Effect of Drought and Abscisic Acid Application on the Osmotic Adjustment of Four Wheat Cultivars

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Summary: The accumulation of osmolytes in leaf tissues and the abscisic acid-induced stomatal closure are well-recognized mechanisms associated with drought tolerance in crop plants. We determine the response in terms of osmotic potential and the contents of leaf proline, glycine betaine and soluble sugar at booting and grainfilling stages of four wheat (*Triticum aestivum* L.) cultivars to drought and exogenously applied abscisic acid (ABA) in a pot study. Leaf sample were collected 3, 6 and 9 days after drought induction and at 48 and 72 h of re-watering (recovery). Marked decreases in osmotic potential associated with the accumulation of proline, glycine betaine and soluble sugars occurred under conditions of drought stress. Accession 011320 was most sensitive to drought and showed the largest decrease in osmotic potential and least accumulation of proline, sugar and glycine betaine The inhibitory effects of drought stress were ameliorated by exogenous application of ABA. This ameliorating effect was more pronounced at the booting than at grainfilling stage particularly in the sensitive accession 011320. Upon rewatering the recovery from drought stress was found to be greater in case of abscisic acid application. The leaf praline content is seen to be a suitable indicator for selecting drought-tolerant genotypes.

Introduction

Crop productivity can be improved by studying the nature of adaptation of cereal crops to drought and to find out possible physiological and biochemical markers, which impart tolerance under these conditions. This is essential for identifying the best-adapted and high yielding cultivars for drought prone areas [1]. Osmotic adjustment *i.e.* the active lowering of osmotic potential is regarded as the mechanism which significantly contributes to increase drought resistance [2]. Thereby plants combat the detrimental effect of water loss by synthesizing compatible solutes, typically certain polyols, sugars, amino acids, betaines and related compounds [3]. Although much research has been done on the effect of drought on grain yield of plants and substantial losses in wheat grain yield have been reported due to water deficiency at different developmental stages [4], information is scanty regarding the effect of drought at booting and grainfilling and the effect of application abscisic acid (ABA) [5] on plant's recovery from drought stress, is not well studied. Therefore we evaluated osmotic potential and leaf tissue osmolyte's content of droughted wheat cultivars with and without external ABA application in a pot experiment.

Results and Discussion

Increasing the intensity and duration of drought stress caused a gradual decrease in osmotic potential of wheat leaves, while the content of osmolytes (proline, glycine betaine and soluble sugars) generally increased. The extent of these responses differed among cultivars. At the booting stage the maximum reduction in osmotic potential after short-term drought (3 d) was observed in accession 011393, while within longer duration of drought the maximum reduction was found in accession 011320 (Table-1), which, also showed least accumulation of all the osmolytes during the longer period (6-9 d) of water stress except for proline (Table-1). This suggests that proline might be the principal osmolyte in this accession. Though the proline production increases more than the other osmolytes, the increase may not be sufficient enough to regulate osmotic imbalance due to water stress. Minimum recovery upon rewatering in accession 011320 also indicates its susceptibility to water stress. In contrast, accession 011417 showed maximum increase in proline (Table-1) and GB (Table-2) content as compared to other accessions. The accession 011417 also showed least response to ABA seed soaking and rapid recovery to control levels, suggesting that this accession can tolerate and recover after water stress in a better way than the other accessions. At grainfilling osmotic potential values were significantly ($P< 0.05$) lower as well as the increase in proline and glycine betaine content was also less as compared to that of booting stage. But the sugar content was found to be significantly

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Table-1: Effect of water stress and abscisic acid (ABA) on osmotic potential (-MPa.) of leaves at booting and grainfilling stages of four wheat accessions.

