

Absciscic Acid and Cytokinin-Induced Osmotic and Antioxidant Regulation in Two Drought-Tolerant and Drought-Sensitive Cultivars of Wheat During Grain Filling Under Water Deficit in Field Conditions

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Abstract

Phytohormones play critical roles in regulating plant responses to stress. The present study investigates the effect of cytokinin, abscisic acid and cytokinin/abscisic acid interaction on some osmoprotectants and antioxidant parameters induced by drought stress in two wheat cultivars (*Triticum aestivum* L.) of 'Pishgam' and 'MV-17' as tolerant and sensitive to drought during post-anthesis phase, respectively grown in field conditions. The most considerable effect of the treatments was exhibited 21 days after anthesis. Under drought conditions, the flag leaf soluble carbohydrate content increased in both cultivars while starch content was remarkably decreased in 'Pishgam' as compared to 'MV-17'. Abscisic acid increased total soluble sugar and reduced starch more than other hormonal treatments, although it decreased studied monosaccharides in 'Pishgam', especially. Drought stress induced high proportion of glycinebetain and free proline in 'Pishgam' cultivar. Application of abscisic acid and cytokinin/abscisic acid interaction increased glycinebetain and proline content in both cultivars under irrigation and drought conditions. The tolerant cultivar exhibited less accumulation of hydrogen peroxide and malondialdehyde in relation to significant increase of catalase and peroxidase activities and α -tocopherol content under drought conditions. All hormonal treatments increased the named enzyme activities under both irrigation and drought conditions, while higher accumulation of α -tocopherol was only showed in case of cytokinin application. Also, abscisic acid and cytokinin/abscisic acid could decrease drought-induced hydrogen peroxide and malondialdehyde level to some extent, although abscisic acid increased both of hydrogen peroxide and malondialdehyde content in irrigation phase, especially.

Keywords: Absciscic acid, antioxidant, osmoprotectant, cytokinin, drought stress, wheat cultivar

Introduction

On a global basis, drought stress (including soil and/or atmospheric water deficit), in conjunction with coincident high temperature and radiation is one limiting factor for plant survival and productivity in different conditions such as agriculture (Chaves *et al.*, 2003). Wheat (*Triticum aestivum* L.), as one of the crucially important food, is especially threaten by water deficit during flowering and grain filling period (Lantican *et al.*, 2005). Drought stress induces various biochemical and physiological responses in plants because of Alteration of water content within the plant tissue and oxidative stress such as protein denaturation, lipid peroxidation, MDA accumulation and

pigment degradation due to produced reactive oxygen species (ROS - Chaves *et al.*, 2003; Gill and Tuteja, 2010). Therefore, accumulation of sugars (such as raffinose family oligosaccharides (RFO), sucrose, trehalose and sorbitol), sugar alcohols (such as manitol), amino acids (such as proline (Pro), and amines (such as glycine betaine and polyamines) is for osmoprotection in plant for water preservation (Seki *et al.*, 2007; Yordanov *et al.*, 2000). Also up-regulating of enzymatic and non-enzymatic antioxidants is in order to overcome oxidative stress. The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX). SOD converts ROS into H₂O₂ and O₂, while CAT and POX convert H₂O₂ into H₂O (Chaves *et al.*, 2003; Gill and Tuteja, 2010).

Besides, non-enzymatic antioxidant such as vitamin E, carotenoids (carotene and xanthophylls) and soluble antioxidant including ascorbate and glutathione can also quench ROS and stabilize photosynthetic complexes (Chaves *et al.*, 2003; Gill and Tuteja, 2010; Liu *et al.*, 2011).

Phytohormones create an ability in plants to adapt to abiotic stresses by mediating a wide range of adaptive responses. CK and ABA cause different manners to encounter the drought stress. In water stressed plants, CK synthesis in the roots and delivery to the leaves is usually less but its degradation might be increased. In consequence, decreased CK content in leaves has been observed (Pospisilova *et al.*, 2000; Pospisilova, 2003). In spite of the latter studies, CKs may partially ameliorate negative effects of water stress by stimulating osmotic adjustment (Merevites *et al.*, 2011) and directly or indirectly scavenging ROS (Stoparic and Maksimovic *et al.*, 2008). The involvement of ABA in mediating drought stress has been extensively researched. ABA plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance (Pospisilova *et al.*, 2000; Pospisilova, 2003; Ghassemian *et al.*, 2008).

The present study determines the comparative effect of CK, ABA and CK/ABA interaction on the osmotic adjustment and some components of antioxidant system in two wheat (*Triticum aestivum* L.) cultivars differing in the degree of drought resistance ('Pishgam' as drought-tolerant cultivar and 'MV-17' as drought-sensitive cultivar) during post-anthesis phase.

