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Abscisic acid and cytokinin-induced carbohydrate and antioxidant levels regulation in drought-resistant and -susceptible wheat cultivar during grain filling under field conditions

Mohammad-Reza Sarafraz-Ardakani[1*], Ramazan-Ali Khavari-Nejad[1,2], Foad Moradi[3], Farzaneh Najafi[1]

[1]*Faculty of Biological Sciences, Kharazmi University, Tehran, Iran*

[2]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[3]*Agriculture and Biotechnology Research Institute, Karaj, Tehran, Iran*

Key words: Drought stress, Oxidative stress, Osmotic adjustment, *Triticum aestivum*

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Abstract

The present investigation was conducted to determine the effect of cytokinin (CK), abscisic acid (ABA) and cytokinin/abscisic acid interaction (CK/ABA) on carbohydrate contents, oxidative stress and antioxidant components 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 during grain filling period. The most considerable effect of the treatments was exhibited 21 days after anthesis. Under drought conditions, the flag leaf carbohydrate content including total soluble carbohydrate and reducing sugars increased in both cultivars while starch content was remarkably decreased in Pishgam as compared to MV-17. Although Pishgam exhibited a significant increase in glucose, sucrose and fructose content but MV-17 showed only a significant increase in fructose content. Relatively, CK/ABA increased total soluble carbohydrates, sucrose, and fructose content more than other hormonal treatments. Also, ABA only reduced starch level of Pishgam, significantly. Nevertheless, level of glucose was significantly affected by none of the hormonal treatments. The tolerant cultivar exhibited less accumulation of hydrogen peroxide and malondialdehyde (MDA) in relation to more significant increase of catalase (CAT) and peroxidase (POX) activities and α-tocopherol content under drought conditions. Among hormonal treatments, ABA and CK/ABA resulted in the highest activities of POX and CAT under both irrigation and drought conditions, respectively. But, higher accumulation of α-tocopherol was showed when CK foliar was applied. ABA and CK/ABA could decrease drought-induced hydrogen peroxide and MDA level, to some extent under drought conditions.

* **Corresponding Author:** Mohammad-Reza Sarafraz-Ardakani ✉ sarafraz_ardakani@yahoo.com

Introduction

Among crop plants, wheat (*Triticum aestivum*), which often experiences water-shortage conditions, is an appealing study system because there are so many natural genotypes differing in drought tolerance (Loggini *et al.*, 1999). Wheat as well as the most cereals is especially threatened by water deficit during flowering and grain filling period (Plaut *et al.*, 2004). The typical first response of all plants to water deficit is osmotic adjustment that is by synthesizing and accumulating compatible osmolyte such as proline (Pro), glycine betaine (GB) and reducing soluble sugars including monosaccharaides, disaccharides and oligosaccharides (Chaves *et al.*, 2003; Ashraf and Foolad, 2007). Also up-regulating of enzymatic antioxidant as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) and also non-enzymatic antioxidants as vitamin E, carotenoids (carotene and xanthophyll) and soluble antioxidant including ascorbate and glutathione can is in order to overcome oxidative stress due to drought conditions (Gill and Tuteja, 2010; Liu *et al.*, 2011).

Among plant hormones, ABA and CK-dependent changes of stress responses, such as drought, have been studied at various levels. Extended works exhibited that increased endogenous ABA (Chaves *et al.*, 2003; Ashraf and Foolad, 2007) and exogenous ABA (Wang *et al.,* 2003) improves defensive mechanisms in plants by regulation up to 10% of protein-encoding genes in response to stress, transcriptionally including cases as osmoregulators and both enzymatic and non-enzymatic antioxidants. But, The negative CK-controlled function in plants encounter to drought has been proved in genetic studies in which endogenous CK levels were mostly changed either by loss of the biosynthesis genes isopentyl transferase (IPT) or by overexpression of cytokinin oxidase (CKX)-encoding degradation genes (Nishiyama *et al.*, 2011; Wang *et al.*, 2011). Meanwhile, elevated endogenous ABA and exogenous ABA excited the reduction of endogenous CK (Pospisilova *et al.,* 2005). Nevertheless, in the one manner similar to ABA, CKs may partially ameliorate negative effects of water stress by stimulating osmotic adjustment (Merewitz *et al.,* 2011). Also, Stoparic and Maksimovic (2008) reported directly or indirectly effect of CK on scavenging ROS.