Treatments	Accession 011251					Accession 011417					Accession 011320					Accession 011393				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	1.41 uvw	1.4 wx	1.39 xy	1.42 uv	1.41 uvw	1.49 ij	1.46 jkl	1.5 hij	1.48 ijk	1.49 ij	1.38 mnopqr	1.37 mnopqr	1.38 mnopqr	1.36 nopqrs	1.37 mnopqr	1.48 kl	1.46 kl	1.42 l	1.48 kl	1.45 kl
T_2	1.61 mn	2.01 g	2.47 c	1.73 k	1.62 m	1.67 fg	1.74 e	2.37 b	1.56 h	1.42 lm	1.53 l	2.22 i	2.35 fg	2.11 j	1.83 k	1.8 g	2.03 e	2.36 b	1.66 i	1.51 k
T_3	1.37 z	1.4 wx	1.38 yz	1.41 uvw	1.39 xy	1.4 m	1.38 m	1.42 lm	1.43 klm	1.41 lm	1.32 rs	1.35 opqrs	1.31 s	1.34 pqrs	1.33 qrs	1.45 kl	1.47 kl	1.48 kl	1.45 kl	1.48 kl
T_{0b}	1.58 p	1.99 h	2.43 d	1.7 l	1.59 op	1.65 g	1.72 ef	2.28 c	1.54 hi	1.43 klm	1.56 l	2.32 gh	2.42 de	2.15 j	1.8 k	1.72 h	2.01 e	2.33 b	1.6 j	1.49 k
T4	1.46 qr	1.44 st	1.46 qr	1.47 q	1.45 rs	1.53 hi	1.52 hij	1.54 hi	1.53 hi	1.53 hi	1.4 mnop	1.42 mn	1.4 mnop	1.39 mnopq	1.42 mn	1.51 k	1.5 k	1.51 k	1.49 k	1.5 k
T_5	1.78 j	2.11 f	2.79 b	1.96 i	1.61 no	1.74 e	1.95 d	2.43 a	1.69 efg	1.54 hi	1.85 k	2.32 gh	2.91 a	2.56 c	2.4 ef	1.91 f	2.18 c	2.7 a	2.12 d	1.8 g
T_6	1.42 vwx	1.42 tu	1.44 rs	1.41 wx	1.43 st	1.49 ij	1.52 hij	1.51 hij	1.49 ij	1.53 hi	1.43 m	1.43 m	1.42 mn	1.41 mno	1.42 mn	1.47 kl	1.51 k	1.48 kl	1.5 k	1.51 k
LSD values	0.01626 at alpha= 0.050					0.0514 at alpha= 0.050					0.0514 at alpha= 0.050					0.0514 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 grainfilling, T4 =water stress at grainfilling, T5= ABA seed soaking (grainfilling), T6 =water stress at grainfilling +ABA seed soaking. d= days after induction of water stress. rw= rewatering.
All such means (within each cultivar), which share common letters do not differ significantly.

Table-2: Effect of water stress and abscisic acid (ABA) on proline content (µmole/g) of leaves at booting and grainfilling stages of the four wheat accessions.

Treatments	Accession 011251					Accession 011417					Accession 011320					Accession 011393				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	1.01 rs	1.31 qr	1.21 opq	1.1 qr	0.95 s	0.72 wx	0.74 vwx	0.67 x	0.73 vwx	0.71 wx	0.82 t	0.91 rs	0.82 t	0.93 qrs	0.89 rst	1.59 x	1.6 wx	1.64 vwx	1.72 rstu	1.67 tuvw
T_2	1.48 lm	3.78 de	4.23 c	1.17 pq	1.21 opq	1.39 j	4.95 d	5.51 a	0.75 vwx	0.76 uvwx	0.94 qrs	1.98 f	3.57 b	1.23 gh	0.91 rs	2.65 i	3.53 e	4.12 c	1.85 nop	1.74 qrst
T_3	1.66 k	1.91 hi	1.87 i	1.81 ij	1.69 jk	0.82 stuvwx	0.81 tuvwx	0.72 wx	1.1 lmno	0.94 opqrst	0.87 st	0.91 rs	0.87 t	0.94 qrs	0.91 rs	1.88 mno	1.91 lm	2.1 j	1.93 lm	1.87 mno
T_{0b}	1.79 kl	4.12 c	5.1 a	2.1 g	2.3 f	1.78 h	4.31 f	5.21 bc	1.3 jk	1.03 mnopq	1.13 ijkl	2.21 e	3.81 a	1.21 hi	1.01 nopq	2.95 g	3.47 ef	4.91 a	2.03 jk	1.92 lmn
T4	1.32 no	1.29 nop	1.37 mn	1.31 nop	1.21 opq	0.89 qrstuv	0.95 nopqrst	0.85 stuvw	0.86 rstuvw	0.96 mnopq	0.91 rs	0.97 opqr	0.94 qrs	1.11 jklm	0.95 pqrs	1.61 wx	1.7 tuv	1.65 uvwx	1.71 stuv	1.73 rst
T_5	1.67 jk	3.67 e	4.11 c	1.41 mn	1.42 mn	1.23 jkl	4.76 e	5.31 b	0.92 pqrstu	0.98 nopqrst	1.03 mnop	1.31 g	3.32 c	1.21 hi	1.24 gh	1.98 kl	3.44 f	4.17 bc	1.93 lm	1.91 lmn
T_6	1.71 jk	1.67 k	1.91 hi	1.89 hi	1.93 hi	1.01 mnopqrst	0.97 nopqrst	0.96 nopqrst	1.08 lmno	1.11 lmn	1.09 klmn	1.13 ijkl	1.08 lmno	1.14 ijk	1.01 nopq	1.81 opq	1.79 pqr	1.83 op	1.78 pqrs	1.82 op
LSD values	0.1454 at alpha = 0.050					0.1626 at alpha = 0.050					0.08903 at alpha= 0.050					0.07270 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 grainfilling, T4 =water stress at grainfilling, T5= ABA seed soaking (grainfilling), T6 =water stress at grainfilling +ABA seed soaking. d= days after induction of water stress. rw= rewatering.
All such means (within each cultivar), which share common letters do not differ significantly.