Materials and methods

Plant materials and growth conditions

A homogenous lot of wheat seeds (*Triticum aestivum* L.) of two cultivars, 'Pishgam' (drought- tolerant) and 'MV-17' (drought-sensitive) were obtained from the Seed and Plant Improvement Institute of Iran. The experimental period started on November 22, 2009 and ended on April 25, 2010. Climate temperature and rainfall from sowing to harvest during experimental period are presented in Table 1. A composite soil sample (from 48 points) was collected from 0-30 and 30-60 cm depth during experimental period and was analyzed in the laboratory. Details of soil physical and chemical properties of the experimental site are given in Table 2. Factorial experiment was based on the randomized complete blocks with three replicates under irrigation (hormonal and non-hormonal treatments) and drought (hormonal and non-hormonal treatments) conditions. The seeds were sown on field, in 48 rows 20 cm apart with the density of 400 seeds m⁻². The needed nitrogen for wheat growth based on the field experiment results was 60 kg net nitrogen per hectare from urea source added to the soil in fall (Feyziasl and Valizadeh, 2001 and 2003). The needed phosphorous was supplied on the basis of soil test and phosphorous deficit from critical level in soil (9 mg per hectare-Feyziasl *et al.*, 2004). The experimental field was covered by a shelter. Watering of irrigation treatments were routinely done until the end of growth period while watering of drought stress treatment was interrupted at the start of second week of flowering stage. Also, degree of soil moisture

was weekly measured on by time domain reflectometry (TDR- table 3). Application of CK (BA 150 µL) and ABA (100 µL) treatments was begun as foliar spray in irrigation and drought conditions in the start of second and fourth week after anthesis, respectively. Therefore hormonal treatments were complete at 21 and 28 days after anthesis and the most significant data was obtained at 21 DAA. Then in the treatments (irrigation and drought stress) after the removal of marginal effects, 60 plants were randomly selected and cut in 0, 7, 14, 21 and 28 days after the pollination stage, respectively. Flag leaves of the samples were separated and wrapped in aluminum foil and were immediately put in liquid nitrogen. The samples were then dried in freeze dryer (-120°C) and were kept at -80°C until next measurement, although many of samples were only transferred at -80°C immediately in related to enzyme activity assay.

Tab. 1. Mean monthly temperature and rainfall during crop growth

Month → Conditions ↓	November	December	January	February	March	April
Temperature (°C)	10.3	1.9	11.9	13.3	20.9	23.7
Rainfall (mm)	8.4	11.6	17.8	16.9	8.7	5.8

Tab. 2. Soil physical and chemical properties of the experimental site (0-30 and 30-60 cm depth)

Depth (cm)	pH	EC (dS m ⁻¹)	OC (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	7.44	1.23	1.19	17.06	463	19.6	41.1	29.4	Clay loam
30-60	7.51	1.38	1.04	39.01	791	23.9	45.6	21.2	Loam

Estimation of carbohydrate content

0.03 g freeze dried samples of flag leaf were ground and extracted three times 50 mL hot 80% ethanol (Gill *et al.*, 2003). Soluble sugars were determined based on the method of phenol-sulphuric acid by Dubois *et al.*, 1956. The reducing sugars were quantitatively estimated in the obtained extract, by Nelson's method (Nelson, 1944). Starch content was also determined using the method of phenol-sulfuric acid by Dubois *et al.*, 1956. Levels of glucose, sucrose and fructose were determined using two-dimensional HPLC (controller, Waters 600 s; pump, Waters 626; autosampler, Waters 717; Waters, Massachusetts, USA) using U.V. detector and C-18 column according to Albertson and Grof, 2007). Sugars were quantified from standard curves calculated from external standards preceding and following each group of samples with the concentration of 0, 5, 10, 15, 20, 25 and 30 mM of glucose.

Free proline and glycinebetaine determination

Free proline content was quantified according to the method of Bates *et al.* (1973). Free glycinebetaine was determined according to Grieve and Maas (1984).

Oxidative stress parameters

Lipid peroxidation was measured in terms of content of malondialdehyde (MDA, ε = 155 mmol⁻¹ cm⁻¹), a product

of lipid peroxidation, following the method of Heath and Packer (1968). H_2O_2 content was determined according to Velikova *et al.* (1990). The content of H_2O_2 was calculated by comparison with a standard calibration curve previously made by using different concentration of H_2O_2 .

Estimation of vitamin E (α -tocopherol)

A level of α -tocopherol was determined using two-dimensional HPLC system (detector: fluorescence, mobile phase: flow intensity: 1.3 ml min^{-1} , excited wavelength: 288 nm min^{-1} , excited wavelength: 288 nm , emission wavelength: 322 nm) with C_{18} column by method of Botsoglou *et al.* (1998).

Measurement of antioxidant enzyme activity

After homogenized with liquid nitrogen, 0.3 g of flag leaves were suspended in 3 ml of ice-cold HEPES buffer (25 mM, pH 7.8) which contained 0.2 mM EDTA and 2% PVP. The homogenate was centrifuged at 4°C and $12,000 \times g$ for 20 min, and the resulting supernatant was used for determination of CAT and POD (Ramiro *et al.*, 2006).

