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ROLE OF ABSCISIC ACID (ABA) IN CONTROLLING THE HORMONAL BALANCE AT BOOTING AND GRAIN-FILLING STAGES OF DIFFERENT WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS UNDER DROUGHT

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Abstract

Water shortage seriously influences wheat productivity in various growing regions.The response of two wheat (*Triticum aestivum* L.) accessions *viz.*, 011417 and 011320 to drought and exogenously applied abscisic acid was determined in a pot study at booting and grain-filling stages to study the role of stress hormone abscisic acid (ABA). Changes in endogenous ABA (bound and free) and the growth promoting hormones i.e. trans zeatin ribiside (t-zr), Indole-3-Acetic Acid (IAA) gibberellins (GA) were monitored. Sampling was done 3, 6 and 9 days after induction of water stress and at 48 and 72 h of re-watering. Marked decreases in t-zr, IAA and GA were found to be associated with an increase in the free and bound ABA content in the leaves under water stress. Sensitive accession 011320, showed the greater decrease in growth promoting hormones and less accumulation of ABA. The inhibitory effects of water stress on plant phytohormonal balance were ameliorated by exogenous ABA application specifically at booting stage and particularly in the sensitive accession 011320. There is a cultivar specific threshold ABA level, which is required to exhibit any significant ameliorating effect under water stress.

Introduction

Although wheat breeders have been successful in increasing yield under productive, cultivated conditions, such gains have been more difficult to achieve in production regions where environmental stresses like water stress occur (Rajram *et al.,* 1996). Exploring water stress resistance physiological mechanisms for different wheat genotypes is of prime importance to find out new drought resistant gene resources (Shao *et al*., 2005). Phytohormones are considered as important signals in root-to-shoot communication during stress conditions (Pospisilova *et al*., 2005). The activities of phytohormones depend on their contents, as well as on their interactions. Drought triggers a change in hormonal balance, including an increase in leaf content of ABA and/or a possible decline in cytokinins (CKs), auxins, and GA contents (Pospisilova, 2003).) This change in hormonal balance might play a key role in sequence of events induced by water stress. Gunderson & Taylor (1991) reported that the applied phytohormones could also change the contents of endogenous phytohormones by inhibiting or stimulating their regulation system and several studies have convincingly demonstrated that the application of exogenous ABA provides tolerance to various stress conditions (La-Rosa *et al*., 1985; Flores *et al.,* 1988; Farooq & Bano, 2006). ABA is considered important because it helps to maintain near-homeostasis of leaf water status when plants are subjected to mild water deficits or to changes in evaporative demand (Borel *et al*., 2001). It is well established fact that phytohormones play important role in the regulation of plant growth responses to changing environmental conditions (Veselov *et al*., 2002)

Despite the fact that the effects of water stress on plants have variously been reported the precise mechanism of a hormonal shift by an environmental change is not fully elucidated in the local cultivars of wheat and the information is scanty regarding the effects of ABA seed soaking and water stress imposed at booting and grainfilling stages on different phytohormones so this comparative study was attempted to observe the effects of ABA applied as seed soaking and water stress induced for various periods at successive stages of booting and grainfilling in two accessions differing in tolerance to moisture stress with the perspective of improving water stress tolerance. The recovery response of the accessions on rewatering the pre-stressed plants has also been evaluated with respect to changing balance of phytohormones.

Materials and Methods

Plant Material and Growing Conditions: The experiment was conducted at Quaid-i-Azam University, Islamabad (latitude, 33.44 55.18" N, longitude73.08'10.57'E and altitude 2021 ft) during the wheat-growing seasons of 2005 and 2006. Seeds of two local accessions (011417 and 011320) of wheat (*Triticum aestivum* L.), which are primitive cultivars/land races belonging to different areas receiving rainfall less than 250mm, were obtained from Plant Genetic Resource Institute (PGRI), National Agriculture Research Centre (NARC), Islamabad. The seeds were sown in earthen pots containing soil, sand and farmyard manure in a ratio of 3:1:1. Recommended doses of nitrogen phosphorus, and potassium fertilizers were applied. Pots were arranged in Randomized Complete Block Design (RCBD) and were protected from rain. A week after germination the plants were thinned to five per pot. The plants were watered as required. Surface sterilized seeds (with 10 % chlorox) were sown in the pots. Prior to sowing, seeds were soaked for 8 h in aqueous solution of ABA (10^{-6} M) and for control seeds were soaked in sterilized water for equal period of time. Water stress was imposed by withholding water supply for a period of 9 days and thereafter the plants were re-watered. The first water stress treatment was started at 50% booting (85-95 DAS) and the second at 50 % grainfilling (125-140 DAS). Sampling was done 3, 6 and 9days after the start of water stress treatment. After 48 and 72 h of re-watering intact plants were uprooted and rhizospheric soil was sampled.

