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Analysis of antioxidant enzyme activity during reproductive stages of barley under drought stress

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Abstract
Drought is especially considered as key stress factor with high potential impact on crop yield. Plants mainly adapt to water deficits by alteration in physiological and biochemical processes. A simulation experiment on the responses of barley (*Hordeum Vulgare* L.) from heading stage to ripening stage for different soil water levels (full water supply, light water stress, and severe water stress) was conducted to determine the effects on leaf water status, levels of chlorophyll and protein, lipid peroxidation and antioxidant enzymes activity. The results indicated that drought stress relied on drought intensity and developmental stage, with more severe drought stress creating more serious effects on barley. Relative water content (RWC) significantly decreased ($P < 0.05$) under drought stress in all stages. The content of soluble protein and chlorophyll decreased and membrane lipid peroxidation (measured as malondialdehyde content) increased significantly according to the severity of water stress and reproductive stage. Under water stress, the activities of antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (POX) and superoxide dismutase (SOD) in leaves increased sharply in flowering and milking stages, but then declined towards the lately ripening stage. Furthermore, compared with well watered conditions, changes in the activities of POX and SOD were different between light water stress and severe water stress at flowering and milking stages. However, the increases in the levels of malondialdehyde (MDA) during flowering and milking stages showed that the increased activities of antioxidant enzymes may not be enough to prevent the peroxidation of lipid membranes and to scavenge reactive oxygen species (ROS) under drought stress.

Keywords: *Hordeum Vulgare*, Drought, Reproductive stages, Antioxidant enzymes, Lipid peroxidation

INTRODUCTION

Environmental stresses are the most limiting factors for agricultural productivity. Nowadays a big concern is the water deficit leading to drought, which is one of the most limiting factors for better plant performance and the higher crop yield. Under such conditions, resistance to abiotic stresses is becoming one of the most desired traits of crops. Breeding of plant species manifesting drought tolerance is considered to be an economic and efficient means of alleviating agricultural problems in dry areas. The improved plant cultivars to use in these areas must have some adaptive features, which confer drought tolerance and allow the utilization of limited water [1].

Various tolerance mechanisms have been suggested on the basis of the biochemical and physiological changes related to drought. Water deficit may increase the formation of free radicals of oxygen. These reactive oxygen species (ROS) involve superoxide (O_2•), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), and single oxygen ($1O_2$). ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage, particularly when plants are exposed to stress conditions [2]. In order to protect cell membranes and organelles from ROS damaging effects, plants are equipped with an antioxidant system. This system consists of low molecular weight antioxidants, such as ascorbate, glutathione, α_tocopherol, and carotenoids, as well as several enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and glutathione reductase (GR) [3].

Drought can cause an oxidative stress in higher plants through breaking the balance between the production of reactive oxygen species (ROS) and the antioxidant defense [4]. In environmental stresses conditions such as drought, high activities of antioxidant enzymes are important for plants to tolerate stresses [5]. It has been proved that efficient antioxidative characteristics can provide better protection against oxidative stress in leaves under drought stress [6]. A number of studies have shown that soluble sugar, proline accumulation and efficient antioxidative characteristics could enhance the tolerance to drought stress in several plant species, such as sugar beet [7], rice [8], and wheat [9].

Nevertheless, most of the investigations in pot trials were limited to spatial growth of the root conditions, making it difficult to apply the conclusions to an agroecosystem under field conditions. In addition, most experiments applied the stress to seedling stages of plant development. Hence, additional information on understanding possible responses and the physiological mechanisms of barley at reproductive stage to changes of field soil water levels under increasing drought stress conditions is needed.

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The objective of this work was to examine the effects of different soil water levels on leaf water status, contents of chlorophyll and protein and lipid peroxidation as well as change of antioxidant enzyme SOD, APX, POX and CAT activities in the leaves of barley at different reproductive stage under prolonged drought stress and natural field conditions.

MATERIALS AND METHODS

Plants and water-stress treatments

The study was performed at the research farm of Ahar Branch, Islamic Azad University (Ahar, Iran). *Hordeum vulgare* L. cv. Valfajr provided by the seed and plant improvement institute, Karaj, Iran, was used in this study. The experimental scheme was carried out using six-month-old plant planted in October 2009, spaced at 25 cm in the row with 25 cm between rows and fertilized soil with 100 kg/ha biotic fertilizer.