A modification of the method of Aebi (1984) was used to assay catalase activity. Activity of peroxidase was measured according to the method of Chance and Maehly (1955). One unit of catalase activity corresponded to the amount of enzyme that decomposes 1 μ mole of

H_2O_2 /min/g fresh wt. One unit of peroxidase is the amount of enzyme required to oxidize one μ mole of guaiacol by H_2O_2 at test conditions.

Statistical analysis

The mean values of studied parameters were taken from the measurements of three replicates and the "Standard Error" of the means was calculated. One-way ANOVA was applied to determine the significance of the results between different treatments and then Duncan multiple range tests ($p < 0.05$) were performed. All the statistical analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows (version 18.0). All significant different were calculated as percentage with two digits after decimal.

Results and discussion

Carbohydrate content

Earlier reports mentioned that sugars protect the cells during drought by two mechanisms. First, the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration. Thus, sugars interact with proteins and membranes through hydrogen-bonding, thereby preventing protein denaturation. Secondly, sugars are a major

Tab. 3. Measurement of soil moisture as sampling in 0-30 cm and 30-60 cm depths

Conditions	Cultivar	Soil depth	Sampling		Sampling		Sampling		Sampling		Sampling	
			27/02/2010	27/02/2010	4/3/2010	4/3/2010	11/3/2010	11/3/2010	18/03/2010	18/03/2010	25/03/2010	25/03/2010
			TDR	VOL%	TDR	VOL%	TDR	VOL%	TDR	VOL%	TDR	VOL%
Drought	Pishgam	0-30	36.0	28.2	49.2	15.7	54.9	11.6	48.4	15.4	46.4	14.3
Drought	Pishgam	30-60	37.5	23.9	38.9	20.9	36.8	16.1	38.2	19.7	31.1	19.8
Drought	MV17	0-30	35.3	21.6	50.7	14.1	59.9	9.8	50.7	12.2	50.7	12.1
Drought	MV17	30-60	41.4	21.1	42.5	18.1	42.2	14.3	39.8	17.4	35.7	17.6
Irrigation	Pishgam	0-30	32.5	28.8	37.5	29.8	45.5	17.1	37.9	29.7	38.5	21
Irrigation	Pishgam	30-60	33.9	25.3	34	25.3	34.5	18.4	37.2	22.7	29.2	22.6
Irrigation	MV17	0-30	34.9	19.6	41	27.4	43.0	17.1	34.2	30.3	36.4	21
Irrigation	MV17	30-60	37.9	24.1	36.7	22.3	39.3	17.1	36.7	20.6	33.2	21

contributing factor to vitrification, which is the formation of a biological glass in the cytoplasm of dehydrated cells. Sugars helped to maintain osmotic adjustment during drought conditions. Also, it has been claimed that even sugar flux may be a signal for metabolic regulation (Xue *et al.*, 2008).

Results revealed that, total soluble sugars (TSS) increased during 21 days after anthesis (DAA) by 115.91 and 29.81% in 'Pishgam' and 'MV-17' under drought conditions as compared to normally-watered plants, respectively. According to our experiments, significant increase in the amount of reducing sugars 92.91% was

recorded in tolerant cultivar during the drought conditions, as compared to the irrigation conditions at the time period of 21 DAA. While, the sensitive cultivar showed 28.03% increase in reducing sugars at 21 DAA. This study revealed that, drought stress could reduce starch content of two cultivars especially by 31.06 and 9.78% in 'Pishgam' and 'MV-17' at 21 DAA, respectively. In related to results above, TSS/starch ratio increased under drought conditions in either cultivar that reached the maximum at 21 DAA by 211.0% in 'Pishgam' and by 44.44% in 'MV-17' (Fig. 1).

During drought conditions, fructose content had tremendous increase (107.75%) at 21 DAA. The same

situation was observed in the susceptible cultivar with lesser extent with an increase of 45.79%. Under drought conditions, glucose level increased only in 'Pishgam' by 53.84% at 21 DAA, significantly. Also, sucrose level increased in 'Pishgam' by 255.18% at 21 DAA. 'MV-17' cultivar also showed 22.64% increase in sucrose level at 21 DAA (Fig 2).

The accumulation of sugars in response to drought stress and decrease of starch content due to the decline of photosynthesis and its degradation was studied in wheat leaves during drought (Xue *et al.*, 2008). Increased level of sucrose, glucose and fructose during drought conditions

implies that complex carbohydrates, such as starch, hydrolyze to simple sugars such as hexoses during drought conditions. Studies with a variety of plants demonstrate that drought induces these carbohydrates into sugar alcohols and proline (Wang *et al.*, 1996). Our results are supported by study that reported genes encoding cytoplasmic and vacuolar enzymes in the pathways leading to glucose, fructose and fructan production were up-regulated in the wheat leaves during drought stress although enzymes involved in carbon fixation (Calvin cycle) were Conversely reduced (Xue *et al.*, 2008).

Plant hormones such as; CK and ABA play important roles in conferring tolerance to environmental stress as effect on carbohydrate content (Pospisilova, 2003).

