ABSCISIC ACID (ABA) SEED SOAKING INDUCED CHANGES IN PHYSIOLOGY OF TWO WHEAT CULTIVARS UNDER WATER STRESS

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Abstract

Wheat production is severely limited by water stress. Exploring water stress resistance physiological mechanisms for different wheat genotypes is of prime importance to find out new drought resistant gene resources. During present investigation two local primitive cultivars of wheat Viz. 011251 and 011393 belonging to arid regions of the country, were used to study the response of cultivars evolved in the natural arid environment to water stress and Abscisic acid (ABA) seed soaking at two important growth stages of the crop (booting and grain filling) in a pot experiment. Changes in endogenous ABA (bound and free) and the growth promoting hormones i.e. trans zeatin ribiside (t-zr), Indole-3-Acetic Acid (IAA) and gibberellins (GA) were monitored. Stomatal conductance and transpiration rate were also measured. Re-watering was done to study the recovery process as well. Significant decrease in all growth promoting phytohormones (IAA, t-zr and GA), transpiration rate and stomatal conductance were observed while an increase was noticed in endogenous ABA (bound and free) under water stress condition at both growth stages. ABA seed soaking helped in mitigating the harmful effects of water stress in both cultivars. But this response was genotype specific and diminished with the progression of the developmental stage of the crop.

Introduction

In developing countries 37% of the area is semiarid in which available moisture is the primary constrain to wheat production (Dhanda & Sethi, 2002). Exploring physiological mechanisms of water stress tolerance for different wheat genotypes is of importance to find out new drought resistant gene resources (Shao *et al.,* 2005). Many advances in relation to this key issue, including molecular mechanism of drought tolerace and corresponding molecular breeding have taken place (Patnaitk & Khurana, 2001; Zhu, 2002; Khakwani *et al*., 2011). Although the obtained transgenic crops (mainly, wheat) exhibit tolerance to water stress to some extent, they have many shortfalls related to agronomical performance and/or development (Patnaitk & Khurana, 2001; Shao *et al*., 2004). These results suggest that our present understanding of physiological mechanism of crops under water stress is not enough, the study of which is the bridge between molecular machinery of water stress and anti-drought agriculture, because the performance of genetic potential of crops is expressed by physiological realization in fields (Fiehn, 2002; Chaves *et al*., 2003; Shao, 2003). Thus study of physiological mechanisms of tolerance of wheat to water stress has much work to do. (Shao *et al*., 2006).

Stomatal control of water loss has been identified as an early physiological response of plants to water stress under field conditions leading to limitation of carbon uptake by the leaves (Chaves, 1991; Cornic & Massacci, 1996). And this stomatal limitation was generally accepted to be the main determinant of reduced photosynthesis under water stress (Cornic, 2000). Stomata close in response either to a decline in leaf turgor and/or water potential, or to a low-humidity atmosphere (Maroco *et al.,* 1997). As a rule, stomatal responses are more closely linked to soil moisture content than to leaf water status. This suggests that stomata are responding to chemical signals (e.g. ABA) produced by dehydrating roots (Davies & Zang, 1991).

Phytohormones are considered as main signals during stress conditions (Davies, 1995; Naqvi, 1995; Pospisilova *et al*., 2005). And almost all processes in the life of a plant are directly or indirectly influenced phytohormones (Pospíšilová, 2003). Especially abscisic acid (ABA) has been considered to be one of the main hormone which trigger various acclimations processes under water stress conditions (Zhu, 2002). More over hormonal priming of seeds has been shown to be beneficial to growth and yield of some crop species grown under stress conditions (Khan *et al*., 2009). Exogenous application of ABA has also been reported to significantly increase tolerance (Farooq & Bano 2006; Jiang & Zhang, 2004).

Keeping in view the role of ABA improving tolerance under water stress conditions and beneficial effects of seed priming in various crops, the present study was conducted to investigate the role of ABA priming on physiological attributes of wheat at booting and grain-filling stages under water stress conditions.