Plants were divided in three treatments: full water supply (F), light water stress (L) and severe water stress (S). F plants were maintained in an optimal soil water condition (the soil water potential was controlled at −0.1 MPa) during the whole experimental period, whereas L and S treatments subjected to a water shortage period starting from April 20 to June 9, 2010 (the soils water potential were controlled at −0.6 MPa and −1.2 MPa for L and S treatments respectively). Containers of L and S were covered with plastic shelter in order to avoid rainfall infiltrations and evaporation from the soil surface.

Each treatment was replicated three times and the experiment was carried out as a complete randomized block design. Leaf samples were collected at the heading, flowering, milking and ripening stages. Fresh leaf samples were used for determinations of RWC, chlorophyll and MDA contents, whereas for assay of protein and enzymes, leaves were covered with an aluminium foil and put in a plastic envelope, and then were frozen in liquid nitrogen immediately and stored at -80 °C until enzyme assays.

Relative water content (RWC)

Leaf samples which were collected of treatments were used for RWC assay. Relative water content was determined by drying the leaves at 80 °C for 48 h and calculated using the following formula: [(fresh weight – dry weight) / (saturated weight – dry weight)] × 100

MDA measurements

The level of lipid peroxidation was expressed as MDA content and was determined as 2-thiobarbituric acid (TBA) reactive metabolites. Plant fresh tissues (0.2 g) were homogenized extracted in 10 ml of 0.25% TBA made in 10% trichloroacetic acid (TCA). Extract was heated at 95 °C for 30 min and then quickly cooled on ice. After centrifugation at 10000 × g for 10 min, the absorbance of the supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was expressed as μmol g^{-1} fresh weight by using an extinction coefficient of 155 mM cm^{-1}.

Extraction and assay of total chlorophyll

Fresh leaf (1 g) was immediately ground and mixed with 80% acetone and kept at -20 °C for 1 h, and then absorbance were recorded at 645 and 663 nm, respectively, with a spectrophotometer (LABOMED UVD-3200).

Protein extraction and determination

Frozen leaf samples (1 g of fresh mass) were ground to a fine powder in liquid nitrogen and extracted with extraction buffer containing 50 mM potassium phosphate (pH 7.5) and 1 mM Sodium Metabisulfite. The extracts were centrifuged at 4 °C for 20 min at 15,000 rpm and the resulting supernatants used as crude extracts. The protein concentration in leaf crude extracts was determined using bovine serum albumin as standard.

CAT activity assays

The enzyme extract (20 μl) was added to reaction mixture containing 750 μl of 70 mM hydrogen peroxide (H_2O_2) and 750 μl of 100 mM phosphate buffer (pH 7.0) adjusted to 3 ml with sterile distilled water. The absorbance was read at 240 nm [10].

POX activity assays

Peroxidase (POX) activity was determined specifically with guaiacol at 470 nm following the method of [11]. The enzyme extract (20 μl) was added to the reaction mixture containing 750 μl of 10 mM guaiacol solution, 750 μl of 70 mM hydrogen peroxide (H_2O_2) solution and 1500 μl of 100 mM potassium phosphate buffer solution (pH 7.0).

APX activity assays

Ascorbate peroxidase (APX) was spectrophotometrically assayed following a decrease in the absorbance at 290 nm [12]. The assay mixture contained: 750 μl of 5mM ascorbate, 750 μl of 2 mM H_2O_2 and 1500 μl of 100 mM phosphate buffer (pH 7.0) with 30 μl of the enzyme extract.

SOD activity assays

Superoxide dismutase (SOD) activity was determined by following the photoreduction of Nitrotetrazolium Blue Chloride (NBT). The reaction mixture contained: 100mM phosphate buffer (pH 7.0), 0.1mM EDTA, 13mM methionine, 75 μM Nitrotetrazolium Blue Chloride, 2mM riboflavin and appropriate amounts of the supernatant. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under 15W fluorescent lamp. The reaction was terminated after 5 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. Reaction products were measured at 560 nm.

Statistical analysis

All data obtained was analyzed by One-Way ANOVA and the mean differences were compared by Duncan's multiple range tests by using SPSS program. Each data point was the mean of 9 replicates (3 plants and three measurements per plant). Comparisons with $P < 0.05$ were considered significantly different. In all the tables and figures the spread of values is shown as error bars representing standard errors of the means.

RESULTS

Relative water content

To understand how water status of barley plants were affected by water stressed treatment we monitored relative water content (RWC) of the leaves in well-water (F) and water stressed (L, S) treatments (Table 1). At the heading, flowering, milking and ripening stages, the MDA content for both the L and S was significantly greater than the S control. Furthermore, between L and S treatments, significant difference in MDA content of leaves was observed.