Extraction and purification of phytohormones

IAA and GA: The plant leaves (1g) were ground in 80% (v/v) methanol, at 4°C with butylated hydroxy toluene (BHT), used as antioxidant. The extraction was done at 4°C till 72h in dark with subsequent change of solvent at each 24 h. The extracted sample was centrifuged and the supernatant was reduced to aqueous phase using rotary

film evaporator (RFE) at 35°C. The pH of aqueous phase was adjusted to 2.5-3.0 with 0.1N Hydrochloric Acid (HCl) and partitioned four times with ½ volume of ethyl acetate. The ethyl acetate was dried down completely using rotary thin film evaporator (RFE). The dried sample was re-dissolved in 1mL. of methanol (100%).

Free and bound ABA: After extraction in methanol the extracted sample was centrifuged and the supernatant was reduced to aqueous phase using rotary film evaporator (RFE) at 35°C.The aqueous phase was divided into two equal parts. For the extraction of free ABA the pH of one half of aqueous phase was adjusted to 2.5-3.0 with 0.1N HCl and partitioned four times with ½ volume of ethyl acetate. The ethyl acetate was dried down completely using rotary thin film evaporator (RFE). The dried sample was re-dissolved in 1mL. of methanol (100%).

Determination of bound ABA was made by hydrolyzing the aqueous phase at pH 11 [1N Sodium Hydroxide (NaOH)] for 1h at 60°C. Under these conditions ABA is librated from ester linkages. After alkaline hydrolysis these samples were adjusted to pH 2.5 with 0.1N HCl. The aqueous phase was extracted three times with an equal volume of ethyl acetate. The three ethyl acetate fractions were combined and reduced to dryness on rotatry evaporater (at 35°C). The dried sample was re-dissolved in 1mL. of methanol (100%). This sample was analyzed on HPLC for total ABA. Bound ABA was calculated as follows:

Bound ABA = Total ABA – Free ABA

Trans Zeatin Riboside (t-zr): The t-zr was extracted and analyzed following the method of Tien *et al*., (1979). The leaves (1g) were ground in 80% methanol, at 4°C with an antioxidant butylated hydroxy toluene (BHT) and extracted at 4◦C for 72hr with subsequent change of solvent. The extracted sample was centrifuged at 8000 rpm for 20min at 4°C and supernatant was acidified to pH of 2.5 with acetic acid solution (1% v/v). Each sample was partitioned four times with the same volume of acetic acid-saturated ethyl acetate (1% v/v). The acidic ethyl acetate was evaporated to dryness at 35 °C using rotary thin film evaporator (RFE). The residues were dissolved in 100µl methanol/ water (30:70).

HPLC operating conditions: Samples were analyzed on HPLC using U.V. detector and C-18 column. For identification of hormones, samples filtered through 0.45-millipore filters were injected into column. Methanol, acetic acid and water (30:1:70) were used as a mobile phase. The wavelength used for the detection of IAA was 280nm (Sarwar *et al.*, 1992), whereas for GA analysis it was adjusted at 254nm (Li *et al*., 1994). For ABA the injected sample was eluted with 0.1% acetic acid and methanol (30-70 % methanol, linear gradient over 30 min) at 254nm wavelength. For cytokinin elution was performed at a flow rate of 1mL min^{-1} and UV detector was adjusted at 254 nm. These growth hormones are identified on the basis of retention time and peak area of the standards. Pure IAA, GA, t-zr and ABA were used as standard for identification and quantification of plant hormones.

Yield parameters: Plants were harvested (150-170 DAS) from all pots and 100-grain weight and No. of grains / spike were determined in triplicate.

Statistical analysis of data: The data were subjected to factorial ANOVA and the mean values were compared with Duncan's Multiple Range Test (DMRT) using MSTAT-C version 1.4.2.