Materials and Methods

Plant material and growing conditions: Seeds of two local primitive cultivars (011251 and 011393) of wheat (*Triticum aestivum* L.), obtained from Plant Genetic Resource Institute (PGRI), National Agriculture Research Centre (NARC), Islamabad were sown after surface sterilization with 10% chlorox 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. 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. A week after germination the plants were thinned to five per pot. 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 when at 50% of plants have attained booting stage (85-95 DAS) and the second at

50% grain-filling (125-140 DAS). Sampling was done 3, 6 and 9 days after the start of water stress treatment and after 48 and 72 h of re-watering.

Stomatal conductance and transpiration rate: Measurements of stomatal conductance and transpiration rates were made on flag leaves with the help of steady state porometer (EGM-4 version 4.13 equipped with PMR-5 version1.00, PPsystems) after calibration. The measurements were made from 10:30 a.m. to 12:00 p.m. Later on data were transferred to computer using EGM4 data software Version= 1.04

Phytohormones analysis: The extraction and purification of IAA, GA and ABA was made according to the method of Kettner & Doerffling (1995). The t-zr was extracted and analyzed following the method of Tien *et al.*, (1979). 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.

Results and Discussion

Significant (p<0.05) and gradual decrease in the stomatal conductance (Table 1) and transpiration rate (Table 2) of leaf was recorded in both cultivars with the progression water stress treatment. ABA seed soaking significantly (p< 0.05) reduced the stomatal conductance and transpiration rate of flag leaves in both well watered and stressed plants in cultivar 011393 at booting stage but cultivar 011251 was found to be less responsive to ABA seed soaking treatment. But more and rapid recovery after the end of stress period was observed in this cultivar. At grain-filling stage significantly (p<0.05) less stomatal

conductance and transpiration rate was recorded in both the cultivars as compared to that of booting stage under both well watered and stressed condition. Reduction in stomatal conductance and transpiration rate was observed under water stress at grain-filling. ABA seed soaking treatment had no significant (p<0.05) effect on the changes in stomatal conductance. While all other changes in stomatal conductance and transpiration rate followed the pattern similar to that of booting. Stomatal closure under water stress is one of the most important factors affecting photosynthesis. Genotypic differences related to water stress tolerant traits were reported in wheat previuosly (Labhilli *et al.,* 1995). Loggini *et al.*, (1999) reported that photosynthesis of drought sensitive and drought tolerant wheat cultivars responded differently to water stress imposed. Improvement of stomatal conductance and transpiration rate upon re-watering was also noted by other workers (Subrahmanyam *et al*., 2006). With leaf age, the ability to recover after re-watering decreased (David *et al*., 1998) as found at grain-filling in comparison with booting stage during the present investigation. This could be due the changes in the water stress induced the hormonal balance as depicted by decrease in ABA/ t-zr ratios upon re-watering. Our investigation revealed the positive effect of ABA seed soaking on stomatal conductance and transpiration rate. It was suggested that the ABA treatment minimized the water loss in cotton by lowering the stomatal conductance (Gadallah, 1995). Stomatal conductance is known to be associated with endogenous ABA content (Socias *et al.,* 1997). A strong effect of ABA application on stomatal closure was also observed by Anan *et al*., (2006). The water stress-tolerant species control stomatal function to allow some carbon fixation at stress, thus improving water use efficiency, or open stomata rapidly when water deficit is relieved. The closure of stomata under water stress has also been implicated due to changes in plant nutritional status, xylem sap pH, xylem hydraulic conductivity, and leaf-to-air vapor pressure deficit (Oren *et al*., 1999). Sensitivity of stomata to ABA varies in different species and genotypes, with age of leaf, nutritional status, ionic composition of xylem sap and particularly the leaf water status (Borel *et al*., 1997; Niinements *et al*., 1999; Auge *et al*., 2000; Wilkinson & Davies, 2002) that may be the cause of differences observed between these cultivars.

Table 1. Effect of water stress and abscisic acid (ABA) on stomatal Conductance (mmolm^{-2}s^{-1}) of leaves at booting and grain-filling stages of two primitive wheat cultivars.