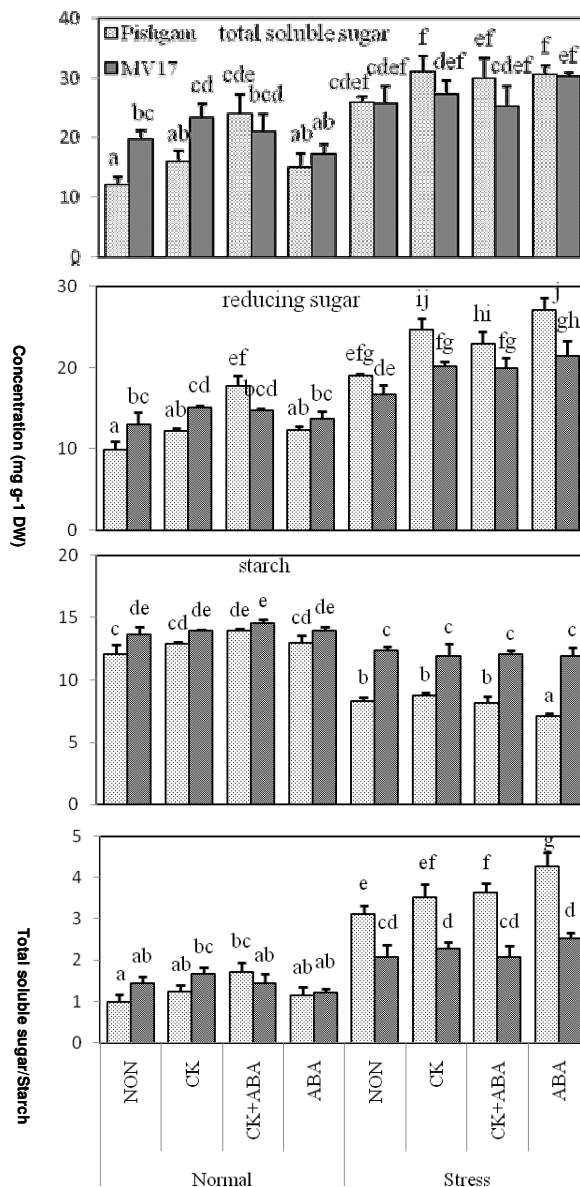


Fig. 1. Effect of hormonal treatments of CK, ABA and CK/ABA on total soluble sugar, reducing sugar, starch content and total soluble sugar/starch ratio under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. 'Pishgam' (white columns) and drought sensitive cv. 'MV-17' (black columns). Data are shown as mean \pm SD of three independent

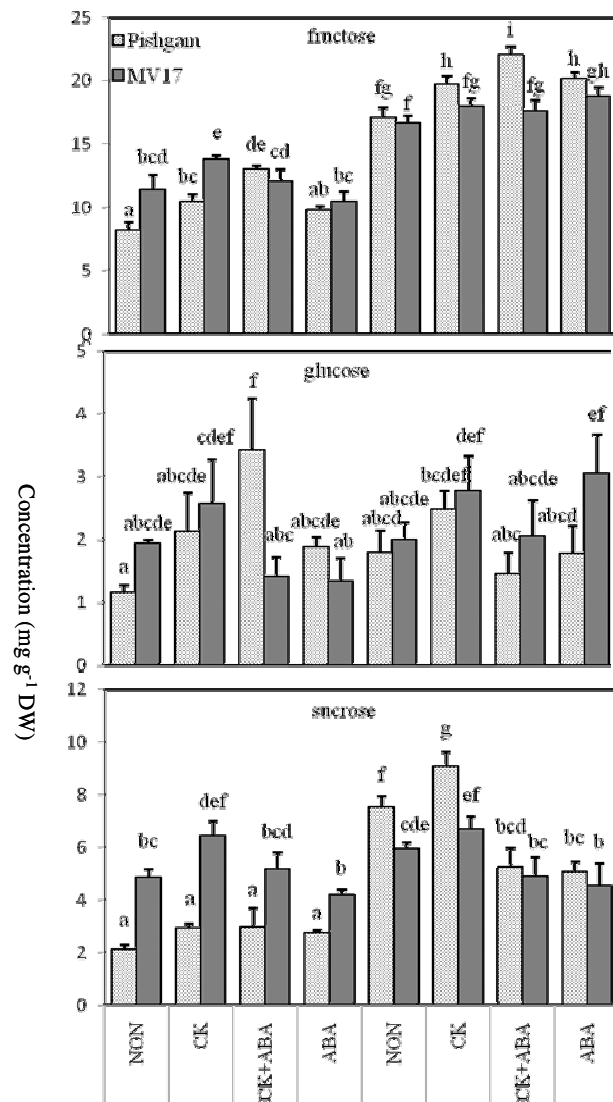


Fig. 2. Effect of hormonal treatments of CK, ABA and CK/ABA on fructose, glucose and sucrose content under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. 'Pishgam' (white columns) and drought sensitive cv. 'MV-17' (black columns). Data are shown as mean \pm SD of three independent measurements

