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*Hamid MOHAMMADI, Foad MORADI*¹**EFFECTS OF PLANT GROWTH REGULATORS ON ENDOGENOUS HORMONES IN TWO WHEAT CULTIVARS DIFFERING IN KERNEL SIZE UNDER CONTROL AND WATER STRESS CONDITIONS****SUMMARY**

In arid and semi-arid regions, wheat productivity is limited by terminal water stress during grain-filling. An experiment which repeated twice was conducted to determine of the changes in the levels of endogenous IAA and ABA during grain filling of two wheat cultivars with different kernel size. Treatments were two Iranian wheat cultivars, water stress levels and hormones. Plants were exposed to water stress after anthesis. Benzylaminopurine (BAP) and Abscisic acid (ABA) were applied at two stages either at initial grain-filling starting 2 days post-anthesis or at early grain-filling starting 11 days post-anthesis. Water stress resulted in reduction of grain yield, biological yield, and HI in both cultivars. Water stress caused significant reduction in 1000 grain weight in two cultivars but the rate of drop was lower in high yield cultivar. BAP application after anthesis increased grain weight, although the rate of increase was varied in cultivars. At the initial grain-filling period, exogenous application of ABA increased HI and 1000-grain weight under two conditions. Indole-3-acetic acid (IAA) levels of grains were high at the early stages of grain-filling, whilst ABA level was greater at the linear grain filling stages. Results suggested that differences between cultivars in case of grain yield and its component substantially resulted from variations of hormonal levels (IAA and ABA). Consequently, Increasing of sink strength and HI with hormonal application might be key factors for improving of wheat grain yield.

Key words: Indole-3-acetic acid, Abscisic acid, Drought, Wheat, Sink strength, Hormonal application

INTRODUCTION

Drought stress is one of the most important abiotic stresses which can affect the growth of a plant, thereby curbing crop yield (Chaves and Oliveira 2004). In cereals like wheat (*Triticum aestivum* L.) the stress induced due to drought at the stage of grain-filling usually shortens the grain-filling period and reduces the grain-filling rate, eventually reducing grain yield (Nicolas et al. 1985a, b ; Ahmadi and Baker 2001a). During seed development, appropriate soil water status is of critical importance for accumulation of starch in grains and thereby formation of grain yield (Ahmadi and Baker 2001b). Previous literature

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shows that endogenous hormones are the crucial regulators for translocation and partitioning photo-assimilates for grain-filling in cereals (Brenner and Cheikh 1995; Ahmadi and Baker 1999). Therefore, they can play a role in regulating of grain weight and yield. Under drought stress, these endogenous hormones act as responding signals (Bano et al. 1993), so they can have the key role in the growth and development process, such as starch synthesis and accumulation in the seed (Yang et al. 2004). In cereals, peas and beans, high levels of cytokinin has been reported for endosperms in course of growth, which is an essential condition for cell division during the first phase of seed setting (Yang et al. 2000). There are numerous reports that the auxins, gibberellins and abscisic acid are also thought to be involved in regulating grain development (Hansen and Grossmann 2000). ABA hormone plays an important role in the acclimation to water stress among other stress, and guarantees survival of the plants by changing their physiology and growth (Zhang et al. 2006; Christmann et al. 2007). ABA is also believed to be the major regulators of senescence and remobilization of assimilates to the grain (Yang et al. 2003b). Dry matter partitioning, the final destination for assimilate streaming from source organs, happens via accessing the transfer route of sink organs, and sink strength is considered as the determiner of dry matter distribution throughout the whole plant (Marcelis, 1996). Understanding this issue could be crucial to improving grain yield in wheat through external application of growth regulators. Overall, there is little information on concentration changes of IAA and ABA during different growth periods of wheat under water stress when externally applying cytokinin and ABA. In arid and semi-arid regions (such as Iran) with sufficient illumination, day length and terminal drought stress, sink limitation under mentioned conditions is more acute, and in order to use the plant dry matter more efficaciously, this experiment was designed to improve sink strength.