Treatments	011251					011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
Control at booting	306abc	310ab	302abc	304abc	315a	324bc	330ab	326abc	332a	323c
Water stress (booting)	230f	192fg	154h	295bcd	302abc	251o	202r	164u	310d	308def
ABA seed soaking (booting)	289cd	292bcd	290bcd	293bcd	290bcd	302fghi	306defg	303efgh	309de	302fghi
ABA seed soaking +water stress (booting)	222f	185fg	149h	278d	289cd	245p	194s	155v	294klm	298hijkl
Control at grain-filling	295bcd	290bcd	292bcd	294bcd	297abcd	303efgh	310d	298hijkl	302fghi	306defg
Water stress at grain-filling	221e	181fg	143h	280d	294bcd	229q	183t	147w	289mn	292lmn
ABA seed soaking grain-filling)	294bcd	290bcd	291bcd	296abcd	295bcd	295jklm	302fghi	296ijkl	301ghij	300ghijk
ABA seed soaking water stress (grain-filling)	218e	178g	140h	278d	290bcd	231q	179t	144w	287n	294klm

d= days after induction of water stress, rw= re-watering
All such means which share common letters do not differ significantly

Table 2. Effect of water stress and abscisic acid (ABA) on transpiration rate (mmolm^{-2} s^{-1}) of leaves at booting and grain-filling stages of two primitive wheat cultivars.

Treatments	011251					011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
Control at booting	3.33abc	3.27abcd	3.4ab	3.23abcde	3.43a	3.6bcd	3.7ab	3.63abc	3.8a	3.5cde
Water stress (booting)	2.33o	1.9q	1.57rs	2.9ijklm	3.2bcdef	2.57k	2.03m	1.73n	3.27fgh	3.47cdef
ABA seed soaking (booting)	2.9ijklm	2.8jklmn	2.9ijklm	2.77klmn	2.8jklmn	3.27fgh	3.3efg	3.33efg	3.37efg	3.23ghi
ABA seed soaking +water stress (booting)	2.1p	1.8q	1.43st	2.63n	2.7mn	2.17lm	1.63no	1.4p	2.97j	3.03j
Control at grain-filling	3fghij	2.97ghijk	3.03efghi	3.2bcdef	3.13cdefgh	3.37efg	3.47cdef	3.43defg	3.4efg	3.3efg
Water stress at grain-filling	2.17op	1.73qr	1.33u	2.73lmn	3.03efghi	2.33l	1.8n	1.53op	3j	3.1hij
ABA seed soaking (grain-filling)	3.07defghi	3.1defghi	3.17cdefg	2.97ghijk	3fghij	3.4efg	3.37efg	3.43defg	3.33efg	3.4efg
ABA seed soaking water stress (grain-filling)	2.13op	1.7qr	1.27tu	2.67n	2.93hijkl	2.27l	1.77n	1.5op	2.93j	3.07ij
	LSD value = 16.6 at alpha = 0.050					LSD value = 5.86 at alpha = 0.050				

d= days after induction of water stress. rw= re-watering

All such means which share common letters do not differ significantly

Water stress is known to change the balance of phytohormones, causing an increase in the growth inhibitory hormones concomitant with a decline in growth promoting hormones. Water stress caused a significant ($p<0.05$) decrease in the IAA (Table 3) and GA content (Table 4) of leaves in both the cultivars. ABA seed soaking treatment was found to be effective only at booting stage in all the accessions and it caused a decrease in the IAA and GA content under unstressed (control) condition while under stress condition it was able to maintain higher IAA content as compared to non-ABA-treated plants, but in case of cultivar 011393 to maintain higher GA content as compared to untreated water stressed plants. At grain-filling stage significantly ($p<0.05$) less IAA and GA content was found than that of booting stage but water stress resulted in less decrease in GA content than that of booting stage. Similar decrease in IAA and GA was recorded previously by others (Yang *et al*., 2001; Xie *et al*., 2003, Farooq & Bano, 2006), which is possibly due to decreased IAA and GA synthesis (Xie *et al*., 2004) or due to destruction of IAA and GA as a result of increased activity of oxidase (Davenport *et al*., 1980). Effect of ABA differed in well watered and stressed conditions. Under control (well watered) condition a decrease in IAA and GA content might be due to the antagonism between ABA and IAA and GA as there is some evidence that ABA can inhibit their biosynthesis. But under stress condition exogenous ABA caused an increase in the IAA and GA content compared to untreated plants these findings are in accordance with the previous ones (Maiti *et al*., 2000; Farooq & Bano,

2006). At grain-filling stage lower IAA and GA contents in the flag leaves may be attributed to the decreased metabolic activity of flag leaf as it proceed towards senescence (Yang & Zhang, 2006).