In well-watered plants of 'Pishgam', CK/ABA treatment increased TSS at 21 DAA by 100.25%. Hormonal treatments had no significant effect on TSS of sensitive cultivar during irrigation conditions. None of hormonal treatments had significant effect on TSS content in 'Pishgam' and 'MV-17' under drought conditions. At the 21 DAA, during irrigation conditions, the hormonal treatment of CK/ABA increased the reducing sugars by 79.75% as compared to the non-hormone treatment in 'Pishgam'. While, significant effect of hormonal treatments was not seen on its of 'MV-17' in latter conditions. The results have shown that ABA increased reducing sugar content during drought conditions more effectively than other hormonal treatments by 42.07 and 27.98 % in 'Pishgam' and 'MV-17' at 21 DAA, respectively. Under irrigation conditions, only CK/ABA increased the starch content by 15.99 and 6.05% at 21 DAA in tolerant and sensitive cultivar, respectively. Interestingly, under drought conditions, only ABA decreased starch content by 14.28% in 'Pishgam'. Although, ABA decreased starch level of 'MV-17' but it was not a significant reduction. This shows the

significantly effective behaviour of ABA on starch content in the stems of wheat cultivar. In well watered plants, only CK/ABA increased significantly TSS/starch ratio at 21 DAA by 71.0% in 'Pishgam'. In drought conditions, effect of ABA was much pronounced which was able to elevate TSS/starch ratio by 37.62 % at 21 DAA in tolerant cultivar as compared with non-hormone treatment effect of other hormonal treatments was not significant in both cultivars (Fig. 1).

CK/ABA caused the most significant increase in fructose content of 'Pishgam' at 21 DAA by 57.69 and 28.64% under irrigation and drought conditions, respectively. Fructose content of sensitive cultivar increased remarkably by 21.36% under irrigation conditions at 21 DAA when it applied CK in compared to other hormonal treatments. But under drought conditions, ABA caused the most increase in fructose content by 13.03%. Under well-irrigated conditions, CK/ABA caused the most significant increase by 193.16% in glucose content of 'Pishgam' at 21 DAA, while hormonal treatments did not create its of 'MV-17'. Under drought conditions, hormonal treatments of CK and ABA increased significantly glucose level by 37.77 and 52.5% in 'Pishgam' and 'MV-17' at 21 DAA, respectively. Application of ABA caused a decrease in sucrose level of 'Pishgam' and 'MV-17' cultivars by 42.82 and 63.36% at 21 DAA, respectively under irrigation conditions. The interaction of CK and ABA also decreased sucrose level in 'Pishgam' and 'MV-17' although its effect was less than individual ABA treatment. Under drought conditions, ABA caused the most significant effect on sucrose level by decreasing it 32.66 and 23.82% in 'Pishgam' and 'MV-17' at 21 DAA, respectively (Fig. 2).

CKs are important in the development of plants' photosynthetic apparatus by directly effecting on chloroplast, increasing the photochemical activity of photosystem II (PS II) and reducing chlorophyll degradation (Goltsev et al. 2001). Therefore, it can be presumed that the foliar application of CK had the ability to increase carbohydrate content due to a good effect on photosynthesis system in our work. In agreement with our study, foliar application of kinetin increased total carbohydrate in leaves of *Codiaeum variegatum* in normal condition (Mazher et al., 2011) and also in water-logged or seawater-treated *Vigna sinensis* and *Zea mays* plants (El-Shahaby et al., 2003). ABA-induced decrease in starch content and increase in reducing sugars in 'Pishgam' especially which may partly be responsible for improving relative water content of plant leaves because make osmotic potential more negative (Pospisilova, 2003). This can be explained by the studies of Lee et al. (2008), suggesting that exogenous application of ABA increased the activity of certain enzymes like-amylase and ribonuclease in white clover leaves. These enzymes might breakdown starches and other materials to make osmotic potential more negative. Moreover, ABA excited many of metabolism pathways including biosynthesis of osmolytes that need carbon framework of sugars. Therefore, ABA can be effective on utilization of TSS in different metabolism pathways (Verslues and Bray, 2006) and a probable reason that we did not show significant change or increase of TSS, glucose and sucrose in hormonal treatment of ABA under drought, although fructose level increased.