Results

Water stress caused a significant ($p<0.05$) decrease in the IAA content of leaves. In case of accession 011320 (Table 1) early and marked decrease (51-57%) in IAA content at both booting and grain filling was noted within first 3d of water stress, in contrast to short term water stress (3d) decreased IAA content by 20 % whereas up to 44 % decrease was recorded in long term (9d). Rewatering restored the decrease in IAA content, accession 011417 (Table 1) restored the IAA content within 48h of rewatering but in accession 011320 (Table 1) very little recovery was observed. ABA seed soaking treatment was found to be effective only at booting stage in both the accessions causing a decrease in the IAA content under unstressed (control) condition while under water stress condition the endogenous IAA content was greater than non-ABA-treated plants. Response to ABA was greater in accession 011320 (Table 1). At grain filling stage significantly ($p<0.05$) less IAA content was found than that of booting stage.

Significantly ($p<0.05$) higher decrease (52%) in GA content was observed in accession 011320 (Table 2). In this accession major decrease (47%) was recorded within first 6d of water stress, while in other accession a progressive decrease occurred in GA content with the period of water stress. No significant ($p<0.05$) effect of rewatering was observed in accession 011320 (Table 2). Under unstressed condition, ABA seed soaking treatment resulted in a significant ($p<0.05$) decrease in the GA content of leaves in both the accessions at booting stage and maximum decrease (30%) occurred in accession 011320 (Table 2). But under water stress treatment ABA assisted to maintain higher GA content as compared to untreated water stressed plants in accession 011320, whereas, no significant effect of ABA seed soaking was noted under water stress in accession 011417. At grain filling stage significantly ($p<0.05$) less GA content was recorded as compared to that of booting stage and water stress resulted in less decrease in GA content than that of booting stage. ABA seed soaking treatment had no significant ($p<0.05$) effect at grain filling stage on GA content.

Within 3 days of water stress significant decrease (50%) in t-zr concentration (the most active and mobile cytokinin) occurred in accession 011320 in contrast to 40 % decrease in 011417. Long-term exposure to water stress decreased the t-zr by 50 % in accession 011417 (Table 3) as compared to 11 % greater decrease in accession 011320 (Table 3). Even on rewatering recovery of t-zr content was greater in accession 011417 (Table 3) showing 26 % less value than control as compared to accession 011320 which exhibited 45 % decrease over control even after rewatering. Under unstressed condition ABA seed soaking treatment caused a significant ($p<0.05$) decrease (13%) in t-zr content of leaves in both the accessions at booting stage. ABA seed soaking was found to be ineffective under both well watered as well as water stressed condition at grain filling stage. Accessions differed significantly in the content of free and bound ABA even under control (unstressed) condition. Basal levels of ABA in accession 011320 were higher as compared to that of

accession 011417. Maximum bound ABA under water stress treatment was found in accession 011417 (Table 4). On imposition of water stress significant increase in free ABA content was recorded in accession 011320 after 6d of water stress while significant increase in ABA content was delayed in accession 011417 till 9d of water stress (Table 4). Rewatering caused a significant decrease in free ABA content, such that the value was close to that of control level, greater decline was recorded in accession 011417 (Table 4) although bound ABA content remained significantly ($p<0.05$) higher than that of control. Seed soaking treatment with ABA caused significant ($p<0.05$) increase in the endogenous free as well as bound ABA content both under unstressed and water stressed condition at booting stage and maximum response was noted in accession 011320 (Tables 4, 5). At grain filling stage significantly ($p<0.05$) higher free and bound ABA contents were recorded as compared to that of booting stage. Greater content of free and bound ABA at each level of water stress was observed in accession 011417 (Tables 4, 5). ABA seed soaking was found to be ineffective in the accession 011417 under unstressed condition but in accession 011320 it caused an increase in free ABA content (Table 4). Under water stress condition free ABA content increased significantly ($p<0.05$) in response to ABA seed soaking in both the accessions (Table 4).

Table 1. Effect of water stress and abscisic acid (ABA) seed soaking (10^{-6} M) on IAA content (ng/g) of leaves at booting and grain filling stages of wheat accessions 011417 and 011320.

