (data not shown).

Plant height and crop yield

Results obtained from mean comparison analysis of plant height and crop yield is shown in Table 3. The drought stress significantly decreased plant height in four genotypes (P< 0.05). Drought stress at the vegetative growth stage caused 54% and 22% reduction in crop yield and plant height (Table 3). In this study, 'Sivand' (2.36 g/plant and 43.4 cm) had the lowest and 'Pishtaz' (2.86 g/plant and 52.7 cm) had the highest crop yield and plant height, respectively (Table 3). Under drought stress, the lowest and highest reductions in crop yield were observed in 'Marvdasht' and 'DN-11', respectively. Marvdast and 'Pishtaz' genotypes with lower crop yield loss had higher resistant to drought stress applied during stem elongation stage than the other genotypes (Fig. I, C). Also, evaluation of genotypes using yield stability index (YSI) allowed selecting susceptible and tolerant genotypes regardless of their yield potential. Based on the results, 'Marvdasht' and 'Pishtaz' had the highest YSI and can consequently be considered as tolerant to drought stress (Fig. IV).

Discussion

Antioxidant capacity evaluation is an important indicator in plant physiology. In the present study, applying drought stress increased SOD enzyme activity, but it did not have any significant effects on CAT and POD enzymes activity (Table 3). The relationship among drought stress and enzymatic antioxidant systems has been studied in some crop species such as wheat (Naderi et al., 2014) and maize (Kolarovic et al., 2009). It has been reported that antioxidant enzymes activities help in the resistance of plants against desiccation or drought. Earlier studies have shown the association of increased antioxidant enzymes under drought stress with the growth of plants and crop production. For example, Shao et al. (2005) observed variation in POD activity in wheat genotypes under soil water deficits at maturation stage and suggested that water stress tolerance was closely associated with POD activities. Similar to our findings, Habibi (2013) and Esfandiari et al., (2011) reported that the activity of antioxidant enzymes was influenced by salinity and drought stress significantly so that drought stress caused a significant increase in SOD, POD, CAT, and APX activities compared with control plants. However, reports on CAT activity under stress condition are heterogeneous. CAT activity has been shown to increase in maize (Kolarovic et al., 2009) and also to remain unchanged or even decrease under drought stress in sunflower (Zhang and Kirkham, 1992). Then, one reason for the unchanged SOD activity in the present study may be that the stomata were open during drought stress, lowering the level of ROS formation. Terzi and Kadioglu (2006) found no significant changes in SOD activity in plant exposed to drought stress.

Results showed a considerable variations among genotypes for antioxidant enzymes activity when grown under drought stress and non-stress conditions (Figs. I and II). In line with our results, Kumar Patel et al. (2011) also reported that drought tolerance genotypes had higher RWC, proline accumulation, enzymatic activities such as SOD, APX, CAT, and POD and lower level of malondialdehyde (MDA), membrane permeability (MP), and H_2O_2 in comparison to drought susceptible genotypes. Under drought stress, the activities of SOD, CAT, and APX enhanced to a greater extent, resulting in lower levels of plasma membrane degradation and electrolyte leakage, in a drought-tolerant clone compared with a drought-sensitive plant (Lima et al., 2002). Moreover, Terzi and Kadioglu (2006) stated that the tolerance of drought stresses apparently is closely associated with the antioxidant enzyme system as well as leaf rolling.

According to our results, drought stress had negative effects on soluble protein content and membrane stability (Table 3). Likely, a reduction in plasma membrane stability reflects the extent of lipid peroxidation caused by ROS (Esfandiari et al., 2011). Our results are in agreement with Sanjeeta et al. (2014) who reported that drought stress resulted in 24% and 37% decrease in leaf soluble protein content on fresh weight and dry weight basis, respectively.

Moreover, Kabiri and Naghizadeh (2015) reported that water stress increased the electrolyte leakage from leaf cells of barley by 55%. The content of soluble proteins in roots and leaves of maize and chickpea have been reported to decrease with increased drought stress (Tida et al., 2006; Mafakheri et al., 2011). The decreased protein content in plants under water stress may reflect the inhibition of protein synthesis, enhanced degradation, or the inhibition of amino acid incorporation into proteins, resulting in the accumulation of free amino acids.

Water deficit is one of the most significant environmental factors that inhibits the regular plant growth and enlargement and limits plant production. Results showed that water stress decreased plant height and crop yield (Table 3).

Reduction in plant height under drought stress is probably because of reduction in internodes length. Cell division (meristem activity), enlargement, and differentiation are affected by water stress. These observations are in agreement with the findings of various researchers (Habibi, 2013; Saeidi and Abdoli, 2015). In addition, Mirzaei et al. (2011) reported that drought stress at all growth stages induced reduction in yield and morphological traits. Drought stress at stages of stem elongation, flowering, and grain filling induced 32, 32, and 35% reduction in grain yield, respectively. Generally, on the basis of antioxidant activity and crop yield, genotype 'Pishtaz' appears to be better adapted to drought stress tolerance.

Conclusions

In summary, results showed that genotypes respond differently to oxidative damage as a result of variations in their antioxidant defense systems. Also, drought stress during the vegetative stage decreased crop yield, plant height, membrane stability index, and soluble protein content. According to the results, the better upregulation of the protective mechanism in 'Pishtaz' and 'Marvdasht' probably induced higher drought resistance. It is suggested that the cellular protection enzymes such as superoxide dismutase, peroxidase activity, and catalase in leaves play significant physiological roles in the primary growth stage under water stress.

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