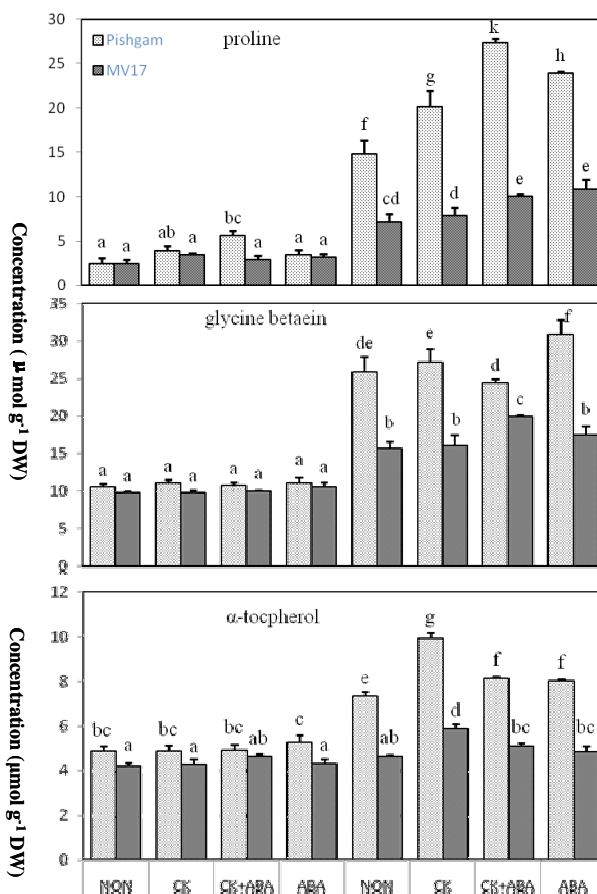


Fig. 3. Effect of hormonal treatments of CK, ABA and CK/ABA on glycinebetaine and proline content under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. 'Pishgam' (white columns) and drought sensitive cv. 'MV-17' (black columns). Data are shown as mean \pm SD of three independent measurements

Proline and glycine betaine content

Accumulation of proline (Pro) and glycine betaine (GB) may be a response characteristic of cultivars under water deficit which it works as osmotic adjustor. Moreover, GB is essential in stabilizing the macromolecules, such as PSII complex and rubisco, and to protect membranes from lipid peroxidation. Pro is also considered as a potent antioxidant and inhibitor of protein denaturation, and preservation of enzyme structure (Chaves *et al.*, 2003). The present results have shown that Pro content increased 488 and 187% in 'Pishgam' and 'MV-17', respectively under drought conditions at 21 DAA. Under drought conditions, GB content also showed an increase of 138.37 and 60.82% in 'Pishgam' and 'MV-17' cultivars, respectively, at 21 DAA (Fig. 3). In agreement with our study, the rate of Pro accumulation was significantly higher in drought-tolerant cultivars than drought-sensitive cultivars of wheat and olive. Also, in species such as wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*), tolerant genotypes normally accumulate more GB than sensitive genotypes in response to stress. This relationship, however, is not universal in salt and drought tolerance (Ashraf and Foolad, 2007).

As well as carbohydrates, phytohormones can alternate levels of other osmoprotectants as Pro in plants under water stress (Pospisilova, 2003). Hormonal treatments had more effect on tolerant cultivar than the susceptible one under well-irrigated and drought conditions. CK/ABA caused 123.71 and 82.74% increase in Pro level of 'Pishgam' under irrigation and drought conditions, respectively. But in sensitive cultivar, under well-irrigated conditions, CK caused 38.95% increase in Pro at 21 DAA. Under drought conditions, ABA also increased Pro content of 'MV-17' by 51.88% at 21 DAA. Under irrigation conditions, hormonal treatments did not show significant effect on GB content in either two cultivars during DAA. Under drought conditions, CK/ABA caused the most effect on GB content so that increased it by 26.78% in 'MV-17', while ABA treatment increased GB by 19.23% in 'Pishgam' more than other hormonal treatments (Fig. 3).

The light-dependence of stress-induced Pro accumulation, which is also CK-inducible, has been demonstrated in several higher plants (Hare *et al.*, 1997). Elevated CK levels promoted survival under water-stress conditions, inhibited leaf senescence and induced increased Pro levels (Nishiyama *et al.*, 2011). In light of the previous information, proline accumulation in plants is mediated by both ABA-dependent and ABA-independent signaling pathways. ABA is known to regulate expression of *P5CS* gene which is involved in proline biosynthesis (Ashraf and Foolad, 2007). In some plants as *Arabidopsis* causal link between ABA and proline accumulation was suggested and it was shown that exogenous application of ABA increases the level of *AtP5CS1* and *AtP5CS2* transcripts (Strizhov, 1997). Although this phenomenon is common, the role of ABA in regulating Pro accumulation has still been a matter of confusion and debate, since contradictory data was obtained in different species by different researchers (Verslues and Bray, 2006).

Malondialdehyde and hydrogen peroxide content

lipid peroxidation and H_2O_2 accumulation not only directly affecting normal cellular functioning, but also aggravating the oxidative stress through production of lipid-

derived radicals in abiotic stresses such as drought. Although, H_2O_2 at low concentrations acts as a signal molecule involved in acclimatory signaling triggering tolerance to various biotic and abiotic stresses (Gill and Tuteja, 2010).

H_2O_2 content increased significantly under drought condition in 'Pishgam' and 'MV-17' by 254.61 and 437.40%, respectively during 21 DAA. According to increased H_2O_2 level under drought conditions, Malondialdehyde (MDA) content increased by 503.43 and 750% in tolerant and sensitive cultivars, respectively (Fig. 4). Many works proved increasing of H_2O_2 and MDA under drought stress in cultivars, while susceptible cultivars elevated H_2O_2 and MDA more than tolerant cultivars (Chaves *et al.*, 2003).