Especially important is the question to how do we profit the CK characters in alleviating of water-deficit damages, while drought and drought-induced endogenous ABA reverse CK accumulation and its effects. Consequently, the present investigation was conducted to determine whether exogenous application CK and ABA combination (interaction) can improve drought tolerance more than individual application of ABA and CK while two named hormone operate antagonist together in different levels of plant development and plant challenge to abiotic stress and if such tolerance correlated with changes in carbohydrate content, as important candidates in osmotic adjustment, and some components of antioxidant. Also, our previous studies are extended to address the following question:

Which cultivar can be more beneficiaries by exogenous application of hormonal treatments? Drought-tolerant or drought-sensitive.

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. 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 2nd week of flowering stage. Also, degree of soil moisture was weekly measured by time domain reflectometry (TDR). 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 2nd and 4th 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.

Extraction and estimation of carbohydrate content

Estimation of carbohydrate content

A 0.03 g freeze dried samples of flag leaf were ground and extracted three times 50 mL hot 80% ethanol (Gill *et al*., 2003). The extract was evaporated to the water phase, and the volume adjusted to 100 mL with distilled water after 0.47 mL 0.3 N $Ba(OH)_2$ and 0.5 mL 5% $ZnSO_4$ was added to remove proteins. Total soluble carbohydrates were determined based on the method of phenol-sulfuric acid by Dubois *et al* (1956). The reducing sugars were quantitatively estimated in the obtained extract, by Nelson's method (1944). Starch content was also determined using the method of phenol-sulfuric acid by Dubois *et al* (1956). After total soluble sugars and reducing sugars assay, extracts were pooled and dried under vacuum, redissolved in one milliliter of HPLC purity-grade H_2O was added to the obtained extract. 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.

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, MDA was extracted with 10% trichloro-acetic acid and was determined following the method of Heath and Packer (1968). A 0.03 g of freeze dried samples were homogenized in 2 ml of refrigerated an ice bath with 5 ml of 0.1% (w/v) trichloro-acetic acid (TCA), the supernatant was used for the H_2O_2 content assay as described by H_2O_2 content was determined according to Velikova *et al* (2000). 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)

0.01 g flag leaf sample (freeze dried) was poured in tube under dark conditions (tube was covered in aluminum foil) and 200 µl pyrocatecole and 5 ml of 0.5M KOH (in methanol) were added and placed in water at 80°C . Then, 1 ml deionized water and 5 ml hexane were added to the mixture and centrifuged at 373 rpm for 2 min. Three milliliter supernatant was taken and freeze dried. Residue was dissolved in 1.5 ml methanol-acetonitrile (50:50 v/v) mobile phase, filtered and 20 µl of each sample was injected to HPLC system (detector: florescence, mobile phase: flow intensity: 1.3 ml min^{-1}, excited wavelength: 288 ml min^{-1}, excited wavelength: 288 nm, emission wavelength: 322 nm) with C_{18} column (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 rpm for 20 min, and the resulting supernatant was used for determination of CAT and POX (Ramiro *et al*., 2006). A modification of the method of Aebi (1984) was used to assay CAT activity. Activity of POX was measured according to the method of Chance and Maehly (1955). One unit of CAT activity corresponded to the amount of enzyme that decomposes 1 µmole of H_2O_2/min/g fresh wt. One unit of POX 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

Effect of hormonal treatments of CK, ABA and CK/ABA interaction on Carbohydrate content under irrigation and drought conditions