MATERIAL AND METHODS

Plant Materials

Based on two-year field experiment in which a wide spectrum of 81 cultivars with diverse acclimations and characteristics were examined (Mohammadi et al. 2011). The cultivars were ranked based on sink properties and from each extreme (high- and low-yielding) of the spectrum, one cultivar was selected for pot experiment in a greenhouse. A factorial experiment based on randomized complete block design was carried out with three replications at Research greenhouse of Agriculture College, University of Tehran, Karaj, during two consecutive growing seasons of 2008-2010 and all of measurements were done in Agricultural Biotechnology Research Institute of Iran (ABRII). Treatments were cultivar [two Iranian cultivars of wheat (high- and low-yielding cultivars, Pishtaz and Karaj3, respectively)], water stress (two levels) and hormone (five levels). Plants were exposed to water stress (WS) after anthesis (50% of anthesis) with daily weighting pots. Plants were irrigated every 2 days to achieve field capacity (FC) and 50 % FC, for control and water-stressed plants,

respectively. Exogenous plant growth regulators and their rate of application were used according to method of Yang et al. (2003a). Plant growth regulators were obtained from Sigma Chemical Company. 6-benzylaminopurine (BAP, a synthetic cytokinin) and ABA were applied at two stages either at initial grain-filling starting 2 days post-anthesis (T1) or at early grain-filling starting 11 days post-anthesis (T2). At each stage, either 25×10^{-6} M ABA, or 50×10^{-6} M BAP were sprayed at the rate of 50 ml per pot on the leaves and spikes daily for 4 days with 0.5% Teepol as surfactant. The plants sprayed with the same volume of 0.5% Teepol were taken as a control. Plants were grown in pots, containing approximately 4 kg of soil comprising a mixture of clay, silt and sand in the ratios of 15.6%, 35.6% and 48.8%, respectively, with an electric conductivity of 1.63 dS.m^{-1} and pH 7.2. The concentrations of total N, P, and K were 0.08 %, 22.9 mg kg⁻¹, and 181 mg kg⁻¹, respectively. At the 3-4 leaf stage, the number of plants was reduced to five per pot by thinning twice. Supplementary light was provided in the greenhouse for 16h per day. The daytime and night-time temperatures of the greenhouse were 28 and 18 °C, respectively. Spikes from each treatment were sampled at 7-day intervals from anthesis to maturity. All grains from each spikelet were removed. The sampled grains were frozen in liquid nitrogen for 2 minutes then stored at -80°C for hormone measurements. In each treatment, plants were harvested at maturity, and were oven dried at 70°C for determining grain yield (GY), biological yield (BY) and harvest index (HI) (%).

Hormonal extraction and purification

Hormonal extraction was done according to method of Kelen et al. (2004) with a little modification. Indole-3-acetic acid (IAA) and Abscesic acid (ABA) in grains was extracted by grinding 1g fresh weight of grain in 20 ml 80 % methanol (HPLC grade) containing 0.25 mg ml^{-1} butylated hydroxytoluene and 0.5 mg ml^{-1} sodium ascorbate (Dunalp and Guinn, 1989) using mortar and pestle. The ground tissue was filtered with suction through Whatman No.1 paper. The residue on the filter paper was rinsed three times with extracting solution. Methanol in the filtrate was removed by rotary flash evaporation (RFE) at 35°C, and the pH of the aqueous residue (about 3 ml) was adjusted to 8.0 with 0.2 N KOH and partitioned twice with an equal volume of ethyl acetate (HPLC grade) to remove some impurities. The ethyl acetate fraction was discarded. Residual ethyl acetate in the aqueous phase was removed by RFE. The pH of the aqueous fraction was adjusted to 2.5 with 0.2 N HCl, and then partitioned twice with the same volume of ethyl acetate. The residual water in the acidic ethyl acetate fraction was removed by addition of anhydrous sulfate. The acidic ethyl acetate fraction was evaporated to dryness by RFE. The residue was immediately dissolved in 1.0 ml Methanol. Sample was filtered with suction through $0.45 \mu\text{m}$ poly tetra flour ethylene filter. Then, the solution was injected to C₁₈ column of HPLC (Knauer, Germany). The separation was carried out with a flow rate of 0.8

ml min⁻¹, UV Detector and 0.2% acetic acid- 100% Methanol (50:50; v/v) were used as mobile phase.