($P< 0.05$) higher at grainfilling indicating the increase in the hydrolysis of stored carbohydrates in the shoots which might contributed to accelerate the rate of re-translocation of pre stored reserves to developing grains [6]. Minimum accumulation of sugar in accession 011320 suggested impaired remobilization and thus grainfilling. Moreover magnitude of recovery observed after rewatering the stressed plants and the response to ABA seed soaking was also significantly ($P< 0.05$) less at grainfilling perhaps the effects of water stress and response to ABA are dependant on the developmental stage and grainfilling appears to be more sensitive to water stress.

Osmotic potential of leaves (Table-1) was decreased significantly ($P< 0.05$) under water stress treatment in all the accessions. At booting stage within first 3 d of water stress maximum decrease (21 %) in osmotic potential was recorded in accession 011393 (Table-1) whereas after 6 d maximum decrease was observed in accession 011320 (Table-1). But after 9 d of water stress treatment maximum reduction was found in accession 011251 (Table-1). Rewatering caused an increase in the osmotic potential of leaves and maximum increase after rewatering was noted in 011417 (Table-1) reaching to the control level within first 72 h after rewatering. Minimum recovery after rewatering was observed in accession 011320 (Table-1). ABA seed soaking treatment had significant ($P< 0.05$) effect on leaf osmotic potential only under stress treatment in accession 011320 (Table-1). While in other accessions no significant ($P < 0.05$) effects ware observed. At grainfilling stage the leaf osmotic potential was significantly ($P < 0.05$) lower as

compared to that of booting stage. Under water stress treatment maximum decrease in osmotic potential was observed in accession 011320 (Table-1) at all the sampling dates. Upon rewatering accession 011417 (Table-1) was able to show marked recovery among all the accessions. ABA seed soaking treatment was found to be ineffective under both the control and drought stress treatments except for accession 011320 (Table-1) which showed some response to ABA seed soaking after 9 d of water stress. The decrease in osmotic potential under water stress was also reported earlier [7]. The plants decrease osmotic potential (Ψs) in order to maintain pressure potential [8]. The decline in Ψs could be a result of either simple passive concentration of solutes due to dehydration or net accumulation of osmolytes as suggested by Jain *et al.* [9]. Gupta *et al.* [10] noted that water stress was found to reduce the leaf osmotic potential but it remained significantly higher in the water stress tolerant cultivar than the sensitive one.

There were significant ($P < 0.05$) differences in the basal level of proline among all the accessions being minimum in accession 011417 (Table-1). At booting stage within first 6d of water stress minimum increase (117 %) in proline content was noted in accession 011320 (Table-1) whereas, after 9 d of water stress minimum increase (152 %) was observed in accession 011393 (Table-1). Sharp decrease occurred in proline content within first 48 h of rewatering, which became slow afterwards, that is at 72 h of rewatering. Seed soaking treatment with ABA had a significant effect on the proline content of leaves in accession 011251, 011320 and 011393; accession 011251 was the most responsive among these three accessions to the ABA seed soaking treatment. Significantly ($P< 0.05$) higher proline contents were recorded in ABA treated plants under unstressed (control) conditions as compared to non-ABA-treated plants. At grainfilling stage, proline content of leaves was significantly ($P< 0.05$) higher as compared to that of booting stage under control condition but on induction of stress condition less increase in proline content occurred at grainfilling stage in comparison with the booting stage. Maximum increase in leaf proline content was found in 011417 (Table-1). After 3-6 d of water stress minimum increase (13-35%) was found in accession 011320 (Table-1). But after 9d of water stress accession 011393 (Table-1) showed minimum increase (152%). Significant effect of ABA seed soaking was also noticed at grainfilling stage. Increased proline accumulation was reported in water-stressed plants [11, 12]. Exogenously applied ABA is known to induce the accumulation of proline under water stress [13]. Increased proline in the stressed plants may be an adaptation mechanism, the purpose of which is to overcome the stress conditions. Proline not only acts as an osmolyte but it has other functions as well. Proline accumulated under stressed conditions supplies energy for growth and survival, might have a scavenger function or act as an osmolyte and thereby helps the plant to tolerate stress [14]. Rapid reduction in proline after rewatering might be an indication of recovery from stress as it was suggested by Hare and Cress [15] that rapid breakdown of proline upon relief from stress might provide sufficient energy for recovery from stress which might help in repairing the stress induced damages.