Data analysis revealed that t-zr content of leaves (Table 5) decreased in both the cultivars at both the growth stages. The major decrease (40-50%) in t-zr content occurred within first 3 d of water stress in both the cultivars and after 6-9 d of water stress about 10% more decrease was observed. Under control (unstressed) condition more t-zr content was found in 011393 as compared to 011251 ABA seed soaking treatment under unstressed condition caused a significant decrease in t-zr content of leaves in all the accessions under control condition. No significant ($p<0.05$) effect, at most of the sampling dates, of ABA seed soaking was observed on leaf t-zr content under stress condition. Reduced CK contents are known to contribute in turgor maintenance by stomatal regulation (Veselov *et al*., 2002). The major decrease in t-zr content under water stress occurred earlier than other hormones indicated the sensitivity of CKs to the smaller changes in water status of leaves. It was reported earlier that the decrease in the CKs content occurred before the initiation of 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. Lack of significant ($p<0.05$) effect of ABA seed soaking on leaf t-zr content under stress condition was perhaps due to stress-induced decrease in t-zr content which occurred earlier than the changes in other hormones in the stress treatment.

Table 3. Effect of water stress and abscisic acid (ABA) on IAA content (ng/g) of leaves at booting and grain-filling stages of two primitive wheat cultivars.

Treatments	011251					011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
Control at booting	53.2a	50.1b	52.4a	49.3bc	54a	49.5bc	51.9a	52.3a	50.7ab	51.7a
Water stress (booting)	37.5k	29.4m	25.3o	45.3fg	47.4cdef	33.2pq	30.1r	24.9u	47.5de	48.1cd
ABA seed soaking (booting)	47.2cdef	48.3bcde	46.5def	47cdef	46.4def	46.2efgh	46.1efgh	47defg	45.9efgh	45.7fgh
ABA seed soaking +water stress (booting)	39.4jk	32.3l	28.2mn	45.7efg	46.2defg	36.3o	33.1pq	27.5st	45.5gh	44.9h
Control at grain-filling	48.5bcd	46.2defg	46.3def	43.7gh	42.2hi	47.4def	46.1efgh	44.7hi	42.3jk	40lm
Water stress at grain-filling	34.1l	27.3mno	22.4p	41.3ij	40.9ij	33.9p	28.7rs	22.5v	38.9mn	37.2o
ABA seed soaking (grain-filling)	47.4cdef	45.9defg	45.4fg	44.9fg	41ij	46.7defg	45.3gh	43.2ij	41kl	39.5lm
ABA seed soaking water stress (grain-filling)	33.7l	27no	21.4p	41.4hij	39.7jk	31.9q	26.4tu	22.1v	37.5no	36.2o
	LSD value= 2.192 at alph= 0.050					LSD value= 1.528 at alpha= 0.050				

d= days after induction of water stress. rw= re-watering

All such means which share common letters do not differ significantly

Table 4. Effect of water stress and abscisic acid (ABA) on GA content (ng/g) of leaves at booting and grain-filling stages of two primitive wheat cultivars.

Treatments	011251					011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
Control at booting	60.3b	64.4a	59.7bc	63.2a	65.2a	70.2cd	71.4bc	73.5ab	74.3a	73.9ab
Water stress (booting)	55.3efg	47.2lmno	40.1p	45.2o	46.7mno	65.4efg	57.7lmnop	50.2r	54.3q	56.5mnopq
ABA seed soaking (booting)	57.2cde	58.6bcd	56.2def	55.3efg	58.2bcd	68.2de	67.4def	65.4efg	67.7def	64.9fg
ABA seed soaking +water stress (booting)	57.3cde	48.1klmn	42.3p	47.2lmno	49.1klm	58.9jklmn	59.2jklm	54.3q	56.4mnopq	58.4klno
Control at grain-filling	54.2fgh	53.1ghi	52.4hij	50.7ijk	54.1fgh	63.9gh	62.7ghi	60.5ijkl	61.5hij	60.1ijkl
Water stress at grain-filling	50.2jk	49.4klm	46.3no	46.2no	47.1lmno	59.2jklm	57.7lmnop	55.2pq	56.3mnopq	55.9nopq
ABA seed soaking (grain-filling)	55.1efgh	50.2jk	49.7kl	54.2fgh	53.3gh	62.7ghi	61.2hijk	60.5ijkl	60.6ijkl	59.4jklm
ABA seed soaking water stress (grain-filling)	48.2klmn	46.2no	45.1o	46.2no	46.3no	58.1klmnop	56.5mnopq	53.9q	55.4opq	55.5opq
	LSD value= 2.363 at alpha= 0.050					LSD value= 2.607 at alpha= 0.050				