The data were analyzed using the SAS statistical package. Data from each sampling date were analyzed separately.

RESULTS AND DISCUSSION

The average grain yield and its components for each cultivar are shown for both control and water stress conditions in Table 1. As an expectance, GY, BY, and HI of Pishtaz were significantly higher than those of Karaj3 under control conditions indicating higher yield potential in Pishtaz. Water stress resulted in reduction of GY, BY, and HI in both cultivars (Table 1, 2). The amount of GY, BY, and HI reduction in Pishtaz were significantly lower than those in Karaj3 (Table 1). This indicates that Pishtaz has higher tolerance and yield stability under water stress conditions. High yield potential and efficient allocation of photo-assimilates are two important properties for yield formation (Reynolds et al. 2009), assuming that the superiority of Pishtaz is related to high yield productivity and better allocation of photo-assimilates to sink organs.

The grain number per spike and grain weight is two important aspects of grain yield. 1000 grain weight was higher in Pishtaz than Karaj3 under two conditions. On the other hand, there was no significant difference between two cultivars in the grain number per spike as an important parameter for sink size under control condition (Table 1). Thus, the higher grain weight in Pishtaz resulted from higher source strength to supply photo-assimilate or sink strength. Considering that there was no significant difference between two cultivars in grain number per spike under mentioned conditions, differences in yield potential is related to endosperm cell number or sink activity.

Table 1. Effects of water stress on the yield and its components in two wheat cultivars

Cultivar	Water treatment	Yield (g/plant)	Biological Yield (g/plant)	Harvest Index (%)	1000-Grain weight (g)	Grain number per spike
Pishtaz	control conditions	1.52 ± 0.08	3.24 ± 0.13	46.98 ± 1.56	42.4 ± 1.22	35.33 ± 1.29
	water stress	1.08 ± 0.07	2.55 ± 0.11	43.25 ± 1.96	33.17 ± 1.05	31.6 ± 1.34
Karaj 3	control conditions	1.19 ± 0.06	3.01 ± 0.11	39.79 ± 1.91	32.99 ± 0.75	35.27 ± 1.11
	water stress	0.7 ± 0.03	2.04 ± 0.05	34.06 ± 1.24	24.75 ± 0.66	27.53 ± 0.95

Plants were exposed to water stress (WS) days post-anthesis.

Water stress caused significant reduction in 1000 grain weight in two cultivar but the rate of drop was lower in Pishtaz than that of Karaj3 (Table 1). Numerous reports show that lower 1000 grain weight was probably resulted from lesser cell division (Singh and Jenner 1984), lesser storage capacity of photo-assimilates in the grains (Wada et al. 1994), shorter grain growth period (Ahmadi

and Baker 2001a) under water stress. As mentioned previously, water stress causes reduction of grain number per spike. Although the number of grains per spike is determined at the double ridge stage (Hay and Walker 1989), occurrence of water stress at 50% anthesis probably causes abortion of florets as well as serious drop in the photo-assimilates supply.

IAA level in grains was significantly higher at the cell division stage, and then began to decrease (Fig. 1). IAA level till 14 days post-anthesis was higher in Pishtaz than that of Karaj3, then the significant difference between the two cultivars diminished (Fig. 1). IAA level in grains of Pishtaz during early stages of grain growth was significantly higher than that of Karaj3. Considering that the early stage of grain growth is endosperm cell division and sink size formation, it can be said that IAA has an important role in cell division. Thus, one of the causes for high productivity of Pishtaz is high concentration of IAA in cell division stage. It has been reported that IAA content change in grains was similar to the changing pattern of zeatin and zeatin ribosid, and the IAA content was highly correlated with cell division rate during the active cell division period (Zhang et al. 2009). Therefore, it can be concluded that auxin and cytokinin can trigger cell division. Thus, high levels of auxin can cause a triggering force, thereby increasing cytokinin level (Yang et al. 2003a). Water stress resulted in significant drop in IAA level (Fig. 1). IAA level of Pishtaz under this condition was higher than that of the Karaj3 at cell division stage (Fig. 1). Therefore, considering the role of IAA in cell division, one of the possible reasons for lower grain yield in both cultivars is lower IAA level.