Table 1. Effects of different water deficit stress on the water status (RWC %) in leaves at different barley reproductive stages. Results are the means ± standard errors (n = 9). Means followed by the same letter within a column are not significantly different at P < 0.05 level

Treatments [a]	RWC (%) [b]			
	Heading	Flowering	Milking	Ripening
F	86.53±0.518 a	84.50±0.491 a	80.33±0.689 a	71.40±0.658 a
L	61.70±0.656 b	58.27±0.811 b	55.30±0.675 b	48.70±0.645 b
S	36.20±0.518 c	34.03±0.446 c	30.03±0.506 c	21.53±0.554 c

[a] F is full water supply, control; L is light water stress; and S is severe water stress.
[b] RWC is relative water content.

Chlorophyll and protein contents

The changes in chlorophyll contents during water shortages are shown in Table 2. It was declined for S treatment at the flowering, milking and ripening stages but for L treatment at the milking and ripening stages. At the ripening stage, Compared to F treatment, chlorophyll content decreased significantly by 30 and 58% in L and S treatments respectively.

As shown in Table 3, under water deficit stress, the content of soluble protein was reduced and the amount of reduction was related to drought intensity, drought duration, and growth stage. During reproductive stages, the protein content decreased in L and S treatments from 2 to 13% and 14 to 22% respectively when compared with the level of F treatment. Protein content in leaves was lowest in S treatment at ripening stage with 5.64 mg g^{-1} FW

Table 2. Effects of different water deficit stress on the chlorophyll content in leaves at different barley reproductive stages. Results are the means ± standard errors (n = 9). Means followed by the same letter within a column are not significantly different at P < 0.05 level

Treatments [a]	Chlorophyll (mg g^{-1} FW [b])			
	Heading	Flowering	Milking	Ripening
F	4.44±0.59 a	4.17±0.30 a	3.34±0.23 a	3.06±0.23 a
L	4.67±0.42 a	3.89±0.62 ab	2.21±0.39 b	2.15±0.25 b
S	3.48±0.53 a	2.57±0.44 b	1.93±0.35 b	1.30±0.11 c

[a] F is full water supply, control; L is light water stress; and S is severe water stress.
[b] FW is fresh weight

Table 3. Effects of different water deficit stress on the protein content in leaves at different barley reproductive stages. Results are the means ± standard errors (n = 9). Means followed by the same letter within a column are not significantly different at P < 0.05 level

Treatments [a]	Protein (mg g^{-1} FW [b])			
	Heading	Flowering	Milking	Ripening
F	8.07±0.1 a	8.11±0.12 a	7.42±0.17 a	7.17±0.13 a
L	7.88±0.09 a	7.01±0.08 b	6.51±0.2 b	6.24±0.2 b
S	6.94±0.13 b	6.79±0.12 b	6.29±0.26 b	5.64±0.22 c

a) F is full water supply, control; L is light water stress; and S is severe water stress.
b) FW is fresh weight

Membrane lipid peroxidation

When plants were subjected to environmental drought stress, oxidative damage resulted in the level of membrane lipid peroxidation, measured as MDA content, indicating possible damage. At all stages, the MDA content for the severe stress treatments (S) was significantly greater than the control. For L, however, it was not significantly at the heading stage. In addition, between L and S treatments, there was a significant difference only at the milking and ripening stages (Table 4).

APX activity

There were no significant differences in APX activity Between L and F treatments at all stages. However, compared with treatment F, APX activity increased significantly at heading, flowering and milking stages (Fig. 1). For example, at the flowering and milking stages as compared with the controls (F), for the S treatment the APX activity of the leaves increased by 23% and 20%, respectively.

Table 4. Effects of different water deficit stress on membrane lipid peroxidation (MDA content) in leaves at different barley reproductive stages. Results are the means ± standard errors (n = 9). Means followed by the same letter within a column are not significantly different at P < 0.05 level

Treatments [a]	MDA (μmol g^{-1} FW [b])			
	Heading	Flowering	Milking	Ripening
F	4.90±0.17 b	6.04±0.10 a	7.41±0.01 a	8.44±0.31 a
L	5.35±0.13 ab	7.42±0.37 b	8.23±0.12 b	9.23±0.09 b
S	5.83±0.06 a	7.89±0.20 b	8.97±0.43 c	9.72±0.22 c

a) F is full water supply, control; L is light water stress; and S is severe water stress.
b) FW is fresh weight