Results revealed that, total soluble carbohydrate (TSC) increased during 21 days after anthesis (DAA) by 140.91 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. Drought stress could reduce starch content of two cultivars especially by 31.06 in Pishgam at 21 DAA. In related to results above, TSC/starch ratio increased under drought conditions in either cultivar that reached the maximum at 21 DAA by 211.0% in Pishgam and by 45.83% in MV-17 (Fig 1). 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 during the present works (Parida *et al*., 2007; Xue *et al*., 2008). The available results are consistent with a recent report who observed that in many woody plants, two shrub species, which exhibited more active accumulations of soluble sugars and proline, also revealed higher resistance to drought than the other species and showed the positive correlation between contents of osmotic solutes (proline and soluble sugars) and antioxidant enzyme activities (Liu *et al*., 2011). Maintain of hydrophilic interactions in membranes and proteins during dehydration because of probably substitution of hydroxyl groups of sugars for water (Leopold *et al*., 1994) and also contribution in vitrification, which is the formation of a biological glass in the cytoplasm of dehydrated cells (Buitink *et al*., 1998) are two protect mechanisms of sugars in plants exposure to drought.

Sucrose, glucose and fructose are known to play a central role in plant metabolism at the cellular and whole plant levels. They are involved not only in the response to abiotic stresses, but also act as nutrient and metabolite signalling molecules, modulating expression of a large number of metabolic genes through sugar sensing mechanisms (Xue *et al*., 2008). 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%. 56.41% significant increase in glucose level occurred in tolerant cultivar during drought phase than well-water conditions while significant change was not exhibit insensitive cultivar. As well as fructose content, sucrose level increased highly in Pishgam by 255.18% at 21 DAA. MV-17 cultivar also showed 22.64% increase in sucrose level at 21 DAA (Fig 2).

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 (Xue *et al*., 2008). Previous studies demonstrate that drought induces these carbohydrates into sugar

alcohols and proline in different plants (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).

Table 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

Table 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

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

In well watered plants of Pishgam, CK/ABA interaction treatment increased TSC at 21 DAA by 100.25% more than other hormonal treatments. The hormonal treatments had no significant effect on TSC of sensitive cultivar during irrigation conditions. Under drought conditions, CK/ABA interaction had the most significant effect on TSC at 21 DAA and improved it by 45.34 and 28.95% in Pishgam and MV-17, respectively. At 21 DAA, during irrigation conditions, the hormonal treatment of CK/ABA increased the reducing sugars by 29.40% as compared to the non-hormone treatment in Pishgam, although; significant effect of hormonal treatments was not seen in MV-17 in latter condition. The results have shown that CK/ABA and ABA increased reducing sugar content during drought conditions more effectively than other hormonal treatments by 43.49 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 showed the significantly effective behavior of ABA on starch content in the stems of wheat cultivar. In well watered plants, only CK/ABA increased significantly sugar/starch ratio at 21 DAA by 71.0% in Pishgam. In drought conditions, effect of ABA was much pronounced which was able to elevate the soluble sugar/starch ratio by 63.02% at 21 DAA in tolerant cultivar as compared with non-hormone treatment more than other hormonal treatments, although CK/ABA only improved this ratio by 30% in MV-17. Effect of other hormonal treatments was not significant in both cultivars (Fig 1). CKs excite the carbohydrate import into sink or inhibit carbohydrate export from it so increase the sink power (Morris, 1993). Also, 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, 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 this study, application of exogenous ABA resulted in increased sugar accumulation, which may partly be responsible for improving relative water content of plant leaves because make osmotic potential more negative (Pospisilova, 2003). 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 sea water-treated *Vigna sinensis* and *Zea mays* plants (El-Shahaby *et al.*, 2003). Furthermore, ABA-increased sugar accumulation and starch reduction in *polypodium vulgar* (Bagniewska-Zadworna *et al.*, 2007) and *Oryza sativa* (Pattanagul, 2011) were similar to influence of ABA on level TSC and starch in obtained results.