ABA level in both cultivars was initially low (Fig. 1), and then triggered to increase and in 21 days post-anthesis reached a maximum. Maximum level of ABA in Pishtaz is higher than that of Karaj3, but after 21 days post-anthesis, ABA levels showed significant decrease in both cultivars. The highest of ABA level in both cultivars was simultaneous to linear grain-filling period and it was more obvious in high-yielding cultivar. ABA level in the Pishtaz was high and showed great grain size (Fig. 1, Table 4). This is in agreement with Schussler et al. (1984) who showed that in soybean, a cultivar with higher grain weight has a higher ABA content in seed coat and developing cotyledons. This shows that ABA promotes uploading of assimilates from sieve tubes to the sink appoplast, and or uploaded assimilates uptake by storage cells (Clifford et al. 1986). Ackerson (1985) suggested that ABA does not affect assimilates accumulation in wheat grains. Thus, more information is needed that can say ABA concentration in developing grains can be an internal factor affecting in assimilates accumulation rate.

The results indicated that the cultivars responded differently to hormonal application at different stages of growth (Table 2). When Cytokinin was applied during cell division stage (2-5 days after anthesis, T1) under control conditions, GY and 1000-grain weight increased significantly (Table 2). Increasing of 1000-grain weight was probably resulted from enhanced sink capacity and rate of cell division (Yang et al. 2002). Several studies have emphasized the role of

cytokinin in determining sink strength by increasing cell division (Yang et al. 2002; Yang et al. 2003b). Yang et al. (2002) showed that exogenous application of BAP in cell division stage can probably increase the internal concentration of zeatin and zeatin ribosid in the grain, and their higher concentration during active cell division period coincided with an increase in sink size. Considering that grain weight of Karaj3 was responsive to cytokinin treatment (Table 2) and knowing that this cultivar has genetically low grain weight (Table 1), it may be resulted that one of the factors for reduced grain weight is the lack of hormone. One of the reasons for sink limitations could be the low activity of sink. Hormones play a substantial role in sink activity, thus the lack of endogenous hormones could be one of these limitations. Pishtaz also showed an increase in grain weight under BAP treatment (Table 2). This is in line with the results of (Dua and Bhardwaj 1979), stating that the genotypes of wheat have larger grains containing more cytokinin, and their growth increases with exogenous application of cytokinin.

Table 2. Effects of exogenous BAP and abscisic acid (ABA) application on grain weight in two wheat cultivars

Cultivar	Hormonal treatment	1000-Grain weight (g)
Pishtaz	Control	38.88 ± 2.31
	BAP (T1)	42.53 ± 2.06
	BAP (T2)	36.98 ± 2.37
	ABA (T1)	30.55 ± 1.76
	ABA (T2)	39.98 ± 2.19
Karaj 3	Control	28.75 ± 2.36
	BAP (T1)	32 ± 1.78
	BAP (T2)	28.17 ± 1.91
	ABA (T1)	24.87 ± 1.44
	ABA (T2)	30.55 ± 1.91

Plant growth regulators were applied either at the initial grain filling starting 2 days post anthesis (T1) or at linear grain filling starting 12 days post anthesis (T2). The whole plant were sprayed with 50×10^{-6} M BAP and 25×10^{-6} M ABA daily for 4 days at each stage.

Under water stress conditions, BAP application at cell division period (T1) resulted in increase in 1000-grain weight (Table 3). Increasing in grain weight has been caused by increasing in photosynthetic capacity and delaying senescence (Roitsch and Ehneb 2000). Application of BAP at cell division period (T1) had a significant effect on the HI in both conditions and cultivars (Table 2, 3). Increase in the harvest index as a result of application of BAP is a reflection of changes in the dry matter partitioning by increased sink size. In this study, the effect of BAP on grain number per spike was not significant (Table 2, 3) because it is obvious that potential sink size in terms of grain number per spike is determined in the initial stages of spike primordium (Hay and Walker 1989) and application of BAP after anthesis could not increase it.