Water stress caused a linear increase in the glycine betaine content of leaves coinciding with the increase in water stress period. Maximum increase in glycine betaine content was observed in accession 011417 (Table-2) though this accession had lowest basal level of glycine betaine under control condition. Minimum increase in glycine betaine content under water stress was observed in accession 011320 (Table-2). Rewatering caused a decline in the glycine betaine content. Upon rewatering accessions significantly ($P< 0.05$) differed in recovery to the prestressed level. The accession 011417 exhibited maximum and early recovery, but the glycine betaine content remained higher than control. ABA seed soaking treatment significantly ($P< 0.05$) enhanced the accumulation of glycine betaine under water stress treatment. Accession 011320 (Table-2) was found to be the most responsive to ABA seed soaking treatment, while accession 011417 (Table-2) and accession 011393 (Table-2) were found to be least effected. But under control condition ABA had no significant ($P< 0.05$) effect on glycine betaine content. At grainfilling stage there occurred a significantly ($P< 0.05$) less increase in the glycine betaine content of leaves. Under well watered condition no significant ($P< 0.05$) differences in glycine betaine content ware observed between booting and grainfilling stages and ABA seed soaking treatment was also found to be ineffective at grainfilling stage. Accumulation of glycine betaine under stress condition suggests its involvement in

stress tolerance as it has been proposed that tolerant genotypes normally accumulate more GB than sensitive genotypes in response to stress but GB accumulation and stress tolerance is species- or even genotype specific [16]. It might be due to its role as a protective molecule as it is suggested that glycine betaine has a protective rather than an osmotic effect [17]. Numerous studies have demonstrated that glycine betaine can stabilize macromolecular activity and membrane integrity [18]. The accessions which accumulated more GB also had higher rate of proline and sugar accumulation during the present investigation. The enhanced glycine betaine synthesis may exert protection on the activity of enzymes, including the enzymes associated with sugar and amino acid metabolism, leading to greater increases in total soluble sugars and free amino acids like proline [19].

At booting stage minimum increase (13-16 %) in sugar content was observed in accession 011320 (Table-3) after 9 d of water stress treatment. After 3-6 d of water stress treatment maximum increase (37%) over control was observed in accession 011251 (Table-3) but after 9 d of water stress maximum increase (57%) was noted in accession 011393 (Table-3) followed by accession 011417 (42%) (Table-3). Under unstressed condition ABA treated plants showed no significant ($P< 0.05$) changes in sugar content in all accessions except for accession 011320 (Table-3) which showed higher (8%) sugar content in treated plants than non-treated plants. Under water stress ABA seed soaking treatment had a significant ($P< 0.05$) effect on the accumulation of sugar in all accessions. Accession 011320 (Table-3) was found to be the most responsive and accession 011417 (Table-3) least affected by the ABA seed soaking. Rewatering caused a significant ($P< 0.05$) decrease in sugar content. The magnitude of decrease varied among the accessions. In accession 011417 (Table-3) the sugar content reached the control level within first 48 h of rewatering while in other accessions it remained a little higher than the control level. At grainfilling stage sugar contents were found to be significantly ($P< 0.05$) higher than booting stage. No significant ($P< 0.05$) change in sugar content was observed in accession 011320 (Table-3) within first 6 d of water stress but after 9 d a little (9%) increase was noted. After 3 d of water stress maximum increase (24%) of sugar was found in accession 011251 (Table-3) and after 6-9 d maximum increase (41-45%) was observed in accession 011393 (Table-3). Rewatering caused a decrease in sugar content and in accession 011417 (Table-3) it reached to the level of control plants after 72 h of rewatering. ABA seed soaking treatment had effects similar to that of booting stage on sugar accumulation at grainfilling stage. The results similar to present finding were reported by Sarker *et al.* [20] they found higher amounts of sugars at lower water status of leaves. Accumulation of sugar during water stress was also noted in durum wheat leaves [21].