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%. Obtained results did not reveal the significant influence of hormonal treatment on glucose level in both cultivars. Application of ABA caused a high significant increase in sucrose level of Pishgam by 108.49 and 65.33% at 21 DAA under irrigation and drought conditions, respectively. Sucrose content of MV-17 did not change by hormonal treatment in irrigation and drought conditions. (Fig. 2). Exogenous ABA application could increase soluble carbohydrate as stachyose and raffinose, glucose and fructose in plants in exposure cold (Meng *et al.*, 2008) and drought (Wang *et al.*, 2002), although effects of exogenous ABA application on plant sugar contents are largely dependent on the concentrations of the applied ABA (Wang *et al.*, 2002). Increase of monosaccharides has also been reported in response to CKs application including N^6-furfurylaminopurine (FAP) and N^6-benzylaminopurine (BAP) in *Wolffa arrhiza* (Piotrowska *et al.*, 2005). ABA and CK-induced Changes in invertase activity have also been reported to correlate with reducing sugar content in maize (Piotrowska *et al.*, 2005) and in chickpea (Kaur *et al.*, 2003), respectively. Moreover, it has been observed that water stress-induced ABA was involved in sugar accumulation in peach fruits by activating sucrose synthase, sucrose phosphate synthase, sorbitol oxidase and acid invertase (Kobashi *et al.*, 2000). Wonderfully, glucose content was not affected by hormonal treatments whereas, many studies have supported that glucose might be a bridge between carbohydrate and phytohormone signaling (Halford *et al.*, 2003; Hartig and Beck, 2006).

Effect of hormonal treatments of CK, ABA and CK/ABA interaction on H_2O_2 and MDA content under irrigation and drought conditions

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, MDA content also increased by 503.43 and 750% in tolerant and sensitive cultivars, respectively (Fig. 3).

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 73.23% in MDA content of MV-17 cultivar at 21 DAA. However, under drought conditions, CK/ABA decreased MDA content by 196.62 and 46.98.96% in Pishgam and MV-17 at 21 DAA, respectively (Fig. 3).

Partial 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 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) especially during exposure with stress and effect of CKs on antioxidants (Synkova *et al.,* 2006; Zavaleta-Mancera *et al.*, 2007) and also direct effect of CK on reduction of ROS such as which have been exhibited about inhibition of the xanthine oxidase activity (Leshem *et al*, 1981), reduction of the lipoxygenase activity (Grossman and Leshem, 1978) and decrease of hydroxyl radicals ($OH^{\cdot}$) concentration (Stoparic and Maksimovic, 2008) caused that CK and ABA combination possibly 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.

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

				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
Replication	Conditions	Cultivar	Soil depth	TDR	Voltage%	TDR	Voltage%	TDR	Voltage%	TDR	Voltage%	TDR	Voltage%
3	drought	Pishgam	0-30	36.0	28.2	49.2	15.7	54.9	11.6	48.4	15.4	46.4	14.3
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
3	drought	MV17	0-30	35.3	21.6	50.7	14.1	59.9	9.8	50.7	12.2	50.7	12.1
3	drought	MV17	30-60	41.4	21.1	42.5	18.1	42.2	14.3	39.8	17.4	35.7	17.6
3	irrigation	Pishgam	0-30	32.5	28.8	37.5	29.8	45.5	17.1	37.9	29.7	38.5	21
3	irrigation	Pishgam	30-60	33.9	25.3	34	25.3	34.5	18.4	37.2	22.7	29.2	22.6
3	irrigation	MV17	0-30	34.9	19.6	41	27.4	43.0	17.1	34.2	30.3	36.4	21
3	irrigation	MV17	30-60	37.9	24.1	36.7	22.3	39.3	17.1	36.7	20.6	33.2	21

Effect of hormonal treatments of CK, ABA and CK/ABA interaction on α-tocopherol content under irrigation and drought conditions

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 stabling 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 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. 4).

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 (Hare *et al.*, 1997) that is an important synthesis and accumulation location for vitamin E. 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.

Effect of hormonal treatments of CK, ABA and CK/ABA interaction on CAT and POX activity under irrigation and drought conditions

Undoubtedly, catalase (CAT) and peroxidase (POX) 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 POX activity for Pishgam and MV-17 under drought stress at 21 DAA, respectively. More increased activity of POX and CAT in tolerant cultivar was related to less increased MDA content under drought conditions (Fig. 4). These results are in line with those related to tolerant and sensitive cultivars of maize seedling under water deficit (Chugh *et al.*, 2011).