Table 3. Effects of water stress on grain weight of wheat pot-grown with exogenous BAP and abscisic acid (ABA) application

Water treatment	Hormonal treatment	1000-Grain weight (g)
control conditions	Control	38.9 ± 2.29
	BAP (T1)	41.42 ± 2.54
	BAP (T2)	37.18 ± 2.29
	ABA (T1)	31.22 ± 1.43
	ABA (T2)	39.75 ± 2.24
water stress	Control	28.73 ± 2.36
	BAP (T1)	33.12 ± 2.27
	BAP (T2)	27.97 ± 1.82
	ABA (T1)	24.2 ± 1.19
	ABA (T2)	30.78 ± 2.07

Plant growth regulators were applied either at the initial grain filling starting 2 days post anthesis (T1) or at linear grain filling starting 12 days post anthesis (T2). The whole plant were sprayed with 50×10^{-6} M BAP and 25×10^{-6} M ABA daily for 4 days at each stage.

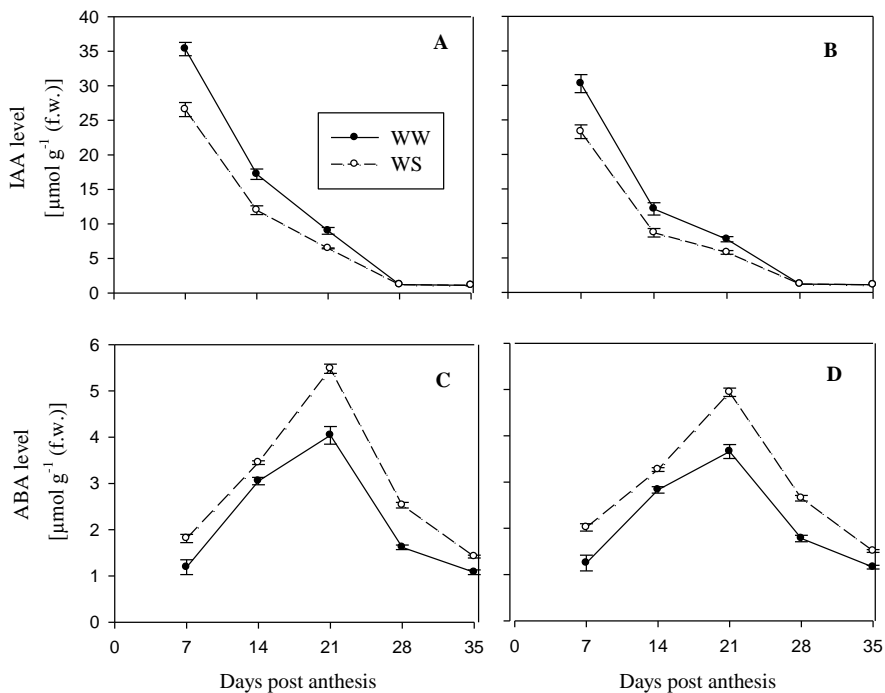


Fig1. Indole-3-acetic acid (IAA) (A,B) and Abscisic acid (ABA) levels (C,D) under control conditions (WW) (Closed circles) and water stress (WS) (Open circles) in Pishtaz (A,C) and Karaj3 (B,D) cultivars. Vertical bars represent \pm S.E. of the means (n=3).