Compared with sugar, proline and glycine betaine accumulated to a higher extent in the leaves during the present study suggesting that osmoregulation may largely depend upon proline and

Table-3: Effect of water stress and abscisic acid (ABA) on glycine betaine content (µg/g) of leaves at booting and grainfilling stages of four wheat accessions.

Treatments	Accession 011251					Accession 011417					Accession 011320					Accession 011393				
T_{0a}	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_1	8.4 m	9.1 m	7.8 m	9.2 m	8.9 m	5.3 mn	4.9 mn	6.3 mn	5.1 mn	6.2 mn	11.3 lm	10.2 m	11.9 lm	10 m	11.5 lm	7.4 no	8.1 no	6.9 no	7.2 no	7.5 no
T_2	22.2 kl	45.3 f	62.1 b	42.5 g	24.5 jk	29.8 ij	53.6 d	68.9 b	39.8 h	15.3 l	18.3 k	27.4 ghi	39.8 b	30.2 cf	25.5 ij	18.7 l	38.2 ef	53.9 a	37.5 f	23.2 j
T_3	8.9 m	9 m	9.4 m	8.7 m	9.5 m	6.3 mn	5.2 mn	6.9 m	5.1 mn	6.2 mn	13.2 l	10.3 m	12.4 lm	11.7 lm	11.2 lm	8.2 no	7.3 no	7.4 no	7.1 no	8.5 n
T_{0b}	26.4 ij	49.5 e	65.3 a	44.6 fg	28.3 i	30.4 ij	50.9 e	71.3 a	42.1 g	14.7 l	24.1 j	32.1 de	44.9 a	34.3 d	28.4 fgh	21.3 jk	42.3 d	55.6 a	39.7 e	25.4 i
T4	9.3m	8.7 m	9.2 m	7.9 m	8.8 m	4.8 mn	5.9 mn	5.2 mn	6.3 mn	5.5 mn	12.4 lm	10.7 lm	12.5 lm	12.1 lm	10.8 lm	6.7 no	7.1 no	6.9 no	7.4 no	6.2 no
T_5	24.5 jk	38.2 h	59.5 c	40.2 h	20.1 l	31.3 i	45.7 f	64.9 c	45.7 f	17.4 k	17.1 k	25.3 ij	37.3 c	32.7 d	26.9 ef	16.9 lm	34.8 h	49.7 b	36.9 f	20.8 k
T_6	7.8 m	8.3 m	8.5 m	8.9 m	7.9 m	6.2 mn	4.6 n	5.1 mn	4.9 mn	6 mn	10.7 lm	11.8 lm	12.4 lm	11.9 lm	12.1 lm	7.1 no	6.4 no	6.8 no	5.9 o	7.4 no
LSD values	2.228 at alpha= 0.050					1.855 at alpha= 0.050					2.180 at alpha= 0.050					0.6824 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 grainfilling, T4 =water stress at grainfilling, T5= ABA seed soaking (grainfilling), T6 =water stress at grainfilling +ABA seed soaking. d= days after induction of water stress. rw= rewatering. All such means (within each cultivar), which share common letters do not differ significantly.

Table-4: Effect of water stress and abscisic acid (ABA) on sugar content (µg/g) of leaves at booting and grainfilling stages of the four wheat accessions.