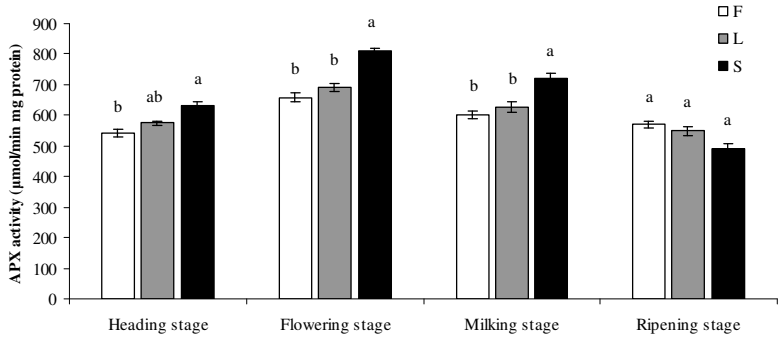

Fig. 1. Effect of drought stresses on activity of APX in leaves of barley at different reproductive stages; data are mean ± S.E. of nine replications; bars with different letters are significantly different at the P < 0.05 level (F – full water supply , L – light water stress, S – severe water stress)

CAT activity

CAT activity at the heading stage compared to the control was significantly higher in S treatment but it was not significant in L treatment. At the flowering and milking stages, compared to the control, CAT activity of both L and S treatments was significantly greater; however, at the ripening stage, it was lower than the control for both L and S treatments. Thereby, under drought stress at the flowering and milking stages, CAT activity increased above the control (F) by 46%, 40% and 49%, 43% respectively, for the L and S treatments (Fig. 2)

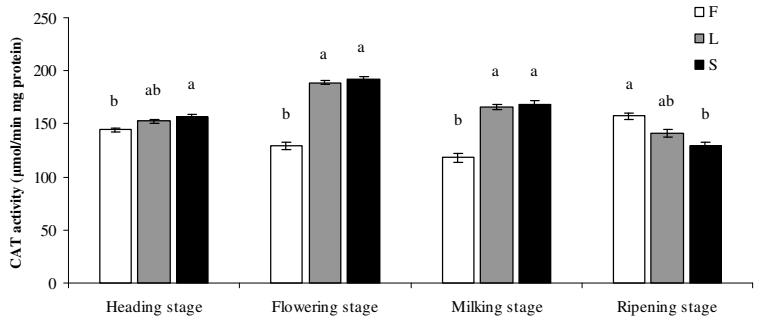

Fig. 2. Effect of drought stresses on activity of CAT in leaves of barley at different reproductive stages; data are mean ± S.E. of nine replications; bars with different letters are significantly different at the P < 0.05 level (F – full water supply , L – light water stress, S – severe water stress)

POX Activity

For the L treatment compared to the control, POX activities at the flowering and milking stages were significantly higher. In addition, at the flowering and milking stages, POX activities of the S treatment was significantly higher than L and F treatments, particularly, which increased by three and half-fold and five-hold respectively, compared with control F. However, sever water stress (S) significantly declined POX activity relative to control at the ripening stage (Fig. 3).

SOD Activity

With SOD activities, during the flowering, milking and ripening stages, with an increase in drought there were significant discrepancies in the three water treatments. Increases in SOD activities were observed in response to drought stress, with a higher

level of SOD activities in severe water stress than in light water stress. In the flowering and milking stages, SOD activities of the three treatments were in the order of S > L > F. however, at the ripening stage, drought stress decreased SOD activity compared with control treatment and the order of SOD activities of the three treatments were F > L > S (Fig. 4). The highest SOD activity with 69.86 units/mg proteins and the lowest SOD activity with 40.02 units/mg proteins were observed in S treatment at milking and ripening stages respectively.

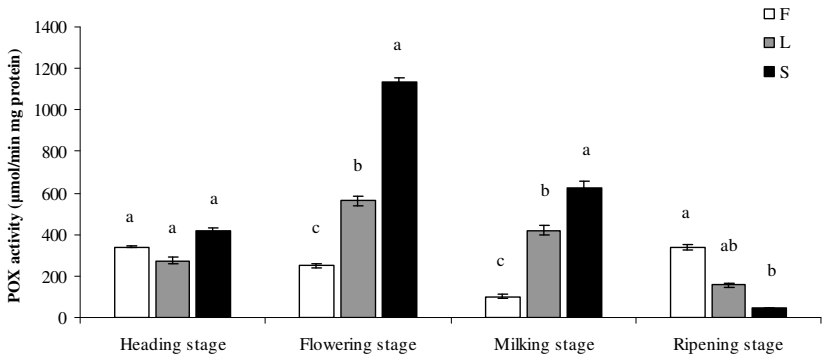

Fig. 3. Effect of drought stresses on activity of POX in leaves of barley at different reproductive stages; data are mean ± S.E. of nine replications; bars with different letters are significantly different at the P < 0.05 level (F – full water supply , L – light water stress, S – severe water stress)