Table 4. Mean values for IAA and ABA levels in two wheat cultivars in response to hormonal application in different stage of grain growth

Hormonal treatment	Cultivar	Days post-anthesis	IAA ($\mu\text{mol g}^{-1}$ FW)	ABA ($\mu\text{mol g}^{-1}$ FW)	
Control	Pishtaz	7	31.83 \pm 1.01	1.32 \pm 0.01	
		14	17 \pm 0.89	3.15 \pm 0.07	
		21	8.76 \pm 0.41	4.43 \pm 0.06	
	Karaj 3	7	28.19 \pm 1.52	1.43 \pm 0.23	
		14	13.38 \pm 0.94	2.83 \pm 0.13	
		21	7.97 \pm 0.52	3.95 \pm 0.36	
BAP (T1)	Pishtaz	7	33.8 \pm 1.98	1.2 \pm 0.18	
		14	17.52 \pm 1.09	2.99 \pm 0.16	
		21	8.99 \pm 1.03	4.2 \pm 0.44	
	Karaj 3	7	29.78 \pm 1.88	1.34 \pm 0.21	
		14	13.76 \pm 1	2.89 \pm 0.16	
		21	8.15 \pm 0.5	4.09 \pm 0.44	
BAP (T2)	Pishtaz	14	14.62 \pm 1.96	3.19 \pm 0.07	
		21	7.58 \pm 0.47	4.53 \pm 0.19	
	Karaj 3	14	9.11 \pm 1.23	3.02 \pm 0.09	
		21	6.63 \pm 0.49	3.93 \pm 0.21	
	ABA (T1)	Pishtaz	7	27.19 \pm 2.04	1.98 \pm 0.08
			14	11.66 \pm 1.14	3.46 \pm 0.06
21			6.63 \pm 0.26	5.11 \pm 0.35	
Karaj 3		7	22.32 \pm 1.32	2.13 \pm 0.08	
		14	7.41 \pm 0.62	3.31 \pm 0.07	
		21	5.82 \pm 0.48	4.59 \pm 0.3	
ABA (T2)	Pishtaz	14	12.19 \pm 0.59	3.48 \pm 0.08	
		21	6.66 \pm 0.12	5.56 \pm 0.16	
	Karaj 3	14	8.17 \pm 0.33	3.23 \pm 0.1	
		21	5.16 \pm 0.16	4.95 \pm 0.14	

Plant growth regulators were applied either at the initial grain filling starting 2 days post anthesis (T1) or at linear grain filling starting 12 days post anthesis (T2). The whole plant were sprayed with 50×10^{-6} M BAP and 25×10^{-6} M ABA daily for 4 days at each stage.

ABA application in cell division stage (T1) had a different effect on yield and its components (Table 2, 3). ABA application in both conditions lowered yield in above mentioned stage (T1) (Table 2, 3). ABA application at cell division period (T1) lead to reduction in yield maybe due to its negative effect on cell division rate of the developing grains and the reduction of sink size (Ahmadi and Baker 1999). ABA application at the initial grain-filling period (T2) increased HI and 1000-grain weight under both conditions in cultivars (Table 2, 3). This is well indicative of different effects of these two regulators on growth. ABA can decrease grain growth, possibly via decreasing the endosperm cell number as well as decreasing the maximal storage capacity, or via inhibiting transport of c^{14} -sucrose (Borkovec and Prochazka 1992). It is observed that exogenous application of ABA can increase its internal concentration in the grains, and its increase can reduce cell division significantly (Yang et al. 2003a).

Table 5. Mean values for IAA and ABA levels in two wheat cultivars in response to hormonal application in different stage of grain growth under water stress