Treatments	011417					011320				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	54.1 ab	53.2 b	52.7 B	50.1 cd	55.1 a	41.9 ab	42.3 a	40.7 b	43 a	42.8 a
T_2	43.4 mno	34.5 qr	29.4 T	50.2 c	50.7 c	20.5 kl	19.3 l	15.4 m	20.2 kl	25.7 i
T_3	48.2 defg	49 cdef	48.1 Efgh	47.9 efgh	48 efgh	37.2 c	36.7 cd	37 c	36.4 cd	36 cd
T_{0b}	45.1 jklm	36.2 pq	33.4 R	47.6 efghi	47 fghij	27.4 h	25.2 i	21.2 k	29.5 g	30.2 fg
T_4	49.2 cde	47.3 efghi	46.1 Hijk	45.3 jklm	43.9 lmno	36.4 cd	35.3 d	33.2 e	31.4 f	30.5 fg
T_5	37.2 p	31.4 s	26.9 U	44.1 klmno	43 no	15.7 m	13.9 no	12.7 op	19.8 kl	22.7 j
T_6	49 cdef	46.9 ghij	45.7 Ijkl	45 jklm	42.3 o	35.4 d	33.7 e	32.8 e	30.9 fg	29.7 g
	LSD value = 1.744			at alpha = 0.050		LSD value = 1.329			at alpha = 0.050	

Table 2. Effect of water stress and abscisic acid (ABA) seed soaking (10^{-6} M) on GA content (ng/g) of leaves at booting and grain filling stages of wheat accessions 011417 and 011320.

Treatments	011417					011320				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	63.1 bc	64.4 ab	66.1 a	65.3 a	65.4 a	30.2 bcd	34.5 ab	36.2 a	37.1 a	31.2 bc
T_2	57.2 fg	50.1 jkl	44.2 n	49.1 jklm	49.3 jklm	20.4 klm	18.3 lmn	17.2 mno	17.4 mno	18.1 lmn
T_3	60.1 de	59.9 de	61 cd	61.2 cd	61.3 cd	27.4 ef	28.4 ef	26.3 efg	25.9 efgh	26.7 efg
T_{0b}	58.1 ef	51.2 jk	45.3 n	50.1 jkl	51.3 j	23.3 hi	23.4 hi	21.3 ijkl	22.4 ijk	22.5 ijk
T_4	55.2 ghi	56.3 fgh	53.9 i	54.7 hi	55.3 ghi	25.4 efgh	26.3 efg	25.3 efgh	24.9 fgh	23.7
T_5	50.1 jkl	48.9 klm	47.7 m	48.2 lm	48.1 lm	21.4 ijkl	20.3 klm	19.7 klmn	19.5 klmn	20.1 klm
T_6	56.2 fghf	53.7 i	55.4 ghi	54.8 hi	55.1 ghi	26.3 efg	25.4 efgh	23.7 ghi	23.2 hi	22.7 ijk
	LSD value = 1.959			at alpha = 0.050		LSD value = 1.576			at alpha = 0.050	

Water stress applied at booting stage significantly ($p<0.05$) reduced the number of grains/ spike and 100grain weight in both the accessions (Figs. 1, 2). Greater reduction in 100 grain weight and number of grains/ spike was observed in accession 011320 (Figs. 1, 2) greater number of grains/ spike (Fig. 1) and higher 100 grain weight (Fig. 2) was noted in accession 011417. ABA seed soaking mitigated the adverse effects of water stress, the effect was more pronounced in accession 011320. Water stress induced at grain filling had caused a significant ($p<0.05$) reduction in 100-grain weight (Fig. 2), which was 2 fold higher in accession 011320 as compared to the accession 01141.

Table 3. Effect of water stress and abscisic acid (ABA) seed soaking (10^{-6} M) on trans-zeatin ribiside content (ng/g) of leaves at booting and grain filling stages of wheat accessions 011417 and 011320.