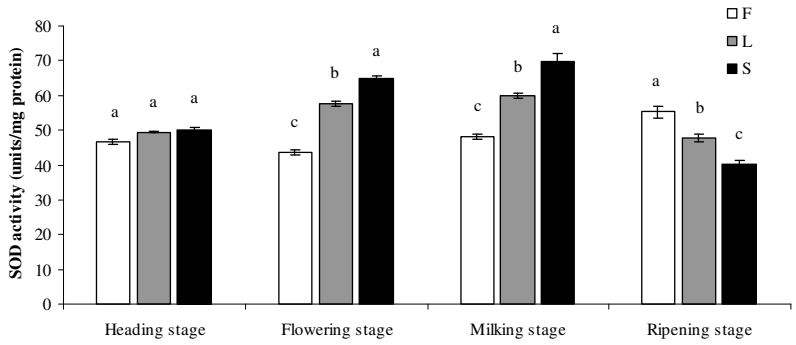

Fig. 4. Effect of drought stresses on activity of SOD in leaves of barley at different reproductive stages; data are mean ± S.E. of nine replications; bars with different letters are significantly different at the P < 0.05 level (F – full water supply , L – light water stress, S – severe water stress)

DISCUSSION

Drought is one of the major limitations to food production worldwide. As the world population continues to grow and water resources for crop production decline, the development of drought-tolerant cultivars and water-use-efficient crops is a global concern. Even the most productive agricultural regions experience short periods of drought within almost any year and occasional years with severe droughts [13].

Investigations on prolonged and progressive drought stress are a very useful way to gain insight into the sudden or punctual responses to drought stress. In particular, the impact of prolonged and progressive drought stress on plants should be assessed by examining drought effects during the time course using a wider range of water availability, since the physiological and biochemical processes of plants depend on the duration and severity of the drought event [14]. In our study, the different physiological and biochemical responses of barley plants investigated under prolonged and increasing drought stress at reproductive stages.

Drought, due to its osmotic effect in natural and agricultural habitats can induce a wide number of responses ranging from increase the osmotic potential of the cell and growth inhibition to leads to the production of ROS such as $O_2^{\bullet}$ and H_2O_2 in plant tissues [15]. The response depends on the species and genotype [16], the length and severity of water loss [17, 18], the age and stage of development [19]. ROS are highly active molecules that can easily damage membrane and oxidize photosynthetic pigments, proteins and nucleic acids [5]. ROS-scavenging mechanisms of the cell include the enzymatic and non-enzymatic antioxidants. Scavenging mechanisms for ROS involve these enzymes: SOD, CAT, APX, POX and GR.

Intensity and prolonged period of drought caused loss of RWC, chlorophyll, protein and lipid peroxidation which could lead to further quality decline. The dramatic decline in chlorophyll and protein and increase in MDA were related closely to decrease in RWC under water deficit stress.

Most research has shown decreased RWC in response to drought stress [20, 21]. In the present study, RWC declined under drought stress during the whole stages (Table 1). Higher RWC has been reported to play a role in the stress tolerance of plants [21], and to be a good indicator of drought stress tolerance [20].

The chlorophyll concentrations decreased in drought-stressed leaves of Barley (Table 2). In general water stress is known to decrease chlorophyll content at all growth stages [22]. Inhibitory effects of water stress on chlorophyll content were also suggested by many researchers [23, 4]. Both reductions in the formation of chlorophyll and increase in breakdown under water stress contributed towards the reduction of chlorophyll under water stress. Increased formation of ROS due to environmental stresses could also be a cause, which is involved in the oxidation of photosynthetic

pigments and the membrane disintegration and damage to chloroplasts [24].

The amount of total soluble proteins reduced in dependence of drought intensity, drought duration, and growth stage. The highest decrease observed in treatment S at ripening stage (Table 3). Similarly, [25] reported that during periods of water stress a decline in total soluble proteins in leaves of spring wheat compared to the control were observed. Water shortage stress enhances proteolytic degradation of Rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase) protein [25]. Hence, decreases in soluble protein contents under water deficit could be largely due to a decline in Rubisco protein. However, Contrasting results were reported on the protein levels in dehydrated leaves of resurrection plants, these being five to six times as high as in control leaves, or reduced from 30% to 40% of the control value [26].