d= days after induction of water stress. rw= re-watering
All such means which share common letters do not differ significantly

Table 5. Effect of water stress and abscisic acid (ABA) on trans-zeatin riboside (ng/g) of leaves at booting and grain-filling stages of two primitive wheat cultivars.

Treatments	011251					011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
Control at booting	74.5c	70.2d	75.4abc	76.2abc	75.3abc	82.5abc	80.4cde	79.5ef	79.4def	81.6abcde
Water stress (booting)	40.2lm	37.3no	35.4o	47.5hi	50.6fg	47.2lm	42.1n	38.2o	50.2jk	55.2i
ABA seed soaking (booting)	68.2de	67.3e	67.5e	66.5e	67.2e	77.5fg	76.4gh	75.4gh	76.2gh	74.9h
ABA seed soaking +water stress (booting)	39.1mn	37.2no	37.3no	49.5gh	52.3f	48.1klm	43.2n	41.4n	51.3j	54.9i
Control at grain-filling	76.2abc	77.3ab	76.1abc	75.3abc	77.5a	80.5cde	83.2ab	84.1a	82.1abcd	80.1cde
Water stress at grain-filling	42.1kl	39.1mn	37.2no	45.3ij	47.4hi	46.3m	43.2n	37.6o	48.2klm	50.1jk
ABA seed soaking (grain-filling)	75.4abc	77.5a	74.9bc	76.3abc	76.9abc	79.5ef	80.2cde	81.3bcde	83.4ab	82.5abc
ABA seed soaking water stress (grain-filling)	44.3jk	39.5mn	36.7o	46.3i	49.2gh	47.1lm	42.9n	35.9o	47.5lm	49.1jkl
	LSD value = 2.084 at alpha = 0.050					LSD value = 2.212 at alpha= 0.050				

d= days after induction of water stress. rw= re-watering
All such means which share common letters do not differ significantly

Both free and bound ABA (Tables 6 & 7) contents increased significantly (p<0.05) under water stress and this increase was linear. At booting stage less increase in both free and bound ABA content was observed in 011251as compared to 011393 (Tables 6 & 7) Significant decrease in ABA content upon re-watering indicated the release from stress In the stressed plants this rapid decrease after re-watering is an indication of resuming the normal processes when the stress is released (Zhang *et al*., 2006) possibly due to degradation or conjugation of ABA as the bound ABA content did not decrease significantly after re-watering. Seed soaking treatment with ABA caused significant (p<0.05) increase in the endogenous ABA content both under well watered and water stress conditions at booting stage At grain-filling stage significantly (p<0.05) higher free and bound ABA contents were recorded as compared to that of booting. At grain-filling stage, ABA seed soaking was found to be ineffective under control as well as stress condition Higher ABA concentrations in the leaves of water - stressed citrus trees than the well watered trees were recently reported by Anan *et al*., (2006). The contents of cytokinins in rice substantially decreased with the decrease in soil moisture but they only slightly increased after re-watering (Bano *et al*., 1993). 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 it was suggested 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. After re-watering the free ABA content showed a rapid decrease during the present study similar observations were made previously by different workers (Liang & Zhang, 1999; Alves & Setter, 2000). The ABA/ t-zr ratios were very high at grain-filling stage as compared to booting in all the accessions. Both ABA and CK are generally believed to be the major regulators of plant senescence at grain-filling though there occurred no significant change in t-zr content the content of ABA was found to be much higher indicating the onset of senescence. Another reason of increase in ABA might be involvement of ABA in the transport of assimilates to the developing grains and it is suggested that the increase in the remobilization of pre stored carbon under water stress can be attributed to the accumulation of ABA (Yang & Zhang, 2006). High ratio of growth inhibitory (ABA) to promotory (t-zr.) hormone was found in cultivar 011251 which may have contributed towards the sensitivity of this accession to water stress.