Treatments	011417					011320				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	77.3 bcd	74.2 e	75.4 de	76.4 cd	77.2 bcd	62.5 bcd	63.6 abc	65.3 ab	63.4 abc	63.9 abc
T_2	46.3 l	40.2 o	37.6 p	53.2 i	56.1 h	30.2 kl	27.4 lmn	25.3 nop	27.2 lmn	30 kl
T_3	69.2 f	67.5 fg	68.1 fg	67.2 fg	66.9 g	57.4 efg	58.5 e	58.7 e	57.2 efg	56.5 efgh
T_{0b}	44.2 m	41.1 no	40.2 o	52.4 i	57.1 h	31.1 k	29.5 klm	28.2 klm	34.5 ij	35.1 i
T_4	79.2 ab	78.5 abc	77.3 bcd	80.1 a	78.2 abc	63.7 abc	65.5 ab	64.7 abc	63.3 abc	62.9 bcd
T_5	47.5 kl	42.7 mn	39.2 op	49.5 jk	53.2 i	31.3 k	26.9 mn	24.3 op	26.4 mno	28.2 klm
T_6	76.9 bcd	77.4 bcd	78.5 abc	76.9 bcd	77.1 bcd	57.3 efg	57.4 efg	56.9 efgh	55.2 fgh	57.5 efg
	LSD value = 2.024		at alpha = 0.050			LSD value = 2.034		at alpha = 0.050		

Table 4. Effect of water stress and abscisic acid (ABA) seed soaking (10^{-6} M) on free ABA content (ng/g) of leaves at booting and grain filling stages of wheat accessions 011417 and 011320.

Treatments	011417					011320				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	6.2 vwx	6.7 vw	5.5 wx	6.3 vwx	5 x	7.2 wx	8.9 uv	7 x	7.5 vwx	8.5 vw
T_2	15.4 q	29.8 i	42.5 f	10.2 s	7.3 uv	20.2 n	35.3 f	38.3 e	26.2 kl	25.3 lm
T_3	9.1 st	8.7 stu	9.3 st	8.4 tu	9.7 st	10.3 tu	11.5 rst	12 qrs	10.5 st	10.7 st
T_{0b}	16.5 pq	32.3 h	46.7 e	13.5 r	10.4 s	27.5 jk	40.2 d	43.3 bc	29.3 hi	26.2 ki
T_4	17.5 nop	18.9 mn	18.7 mno	19.2 mn	19.7 m	10.9 st	11.7 qrst	10.2 tu	12.8 pqr	13 opqr
T_5	34.9 g	50.5 d	62.3 b	25.3 k	23.2 l	28.4 ij	39.7 d	44.1 b	30.2 h	24.3 m
T_6	16.8 pq	17.2 op	18.7 mno	19.2 mn	19.5 n	14.1 op	13.9 op	13.7 op	14.5 o	13.2 opq
	LSD value = 1.514		at alpha = 0.050			LSD value = 1.361		at alpha = 0.050		

Table 5. Effect of water stress and abscisic acid (ABA) seed soaking (10^{-6} M) on bound ABA content (ng/g) of leaves at booting and grain filling stages of wheat accession 011417 (V1).

Treatments	011417					011320				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	1.2 u	2 stu	1.5 u	1.3 u	1.7 tu	1.9 m	2.3 lm	1.8 m	2.7 klm	2.2 lm
T_2	8.3 no	14.7 m	17.4 jk	23.3 fg	24.2 ef	6.2 fg	10.1 gh	13.2 fg	13.5 f	14.1 f
T_3	3.1 rst	3.4 rs	3.2 rst	4.1 r	3.8 r	5.3 jkl	5.7 jk	6 jk	5.4 jkl	6.4 ij
T_{0b}	9.5 n	16.2 kl	19.3 i	26.4 cd	25.3 de	9.7 hi	14.3 f	19.4 de	19.1 e	18.9 e
T_4	5.9 q	6.4 pq	7.2 opq	6.5 pq	7.6 op	7.3 hij	7 hij	7.5 hij	6.9 hij	7.9 hij
T_5	14.2 m	18.4 ij	22.3 gh	26.4 cd	27.2 bc	19.2 e	22.5 cd	27.4 ab	25.9 b	24.2 bc
T_6	6.3 pq	6.7 opq	7.5 opq	7.2 opq	6.8 opq	8.1 hij	7.9 hij	8.4 hij	8.5 hij	8.7 hij
	LSD value = 1.407		at alpha = 0.050			LSD value = 2.981		at alpha = 0.050		

T0a = control at booting, T1 = water stress at booting, T2 = ABA seed soaking (booting), T3 = water stress at booting + ABA seed soaking, T0b = control at grain filling, T4 = water stress at grain filling, T5 = ABA seed soaking (grainfilling), T6 = water stress at grain filling +ABA seed soaking, d = days after induction of water stress and rw = rewatering.
For each cultivar all such means, which share common letters, do not differ significantly.