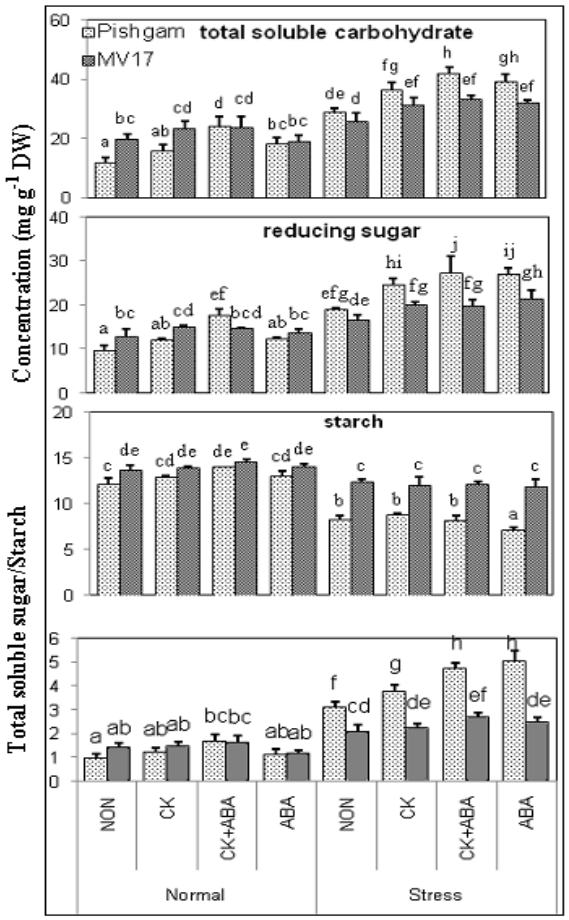

Fig. 1. Effect of hormonal treatments of CK, ABA and CK/ABA on total soluble carbohydrate, reducing sugar, starch content and total soluble carbohydrate /starch ratio under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. Pishgam and drought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

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.

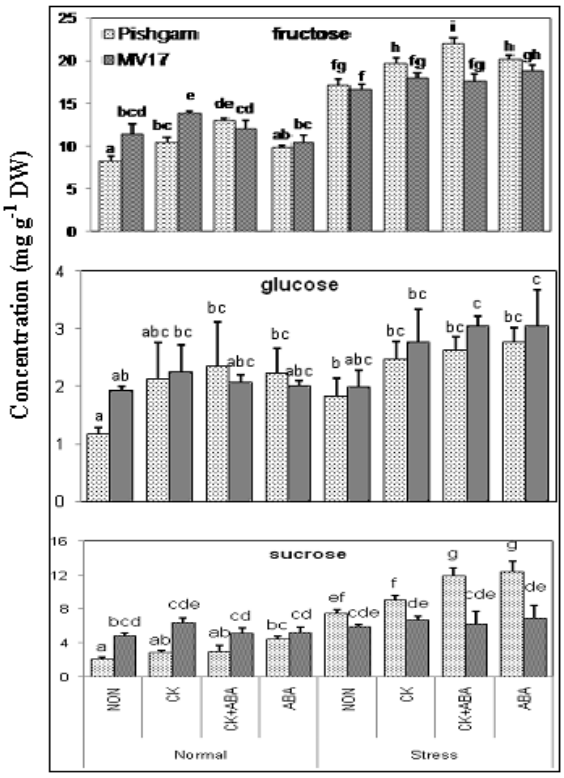

Fig. 2. Effect of hormonal treatments of CK, ABA and CK/ABA on fructose, glucose and sucrose content under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. Pishgam and drought sensitive cv. MV-17). Data are shown as mean ± SD of three independent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

Under both irrigation and drought conditions, ABA caused significant increase in POX activity of Pishgam by 60.41 and 20.30% and MV-17 by 66.66 and 36.11% at 21 DAA, respectively (Fig. 4).