From this discussion it can be concluded that inhibitory effects of water stress on physiological attributes of plants like stomatal conductance, transpiration rate and phytohoromone content can be mitigated by ABA seed soaking and booting stage is more responsive to ABA priming as compared to grain-filling stage. This may either be due to greater sensitivity of the grain-filling stage to water stress or it might be due to the fact that the effects of ABA diminished with the progression of developmental stage.

Table 6. Effect of water stress and abscisic acid (ABA) on free ABA content (ng/g) of leaves at booting and grain-filling stages of two primitive wheat cultivars.

Treatments	011251					011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
Control at booting	5.5pq	5.1q	4.9q	4.4q	5.1q	3.9uv	4.4u	3.5uvw	3.7uv	3.7uv
Water stress (booting)	17.5i	30.2f	37.5d	12.5m	8.2o	14.3j	26.5g	35.4cd	10.2lmn	6.9
ABA seed soaking (booting)	8.3o	7.5o	8.1p	7op	7.9o	6.7opq	6.2pqr	5.6qrst	6.9opq	7.1op
ABA seed soaking +water stress (booting)	19.7h	33.3e	40.1c	16.3ij	9.9n	17i	29.3ef	37.2b	12.4kl	7.7nop
Control at grain-filling	13.5lm	14.3kl	14.5kl	15.4jk	15.7jk	8.3no	9.6mn	9.5mno	10.8lm	11.2klm
Water stress at grain-filling	27.2g	43.1b	47.2a	20.5h	16.5ij	19.7hi	30.6d	40.2ab	19.7hi	13.6jk
ABA seed soaking (grain-filling)	13.5lm	13.3lm	14.9jkl	15.4jk	15.5jk	7.9nop	8.7no	9.3mno	9.4mno	10.9lm
ABA seed soaking water stress (grain-filling)	26.4g	44.1b	46.9a	19.8h	16.7ij	20.1h	29.9de	42.3a	20.3h	14.4ij
	LSD value = 1.567 at alpha = 0.050					LSD value = 1.243 at alpha = 0.050				

d= days after induction of water stress. rw= re-watering
All such means which share common letters do not differ significantly

Table 7. Effect of water stress and abscisic acid (ABA) on bound ABA content (ng/g) of leaves at booting and grain-filling stages of two primitive wheat cultivars.

Treatments	011251					011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
Control at booting	1.3no	1.7mno	1o	0.95o	1.3no	0.87n	0.94n	1.3mn	1.4mn	0.91n
Water stress (booting)	7.9j	12.3h	15.4ef	14.3fg	13.5g	6.2j	11.5h	13.2fg	14.1f	13.9f
ABA seed soaking (booting)	2.7lm	2.3lmn	3.2l	2.4lmn	2.3lmn	2.2lm	2.1lm	2lmn	1.9lmn	2.8l
ABA seed soaking +water stress (booting)	9.3i	14.4fg	17.4cd	16.2de	16.3de	8.9i	13.7f	16.9cde	16.1de	15.9e
Control at grain-filling	6.3k	6.2k	7.1jk	6.4k	7.4jk	5.2jk	5.9jk	5.1jk	4.9k	5.3jk
Water stress at grain-filling	13.4g	16.2de	19.3ab	18.9ab	18.2bc	11.9h	17.1cd	19.7ab	19.2ab	20.1a
ABA seed soaking (grain-filling)	6.4k	7.2jk	6.8jk	7.3jk	6.5k	5.9jk	5k	4.9k	5.7jk	6jk
ABA seed soaking water stress (grain-filling)	14.3fg	17.3cd	20.1a	19.9a	19.4a	12.2gh	17.4c	20.1a	19.8ab	18.8b
	LSD value = 1.082 at alpha = 0.050					LSD value = 1.005 at alpha = 0.050				

d= days after induction of water stress. rw= re-watering
All such means which share common letters do not differ significantly

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