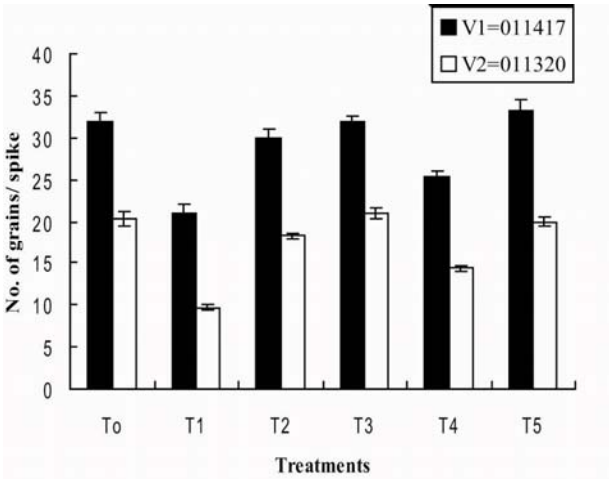

Fig. 1. Effect of water stress and abscisic acid (ABA) on number of grains/spike of the four wheat accessions.

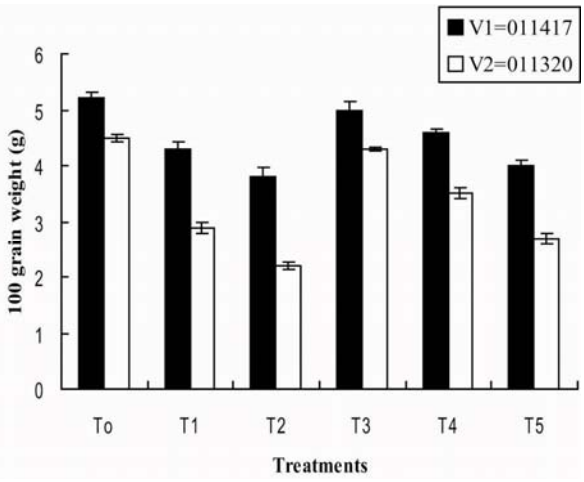

Fig. 2. Effect of water stress and abscisic acid (ABA) on 100grain weight (g) of the four wheat accessions.
T0 = control, T1 = water stress at booting, T2 = water stress at grain filling, T3 = ABA seed soaking, T4 = water stress at booting + ABA seed soaking, T5 = water stress at grain filling +ABA seed soaking.

Discussion

Changing balance of ABA t-zr (Table 6) i.e., lesser increase in ABA/t-zr possibly contributed towards the enhanced tolerance to water stress in accession 011417. Moreover the recovery process was also very efficient in this accession, which was found to be associated with the decrease in the growth inhibitory hormone, ABA close to control levels upon rewatering concomitant with the increase in growth promontory hormones (IAA, GA and t-zr.). The maximum ameliorating effect of ABA seed soaking under water stress was noted in accession 011320, which may be attributed to the improved endogenous ABA content which might have affected metabolic processes in a positive way and ultimately resulting in better yield. This indicates that a threshold of ABA level is required to maintain the growth processes under water stress. Significant decrease in ABA content upon rewatering indicated the release from stress, which was found to be greater in accession 011417 (Table 4). In the stressed plants this rapid decrease after rewatering is an indication of resuming the normal processes when the stress is released (Liang & Zhang, 1999; Alves & Setter, 2000; Zhang *et al.,* 2006) due to degradation or conjugation of ABA. At booting stage seed soaking treatment with ABA caused significant ($p<0.05$) increase in the endogenous ABA content both under well watered and water stress conditions and maximum response was noted in accession 011320 (Tables 4, 5). Maximum percentage increase in free and bound ABA content only in short term water stress (3-6d) in accession 011320 (Tables 4, 5) indicates the inability of 011320 to accumulate free active ABA as an adaptive mechanism to combat long term water stress. Possibly a critical level of ABA is required to exhibit its effects. ABA seed soaking was found to be ineffective in causing any change under control condition in accession 011417 whereas in accession 011320 it caused an increase in free ABA content (Table 4). As variation in soil moisture may influence yield by directly affecting the physiological processes involved in plant growth and grain production (Majeed & Bano, 2008) or due to the inhibitory effects of water stress on the process of cell division (Ahmadi & Baker, 2001). The changing hormonal balance which control all these processes seems quite important The positive effects of ABA on grain yield have been reported previously (Amzallag *et al*., 1990), which are likely to be due to its effects on stomatal aperture thus increasing the water use efficiency (Xie *et al.,* 2004).