Malondialdehyde has been known as the end product of peroxidation of membrane lipids and the MDA content reflects the degree of the peroxidation of membrane lipids. Water deficit stress by increase of generation ROS is responsible for stress-dependent peroxidation of membrane lipids [27]. Increased MDA accumulation has been correlated with reduction of RWC and photosynthetic pigment content under prolonged drought [28]. In the present study, MDA content of barley leaves increased under drought stress (Table 4) similar to what has been found in other cereals plants such as wheat [29], rice [30].

In the ascorbate-glutathione cycle, APX reduces H_2O_2 using ascorbate as an electron donor [31]. According to the results of the present study, only sever water stress (S) caused the increase in APX activity compared with the control. APX activity was higher in S treatment in flowering stage than in the other stages as presented in Fig. 1. Increase in APX activity results from a variety of environmental stresses such as water deficit stress reported by previous studies [32, 6].

Catalase scavenges H_2O_2 by breaking it down directly to form water and oxygen and an increase in its activity is related to an increase in stress tolerance. The CAT activity was elevated significantly under flowering and milking stages and maximum activity was registered in sever water stress (S) at flowering stage (Fig. 2). Increased CAT activity under water stress has been reported by [33] and [34]. However, the CAT activity decreased significantly in S treatment compared with F control at ripening stage. The reduction in CAT activity under drought stress may have been due to either reduced synthesis or enhanced degradation of the enzyme. This is in conformity with the data of [35] who reported that the CAT activity was decreased under long term drought stress compared with control at mature stage of maize.

POX is among the major enzymes that scavenges H_2O_2 in chloroplasts which is produced through dismutation of O_2• catalyzed by SOD [15]. Present investigation revealed that activity of peroxidase (POX) was significantly increased with the increase in the intensity of water stress at both flowering and milking stages. But, at ripening stage the POX activity was reduced below control values under water stress (Fig. 3). Increase in POX activity in response to drought stress has been reported in *Cassia angustifolia*, *Arabidopsis thaliana* [33, 4].

The activity of SOD, which is responsible for scavenging O_2• to produce H_2O_2, increased with both light and sever water stresses (L, S) at the flowering and milking stages (Fig. 4). This increased activity may reflect the enhanced amount of O_2• production and also indicate the possible role for SOD's dismutation effects on O_2• and protection of the photosynthetic apparatus [28]. However, when leaf RWC dropped to about 48% and 21% at ripening stage in L and S treatments respectively, the activity of SOD was inhibited significantly (Fig. 4). The results suggested that severe water deficit in plant could impair O_2• scavenging in the cell and favor accumulation of O_2• [28].

The important components of protective systems are enzymatic defenses such as APX, CAT and POX as well as SOD which scavenge hydrogen peroxide (H_2O_2) and superoxide radical (O_2•), respectively. This research revealed that the rules of APX, CAT, POX and SOD activity change under increasing and prolonged of water stress are approximately similar (Figs. 1, 2, 3 and 4), which indicated that these four enzymes cooperated with each other during water deficit stresses. Generally, compared to control treatments (F) the activities of APX, CAT, POX and SOD first unchanged (for POX and SOD) or lower increased only in sever water stress (S) (for APX and CAT) at heading stage and then increased significantly in both light water stress (L) and sever water stress (S) (for CAT, POX and SOD) and only in sever water stress (S) (for APX) at flowering and milking stages and finally unchanged (for APX) or decreased in S treatment (for CAT and POX) and in both L and S treatments (for SOD) at later ripening stage. This result was similar to the reports of [28], [36], [37] and [35] who found that the activity of the antioxidant enzymes markedly increased during the first days of dehydration at the early stages and then decreased with prolonged dehydration at the lately stages.

Cereals are very sensitive to water stress during the growing season, and is especially vulnerable to soil drought during the flowering and grain-filling periods. In this study, at the flowering and milking stages of barley plants, the activities of APX, CAT, POX and SOD significantly increased under drought stresses compared with the full water supply (Figs. 1, 2, 3 and 4). Furthermore, for POX and SOD, the increase was significantly higher in sever drought stress (S) treatment compared with light drought stress (L) treatment (Figs. 3 and 4). However, the increases in the levels of MDA under drought stresses at flowering and milking stages (Table 4) indicated that the increased activities of antioxidant enzymes may not be enough to break down ROS molecules and to prevent the peroxidation of lipid membranes under drought stress, similarly as detected in wheat [38] and poplar [39].