Under irrigation condition, significant increase of H_2O_2 content was only observed by ABA application at 21 DAA in 'Pishgam' and 'MV-17' by 27.69 and 23.66%, respectively. Generally, all hormonal treatments decreased H_2O_2 content under drought condition. However, during 21 DAA, the most reduction of H_2O_2 content of 'Pishgam' and 'MV-17' was induced by CK/ABA interaction by 140.10 and 33.33%, respectively. Under irrigation conditions, ABA application caused considerably a significant increase of 62.25 and 73.23% in MDA content of 'Pishgam' and 'MV-17' cultivars at 21 DAA, respectively. However, under drought conditions, CK/ABA increased MDA content by 66.28 and 31.96% in 'Pishgam' and 'MV-17' at 21 DAA, respectively (Fig. 4).

Increasing H_2O_2 level during irrigation is due to particular role of ABA on plasma-membrane NADPH oxidase complex and its-produced H_2O_2 (Ghassemian *et al.*, 2008). Therefore, increased level of H_2O_2 caused oxidative damages as MDA production that was more partially in

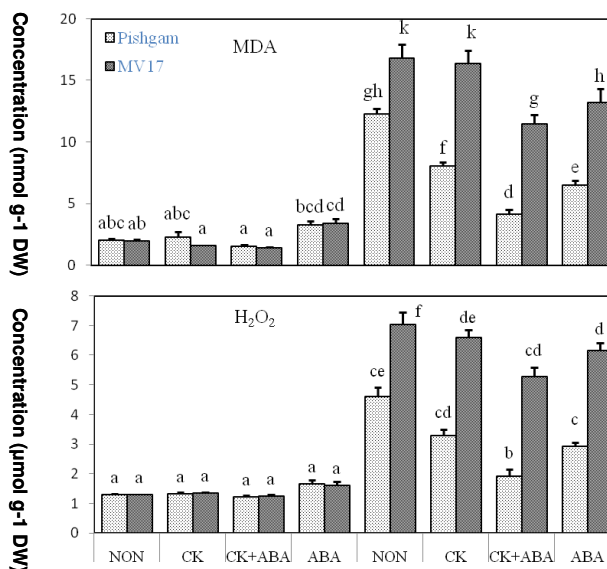


Fig. 4. Effect of hormonal treatments of CK, ABA and CK/ABA on H_2O_2 and MDA content under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. 'Pishgam' (white columns) and drought sensitive cv. 'MV-17' (black columns). Data are shown as mean \pm SD of three independent measurements

'MV-17', although a level of H_2O_2 is necessary for signaling and up-regulation of antioxidants (Ghassemian *et al.*, 2008). ABA effect on up-regulation antioxidant system (Ghassemian *et al.*, 2008) and direct and indirect effect of CK on inhibition of ROS (Stoparic and Maksimovic, 2008) caused that CK/ABA interaction inhibited H_2O_2 and MDA accumulation in drought condition higher than effect of individual ABA or CK treatment on H_2O_2 and MDA content.

α -tocopherol

Drought stress excited the molecule and enzyme antioxidant in different cell compartments. Antioxidant capacity is greatly dependent on the severity of the stress as well as the species and the development stage (Chaves *et al.*, 2003). α -tocopherol as a constitutive component of lipid matrix of thylakoid membrane has a photoprotective and stabilizing function. In agreement with previous study, higher α -tocopherol contents occurred in drought-tolerance species than in susceptible one (Chaves *et al.*, 2003). Also, Overexpression of VTE1 (Tocopherol cyclase) from Arabidopsis in transgenic tobacco plants, showed decrease in LPO, electrolyte leakage and H_2O_2 content in drought stress (Liu *et al.*, 2008).

α -tocopherol content showed a 50.20 and 10.76% increase in 'Pishgam' and 'MV-17' at 21 DAA under drought condition, respectively. Under irrigation conditions, only CK/ABA caused a 10.55% significant increase in α -tocopherol content of 'MV-17' at 21 DAA. Under drought conditions, CK treatment caused the most significant increase in α -tocopherol content of 'Pishgam' and 'MV-17' by 27.69 and 26.99% at 21 DAA, respectively (Fig 3).

It was suggested that, the increased α -tocopherol level in flag leaf tissue under CK treatment might be in related to especial CK effect on the chloroplast development and stability that is an important synthesis and accumulation location for vitamin E (Hare *et al.*, 1997). Nevertheless, the role of ABA proved in increase of α -tocopherol and L-ascorbic acid in transcriptional and post-transcriptional levels (Ghassemian *et al.*, 2008), ABA application had non-significant effect on its of tolerant and sensitive cultivars in this study.