Table 6. ABA/ t-zr in the leaves of accessions 011417 and 011320.

Treatments	011417					011320				
T_{0a}	3d	6d	9d	11d	12d	3d	6d	9d	11d	12d
T_1	0.08	0.09	0.072	0.082	0.064	0.115	0.139	0.107	0.118	0.133
T_2	0.332	0.741	1.13	0.191	0.13	0.668	1.288	1.51	0.96	0.84
T_3	0.131	0.128	0.136	0.125	0.144	0.179	0.196	0.19	0.183	0.189
T_{0b}	0.373	0.785	1.16	0.257	0.182	0.884	1.36	1.53	0.849	0.74
T_4	0.22	0.24	0.241	0.239	0.251	0.171	0.178	0.157	0.2	0.206
T_5	0.734	1.18	1.58	0.511	0.436	0.907	1.475	1.81	1.14	0.861
T_6	0.218	0.222	0.238	0.249	0.252	0.246	0.242	0.24	0.24	0.229

Water stress caused a significant ($p<0.05$) decrease in the IAA and GA content of leaves. Similar decrease in IAA and GA was recorded previously by others (Yang *et al*., 2001; Xie *et al.*, 2003; Farooq & Bano, 2006). Under control condition a decrease in IAA and GA content due to ABA seed soaking might be the ABA inhibition of their biosynthesis. But the major decrease in t-zr content under water stress occurred earlier than other hormones suggesting the sensitivity of CKs biosynthesis/accumulation to the smaller changes in water status of leaves. It was reported earlier that the decrease in the CKs content occurred prior to the inhibition of leaf growth (Kudoyarova *et al.,* 1998) this

early decrease might have contributed towards the maintenance of turgor during the short term changes in water status. Interaction occurs between ABA and CK under stress and a decrease in t-zr content under water stress can also be a cause of ABA accumulation as demonstrated by Cowan *et al.,* (1999) that CK might exert an effect on ABA metabolism by influencing the oxidation of xanthoxal to ABA and its further conversion to PA and DPA. Higher ABA/ t-zr ratios under water stress and its decline on rewatering might be an adaptive process of water stress tolerance. This ratio remained higher in accession 011320 even on rewatering (Table 6). Similar results were recorded previously where this ABA/ Cks ratio was also increased in apoplectic solution of water-stressed cotton and sunflower, but the CK concentration was not significantly changed (Hartung *et al*., 1992; Masia *et al.,* 1994). Similar results were obtained by Bano *et al*., (1993) in rice where they found that the contents of cytokinin substantially decreased with the decrease in soil moisture but they only slightly increased after rewatering. The free to bound ABA ratios also showed differences in these two contrasting accessions and was found to be higher (about 3) in accession 011320 under stress condition in contrast to the tolerant accession 011417 and the recovery process was also defected as upon rewatering there occurred a considerable decrease in free to bound ABA ratio in accession 011417, while in case of susceptible accession 011320 it remained higher (2). The ABA application helped in decreasing the free to bound ABA ratio in susceptible accession 011320. As ABA is known growth inhibitor (Kato-Noguchi & Tanaka, 2008) so its lower content would be required for resuming normal growth and functioning of plant under normal condition. The accession 011417 was less responsive to exogenous ABA as compared to that of 011320, which had lower endogenous ABA content, this lower endogenous ABA content might have made the plant more sensitive towards the exogenous ABA as suggested by Nayyar & Walia (2004).

It is inferred from the present results that the response to ABA and water stress is stage specific in wheat, booting stage being more responsive to ABA and less sensitive to water stress. Thus ABA seed soaking would be more effective under conditions when water stress is likely to occur at booting rather than grain filling stage. ABA/ t-zr ratio may be suggested as a biochemical marker for breeding and selection purpose under water stress. The results revealed that the inhibitory effect of water stress was delayed in accession 011417. The magnitude of inhibition was also lower than that of 011320 thus abling this accession to tolerate water stress in a better way. It can also be inferred that there is a threshold ABA level which is required to exhibit any significant ameliorating effect under water stress and the water stress tolerant genotypes must have the ability to show active ABA catabolism and increase in IAA, GA and t-zr levels once the stress is relieved when they are transferred to normal condition.

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