CONCLUSION

Our results manifested that acclimation to drought stress are not only with the environmental factors of plant's natural habitats but also related with the severity, duration of the drought event and their interaction. Soil drought stress would decrease relative water status (RWC), and thereby declined the content of chlorophyll and protein in barley leaves. When plants were exposed to different soil water stress, the activities of antioxidant enzymes APX, CAT, POX and SOD protecting the plant against deleterious effects of ROS were increased at flowering and milking stages. Moreover, this study indicated that antioxidant protection in barley plants could be attributed mainly to POX and SOD. However, this work demonstrated the increase of antioxidant enzymes activity can not reduced lipid peroxidation and MDA accumulation and thereby improved the growth conditions for plants under water deficit stresses. Furthermore, prolonged severe drought stress decreased the activities of antioxidant enzymes at lately ripening stage of barley that may have been due to either reduced synthesis or enhanced

degradation of the enzyme. Our results suggested that with increasing drought stress the cellular damage from the flowering to the ripening stages was considerably significant. Hence, water supply is important for barley growth during the reproductive stage.

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REFERENCES

[1] Shaheen, R. and R. C. Hood-Nowotny. Effect of drought and salinity on carbon isotope discrimination in wheat cultivars. *Plant Sci.* 168: 901–909.

[2] Yong, T., L. Zongsuo, S. Hongbo and D. Feng. 2006. Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of *Radix astragali* at seeding stage. *Colloid Surface* B, 49: 60–65.

[3] Saglama, A., N. Saruhan, R. Terzia and A. Kadioglua. 2011. The relations between antioxidant enzymes and chlorophyll fluorescence parameters in common bean cultivars differing in sensitivity to drought stress. *Russ. J. Plant Physiol.* 58: 60–68.

[4] Jung, S. 2004. Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. *Plant Sci.* 166: 459–466.

[5] Gong, H., X. Zhu, K. Chen, S. Wang and C. Zhang. 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci.* 169: 313–321.

[6] Ramachandra Reddy, A., K. V. Chaitanya, P. P. Jutur and K. Sumithra. 2004. Differential antioxidative responses to water stress among five mulberry (*Morus alba* L.) cultivars. *Environ. Exp. Bot.* 52: 33–42.

[7] Bor, M., F. Ozdemir and I. Turkan. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L and wild beet *Beta maritima* L. *Plant Sci.* 164: 77–84.

[8] Guo, Z., W. Ou, S. Lu and Q. Zhong. 2006. Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. *Plant Physiol. Biochem.* 44: 828–836.

[9] Khanna-Chopra, R. and D. S. Selote. 2007. Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than -susceptible wheat cultivar under field conditions. *Environ. Exp. Bot.* 60: 276–283.

[10] Margonis, k., I. G. Fatouros, A. Z. Jamurtas, M. G. Nikolaidis, I. Douroudos, A. Chatzinikolaou, A. Mitrakou, G. Mastorakos, I. Papassotiriou, K. Taxildaris and D. Kouretas. 2007. Oxidative stress biomarkers responses to physical overtraining: Implications for diagnosis. *Free Radical Bio. Med.* 43: 901 – 910.

[11] Choi, D. G., N. H. Yoo, C. Y. Yu, B. Reyes and S. G. Yun. 2004. The activities of antioxidant enzymes in response to oxidative stresses and hormones in paraquat-tolerant *Rehmannia glutinosa* plants. *J. Biochem. Mol. Biol*. 37: 618-624.

[12] Sharma, P. and R. S. Dubey. 2007. Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. *Plant Cell Rep.* 26: 2027-2038.

[13] Barnabas, B., K. Jager and A. Feher. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* 31: 11–38.

[14] Kozlowski, T. T. and S. G. Pallardy. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. *Bot. Rev.* 68: 270–334.

[15] Turkan, I., M. Bor, F. Ozdemir and N. Koca. 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci.* 168, 223–231.

[16] Rampino, P., S. Pataleo, C. Gererdi, G. Mita and C. Perrotta. 2006. Drought response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ.* 29: 2143–2152.

[17] Araus, J. L., G. A. Slafer, M. P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C_3 cereals: what should we breed for? *Annal. Bot.* 89: 925–940.

[18] Bartels, D. and E. Souer. 2004. Molecular responses of higher plants to dehydration. In: H. Hirt and K. Shinozaki (Eds.), Plant responses to abiotic stress, Berlin: *Springer-Verlag and Germany: Heidelberg,* pp. 9–38.

[19] Zhu, X., H. Gong, G. Chen, S. Wang and C. Zhang. 2005. Different solute levels in two spring wheat cultivars induced by progressive field water stress at different developmental stages. *J. Arid Environ.* 62: 1–14.