It was shown that increased amounts of ABA induced CAT activity during drought conditions in triploid Bermudagrass that 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 POX. 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). Nevertheless, 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). Therefore, the positive effect of both CK and ABA on enhancement of antioxidants, in related to performed studies, might be one reason for more increasing of CAT activity subjected to CK/ABA application than other hormonal treatments in Pishgam and MV-17 during drought condition, although the highest level of POX activity was exhibited when ABA foliar applied.

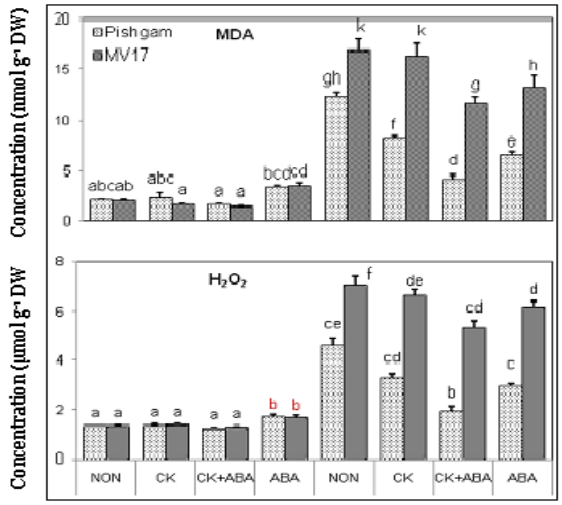

Fig. 3. Effect of hormonal treatments of CK, ABA and CK/ABA on H_2O_2 and MDA content under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. Pishgam and drought sensitive cv. MV-17). Data are shown as mean ± SD of three independent measurements.

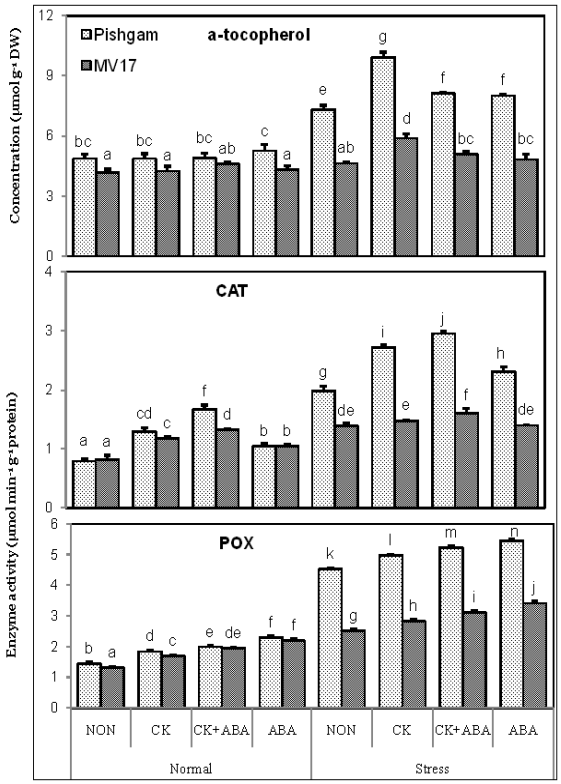

Fig. 4. Effect of hormonal treatments of CK, ABA and CK/ABA on α-tocopherol content andenzyme activity of CAT and POX under irrigation and drought condition in flag leaves during grain filling in two wheat cultivars (drought tolerant cv. Pishgam drought sensitive cv. MV-17). Data are shown as mean ± SD of three independent.

In a general conclusion of obtained results, With regard to different cultivars, the constitutive H_2O_2 scavenging enzyme activity and α-tocopherol as non-enzymatic antioxidant and also carbohydrate content in the flag leaves of drought-tolerant Pishgam were higher than those in drought-sensitive MV-17. This fact is related to lower lipid peroxidation and pigment degradation 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. Also, it seems that exogenous application of CK and ABA combination caused the most effect on assayed parameters in many treatments and so may be a successful experience in relation to utilization of

hormonal treatments.

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Abbreviations

ABA-absisic acid; CAT-catalase; CK-cytokinin; DAA-days after anthesis; MDA-malondialdehyde; ROS-reactive oxygen species; POX-peroxidase; TSC-total soluble carbohydrate.