Hormonal treatment	Water treatment	Days post-anthesis	IAA ($\mu\text{mol g}^{-1}$ FW)	ABA ($\mu\text{mol g}^{-1}$ FW)
Control	control conditions	7	33.79 \pm 1.01	0.94 \pm 0.01
		14	17.46 \pm 0.89	2.69 \pm 0.07
		21	10.02 \pm 0.41	3.26 \pm 0.06
	water stress	7	26.23 \pm 0.65	1.82 \pm 0.06
		14	12.92 \pm 0.74	3.29 \pm 0.08
		21	6.71 \pm 0.08	5.12 \pm 0.17
BAP (T1)	control conditions	7	36.08 \pm 0.99	0.85 \pm 0.02
		14	17.97 \pm 0.89	2.59 \pm 0.03
		21	10.28 \pm 0.46	3.17 \pm 0.05
	water stress	7	27.51 \pm 0.87	1.7 \pm 0.06
		14	13.31 \pm 0.8	3.29 \pm 0.05
		21	6.86 \pm 0.09	5.12 \pm 0.06
BAP (T2)	control conditions	14	15.22 \pm 1.8	2.95 \pm 0.08
		21	8.18 \pm 0.2	3.78 \pm 0.16
	water stress	14	8.5 \pm 0.78	3.26 \pm 0.03
		21	6.04 \pm 0.22	4.67 \pm 0.12
ABA (T1)	control conditions	7	28.49 \pm 1.47	1.88 \pm 0.04
		14	11.4 \pm 1.31	3.29 \pm 0.06
		21	7.05 \pm 0.08	4.12 \pm 0.11
	water stress	7	21.02 \pm 0.75	2.23 \pm 0.05
		14	7.67 \pm 0.66	3.47 \pm 0.06
		21	5.41 \pm 0.29	5.57 \pm 0.14
ABA (T2)	control conditions	14	11.18 \pm 1.03	3.2 \pm 0.09
		21	6.21 \pm 0.32	4.92 \pm 0.13
	water stress	14	9.18 \pm 0.79	3.5 \pm 0.07
		21	5.61 \pm 0.36	5.59 \pm 0.15

Plant growth regulators were applied either at the initial grain filling starting 2 days post anthesis (T1) or at linear grain filling starting 12 days post anthesis (T2). The whole plant were sprayed with 50×10^{-6} M BAP and 25×10^{-6} M ABA daily for 4 days at each stage. Plants were exposed to water stress (WS) days post-anthesis.

Also, contrary to BAP, ABA can close stomata, thereby reducing photosynthesis or by increasing ethylene and internal sensitivity of the plant caused stimulating senescence, reducing the grain-filling period, BY, and the photosynthesis rate and activity and thereby reducing GY (Yang et al. 2003b). Moreover, increased HI is possibly caused by accelerating remobilization of storage from secondary sources, especially stems to developing grains (Yang et al. 2003b). The main

reason for grain number loss per spike can be sterility of florets, because the criterion for hormonal treatments is occurrence of 50% spike anthesis.

As above mentioned, applying of BAP during cell division stage under control conditions caused a significant increase in GY and 1000-grain weight in both cultivars. With respect to the formation of sink size at early stage of grain growth, it is considerable BAP application during this stage would lead to increase in sink size through the increase of cell division rate. To confirm this object, BAP application at cell division stage (T1) leads to rise of IAA level of grains in this stage (Table 4). Considering the effect of IAA in grain cell division, the raise of IAA level and consequent increase in cell division rate and sink size formation is one of the probable mechanism by which BAP can increase the GY in this stage (Table 4). Thus, increasing of IAA level due to exogenous BAP demonstrated that the two hormones (auxin and cytokinin) may work together. IAA level in Pishtaz was significantly higher than that of the Karaj3 (Table 4). It is also reported that increasing of GY due to BAP application at the cell division stage can possibly achieve by increasing cell division and finally sink size (Yang et al. 2003a). Exogenous application of ABA decreased IAA level and this decrease was smaller for Pishtaz than Karaj3 (Table 4). It has been shown that ABA and IAA are involved in sink strength and acts conversely and ABA initially lowers cell division of endosperm, thereby reduces sink size (Zhang et al. 2009). BAP application during cell division and grain-filling period caused no difference in both of cultivars in case of ABA level (Table 4). Application of ABA during cell division and grain-filling period greatly increased ABA level (Table 4, 5). This has been reported elsewhere too (Guóth et al. 2009). Increased ABA levels by decreasing of IAA level and possibly cytokinin in developing grains and finally, lower sink size can limit sink strength formation by negatively affecting cell division and ultimately reducing 1000-grain weight. When ABA was applied at the cell division and filling stages (T1 and T2), ABA level increased significantly (Table 4, 5); this is similar to water stress conditions by which can lower cell division and growth (Ahmadi and Baker 1999; Yang et al. 2003a). Although there are reports indicating a positive relationship between ABA concentration and starch accumulation in grains at well-watered conditions (Xie et al. 2003), higher and continuous concentrations may inhibit grain growth and thereby seed setting (Ahmadi and Baker 1999). It is reported that increased ABA as a result of water stress at the initial of grain-filling period (Kermode et al. 1989) decreases sucrose synthase enzyme activity (Zhang et al. 2009). ABA application at the early grain-filling period increased ABA level in both cultivars (Table 4). This is consistent with results of Yang et al. (2006) who reported that higher concentrations of ABA were necessary for faster grain-filling. The mechanism in which ABA facilitates grain-filling is not understood yet. It is suggested that ABA plays a key role in sugar signaling pathway, and increases responsiveness to signals (Rook et al. 2001).