[20] Shaw, B., T. H. Thomas and D. T. Cooke. 2002. Responses of sugar beet (*Beta vulgaris* L.) to drought and nutrient deficiency stress. *Plant Growth Regul.* 37: 77–83.

[21] Terzi, R. and A. Kadioglu. 2006. Drought stress tolerance and the antioxidant enzyme system in *Ctenanthe setosa*. *Acta Biol. Cracov. Bot.* 48: 89–96.

[22] Vyas, S. P., B. K. Garg, S. Kathju, and A. N. Lahiri. 2001. Influence of potassium on water relations, photosynthesis, nitrogen metabolism and yield of clusterbean under soil moisture stress. *Indian J. Plant Physiol.* 6: 30-37.

[23] Alonso, R., S. Elvira, F. J. Castillo and B. S. Gimeno. 2001. Interactive effects of ozone and drought stress on pigments and activities of antioxidative anzymes in *Pinus halepensis*. *Plant Cell Environ.* 24: 905–916.

[24] Bano, A. and N. Aziz. 2003. Salt and drought stress in wheat and the role of abscisic acid. *Pakistan J. Bot.* 35: 871-883.

[25] Lin, G. S. and G. X. Wang. 2002. Doubled CO_2 could improve the drought tolerance better in sensitive cultivars than in tolerant cultivars in spring wheat. *Plant Sci.* 163: 627-637.

[26] Veljovic-Jovanovic, S., B. Kukavica, B. Stevanovic, and F. Navari-Izzo. 2006. Senescence- and drought-related changes in peroxidase and superoxide dismutase isoforms in leaves of *Ramonda serbica*. *J. Exp. Bot.* 57: 1759–1768.

[27] Upadhyaya, H. and S. K. Panda. 2004. Responses of *Camellia sinensis* to drought and rehydration. *Biol. Plantarum*. 48: 597-600.

[28] Jiang, Y. and B. Huang. 2001. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. *Crop Sci.* 41: 436–442.

[29] Nikolaeva, M. K., S. N. Maevskaya, A. G. Shugaev and N. G. Bukhov. 2010. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. *Russ. J. Plant Physiol.* 57: 87-95.

[30] Fu, G. F., J. Song, Y. R. Li, M. K. Yue, J. Xiong and L. X. Tao. 2010. Alterations of panicle antioxidant metabolism and carbohydrate content and pistil water potential involved in spikelet sterility in rice under water-deficit stress. *Rice Sci.* 17: 303-310.

[31] Arrora, A., R. K. Sairam and G. C. Srivastava. 2002. Oxidative stress and antioxidant system in plants. *J. Plant Physiol.* 82: 1227–1237.

[32] Ozkur, O., F. Ozdemir, M. Bor and I. Turkan. 2009. Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. *Environ. Exp. Bot.* 66: 487-492.

[33] Agarwal, S. and V. Pandey. 2003. Stimulation of stress-related antioxidative enzymes in combating oxidative stress in Cassia seedlings. *Indian J. Plant Physiol*. 8:264-269.

[34] Da, G. T., S. F. Gong, B. L. Ping, L. Y. Yan and Z. G. Sheng. 2005. Effects of water stress on the protective enzyme activities and lipid peroxidation in roots and leaves of summer maize. *Scientia Agric Sinica,* 38: 922-928.

[35] Xin, Z. L., G. Mei, L. Shiqing, L. Shengxiu and L. Zongsuo. 2011. Modulation of plant growth, water status and antioxidant system of two maize (*Zea Mays* L.) cultivars induced by exogenous glycinebetaine under long term mild drought stress. *Pak. J. Bot.* 43: 1587-1594.

[36] Agarwal, S. and R. Shaheen. 2007. Stimulation of antioxidant system and lipid peroxidation by abiotic stresses in leaves of *Momordica charantia*. *Brazil. J. Plant Physiol.* 19:149-161.

[37] Liu, C., Y. Liua, K. Guo, D. Fan, G. Li, Y. Zheng, L. Yu and R. Yang. 2011. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ. Exp. Bot.* 71: 174–183.

[38] Niedzwiedz-Siegien, I., R. Bogatek-Leszczynska, D. Cˆomea and F. Corbineau. 2004. Effects of drying rate on dehydration sensitivity of excised wheat seedling shoots as related to sucrose metabolism and antioxidant enzyme activities. *Plant Sci.* 168: 879-888.

[39] Yang, F. and L. F. Miao. 2010. Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. *Silva Fenn*. 44: 23-37.