Antioxidant enzymes

Undoubtedly, catalase (CAT) and peroxidase (POD) are the most important antioxidant enzymes that scavenge H_2O_2 as well as APX (Liu *et al.*, 2011). During the present study, 151.89 and 64.7% increase in CAT activity was seen for 'Pishgam' and 'MV-17' at 21 DAA under drought stress, respectively. Also, the results have shown that 214.58 and 90.90% increase in POD activity for 'Pishgam' and 'MV-17' under drought stress at 21 DAA, respectively. More increased activity of POD and CAT in tolerant cultivar was related to less increased MDA content under drought condition (Fig. 5). These results are in line with those related to tolerant and sensitive cultivars of maize seedling under water deficit (Chugh *et al.*, 2011). As previous reviews (Chaves *et al.*, 2003), a drought-tolerance cultivar can employ great activities of antioxidant enzymes than a drought-sensitive cultivar under drought. The increased activities of anti-oxidative enzymes, induced by moderate drought stress can protect cell membranes, proteins and metabolic machinery, which would preserve sub-cellular

structure from damage as a result of cell dehydration (Gill and Tuteja, 2010). Consequently, a drought tolerance

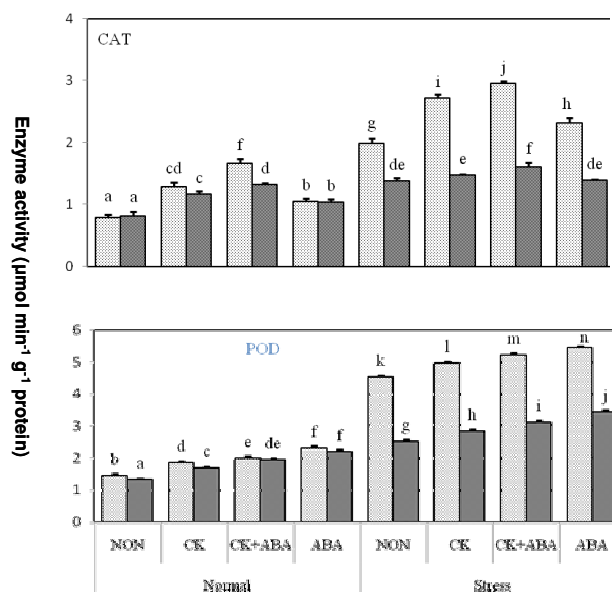


Fig. 5. Effect of hormonal treatments of CK, ABA and CK/ABA on enzyme activity of α -tocopherol content CAT and POX under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. 'Pishgam' (white columns) and drought sensitive cv. 'MV-17' (black columns). Data are shown as mean \pm SD of three independent

cultivar can possess a stronger ability to eliminate excessive ROS and reduce lipid peroxidation (Chaves *et al.*, 2003).

The results revealed that under irrigation conditions, CK/ABA caused considerably 110.12 and 62.96% increase in CAT activity in 'Pishgam' and 'MV-17' at 21 DAA, respectively. Under drought conditions, CK/ABA also caused the highest increase in CAT activity of 'Pishgam' and 'MV-17' by 48.24 and 15.94% at 21 DAA, respectively.

Under both irrigation and drought conditions, ABA caused significant increase in POD activity of 'Pishgam' by 60.41 and 20.30% and 'MV-17' by 66.66 and 36.11% at 21 DAA, respectively (Fig. 5).

A previous study showed that increased amounts of ABA induced CAT activity during drought conditions in triploid Bermuda grass have been accompanied with H_2O_2 and NO production (Lu *et al.*, 2009). It has also been shown that the ABA-induced antioxidant enzyme activities in maize leaves require the participation of H_2O_2 (Jiang and Zhang, 2003). It seems that ABA-induced H_2O_2 accumulation modulated metabolic and redox control pathways in Arabidopsis by influence on many of POD. Moreover, there is an intricate relationship, at the transcriptional and possibly post-transcriptional levels, between ABA biosynthesis and scavenger system of H_2O_2 (Ghassemian *et al.*, 2008). Also, this was proved by Synkova *et al.*, (2006) who found that kinetin inhibited a decline in CAT activity. It was concluded that the mechanism of cytokinin-dependent delay in leaf senescence involves the reduction in H_2O_2 levels due to the hormone's stimulatory

effect on CAT and APX activities (Zavaleta-Mancera *et al.*, 2007). Increased activities of ascorbate/glutathione enzymes were detected in cytokinin over-producing tobacco (Nishiyama *et al.*, 2011). Although, in contrary with latter studied works, it was reported that the CK repress the expression of antioxidant enzymes such as a soybean Fe-containing superoxide dismutase gene (Crowell and Amasino, 1991).

Conclusion

With regard to different cultivars, the constitutive H₂O₂ scavenging enzyme activity and also non-enzymatic antioxidant and osmoprotectant content in the flag leaves of drought-tolerant 'Pishgam' were higher than those in drought-sensitive 'MV-17'. This fact is related to lower H₂O₂ content and lipid peroxidation in 'Pishgam' as compared to 'MV-17'. This study reveals that, as compared to drought-sensitive 'MV-17', drought-tolerant 'Pishgam' could successfully activate defensive system and diminish subsequent damage, under drought. Also, tolerant cultivar could use the hormonal treatment better than susceptible cultivar to improve its defensive system. CK/ABA interaction caused the most effect on assayed parameters in many treatments.

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