There are many reports show that ABA can increase movement of photo-assimilates toward developing grains (Brenner and Cheikh 1995; Yang et al. 2006). Also studies show that grain-filling may be due to the increase in sink capacity via regulation of enzymes involved in converting sucrose to starch, like sucrose synthase, AGPase and starch synthase (Yang et al. 2004).

CONCLUSION

The results show that application of cytokinin and abscisic acid at cell division and grain-filling stages, respectively, can increase grain weight. In arid and semi-arid areas (such as Iran) where sunshine and day length is good enough to support high photosynthesis rate and remobilization of pre and post-anthesis is low, sink size is a major factor limiting the final grain weight, it is important to use the cultivars with naturally elevated cytokinin content during cell division period and abscisic acid at grain-filling stage. Application of hormones for increasing sink strength through reducing sink limitation and increasing pre-stored reserves remobilization under drought stress are recommended.

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**UTICAJ REGULATORA RASTA BILJAKA NA
ENDOGENE HORMONE KOD DVA KULTIVARA
PŠENICE RAZLIČITIH VELIČINA ZRNA POD
KONTROLISANIM I USLOVIMA VODNOG STRESA**

SAŽETAK

U aridnim i poluaridnim područjima produktivnost pšenice ograničava terminalni vodni stres tokom nalivanja zrna. Eksperiment je vršen da bi se utvrdile promjene u nivoima endogenih IAA i ABA tokom nalivanja zrna dva kultivara pšenice različitih veličina zrna. Tretman je obuhvatio dva iranska kultivara pšenice, nivoi vodnog stresa i hormoni. Biljke su izložene vodnom stresu nakon punog procvata. Benzilaminopurin (BAP) i apscisinska kiselina (ABA) primijenjene su nu dvije faze ili nakon inicijalnog nalivanja zrna počev 2 dana nakon punog procvata, ili u ranoj fazi nalivanja zrna, počev 11 dana nakon punog procvata. Vodni stres je imao za rezultat smanjenje prinosa zrna, biološkog prinosa, i HI kod oba kultivara. Vodni stres je izazvao značajno smanjenje mase 1000 zrna kod dva kultivara, ali je stopa pada bila niža kod kultivara visokog prinosa. Primjena BAP nakon punog procvata povećao je masu zrna, iako je stopa povećanja varirala kod kultivara. Prilikom inicijalnog perioda nalivanja zrna, egzogena primjena ABA povećala je HI i masu 1000 zrna pod dva uslova. Nivoi indol-3-sirćetne kiseline (IAA) zrna bili su visoki tokom ranih faza nalivanja zrna, dok je ABA nivo bio viši u linearnim fazama nalivanja zrna. Rezultati sugerišu da su razlike između kultivara u slučaju prinosa zrna i njegove komponente u značajnoj mjeri rezultat varijacija nivoa hormona (IAA i ABA). Iz tog razloga, pojačavanje snage potrošačkih organa („sink strength“) i HI primjenom hormona mogli bi biti ključni faktori za poboljšanje prinosa zrna pšenice.

Ključne riječi: Indol-3-sirćetna kiselina, apscisinska kiselina, suša, pšenica, snaga potrošačkih organa („sink strength“), primjena hormona