Treatments	Accession 011251					Accession 011417					Accession 011320					Accession 011393				
	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)	3d	6d	9d	48h (rw)	72h (rw)
T_{0a}	92.5 klmnop	95.1 op	101.1 nop	99.1 mnop	91.1 p	201.3 rs	209.3 pqr	205.7 qrs	211.8 opq	199.8 s	102.3 pq	101.3 q	103.5 pq	101.5 q	101.6 q	92.5 o	99.8 m	90.1 o	92.3 o	99.3 mn
T_1	127.1 ghijk	131.3 cdef	137.1 bcde	121.1 fghij	103.1 lmnop	235.5 ghi	284.1 cd	291.3 c	213.9 nopq	214.3 mnopq	115.9 mno	117.8 lmno	119.9 klm	111.2 nop	110.7 op	121.3 f	133.9 e	141.3 d	111.3 gh	100.1 m
T_2	103.9 klmnop	99.8 mnop	103.4 klmnop	102.1 lmnop	105.3 lmnop	213.1 nopq	219.3 lmno	221.3 klmn	219.8 klmno	217.5 lmnop	121.3 jklm	121.4 jklm	119.9 klm	122.9 jklm	121.8 jklm	96.4 n	99.9 m	100.1 m	101.3 lm	99.4 mn
T_{3}	133.4 efghi	141.2 bcd	154.1 ab	112.4 defgh	113.3 hijkl	252.4 e	291.4 c	301.9 b	221.3 klmn	201.6 rs	155.7 c	161.2 c	177.3 b	118.9 klmn	121.7 Jklm	135.3 e	144.4 d	151.3 c	113.4 g	112.1 gh
T_{0b}	112.5 hijklm	105.3 jklmn	109.3 klmno	113.4 ijklmn	113.6 hijkl	214.3 mnopq	218.3 lmno	221.3 klmn	211.4 opq	220.8 klmn	123.4 jklm	125.9 hijk	126.1 hijk	125.7 hijkl	124.1 ijkl	107.3 ij	106.2 ijk	105.4 jk	103.9 kl	104.7 jk
T4	139.1 cdefg	142.4 bc	151.1 b	132.1 bcd	120.3 efghi	248.3 ef	291.3 d	302.1 b	224.3 jkl	220.1 klmno	121.5 jklm	128.9 ghij	137.2 ef	131.8 fghi	132.1 fgh	132.4 e	149.3 c	152.5 bc	119.8 f	112.3 gh
T_5	121.1 fghij	129.3 cdefg	121.4 fghij	122.9 fghi	128.4 efghi	223.1 jklm	228.4 ijk	231.2 hij	234.1 ghi	233.7 ghi	134.5 efg	141.3 de	136.9 ef	134.5 efg	135.7 efg	112.5 g	119.8 f	113.6 g	112.7 g	111.6 gh
T_6																				
LSD values	4.807					8.928					7.94					3.286				
	at alpha= 0.050					at alpha= 0.050					at alpha= 0.050					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 grainfilling, T4 =water stress at grainfilling, T5= ABA seed soaking (grainfilling), T6 =water stress at grainfilling +ABA seed soaking. d= days after induction of water stress. rw= rewatering. All such means (within each cultivar), which share common letters do not differ significantly.

GB. In addition, the preferential accumulation of a particular solute might have implication in determining the sensitivity status [22]. It was apparent from the present results that the capacity to accumulate different osmolytes among accessions was markedly different. Possibly due to the inherent control in production of these osmolytes imparting tolerance. Though accession 011417 showed lowest basal level of osmolytes the rate of accumulation (percentage increase over time) was observed to be highest in this accession. The rate of increase of osmolytes rather their content per se may be more important [15]. There are reports that isogenic lines of wheat having higher ABA content have better capacity to osmoregulate and hence perform better in terms of growth and yield under water stress [23], indicating a close association between ABA and solute accumulation. This was corroborated by the present study where exogenous application of ABA to plants experiencing water stress enhanced the accumulation of osmolytes particularly in accession 011320 and thereby plant water status was improved. ABA is reported to increase the endogenous content of proline [8] and other osmolytes [24]. During the present study magnitude of stimulation by ABA was more pronounced in accession 011320, the most sensitive to water stress, it is known that the sensitive genotypes are usually more responsive to exogenously applied ABA [25]. Perhaps the level of sensitivity of a plant varies with the basal level of endogenous ABA this suggests that an optimal ABA level may be required to sustain growth under stress [26] that may not exist in the susceptible genotype because of which its osmoregulation ability is weak and get impaired.

Experimental

The experiment was conducted under natural condition in a net house of Quaid-i-Azam University, Islamabad during the wheat-growing season 2005 and 2006. Seeds of four accessions (011251, 011417, 011320 and 011393) of wheat (*Triticum aestivum* L.) were obtained from Plant Genetic Resource Institute (PGRI), National Agriculture Research Centre (NARC), Islamabad. Prior to sowing surface sterilized (done with 10 % chlorox) seeds were soaked for 8 h in aqueous solution of ABA (10^{-6} M) and in autoclaved distilled water in case of control. The seeds were sown in earthen pots (20 x 30 cm^2) containing soil, sand and farm yard manure in a ratio of 3:1:1. Soil pH and EC were determined with the help of pH and EC meter and were found to be 8.2 and 127µs cm 1respectively. Recommended doses of nitrogen phosphorus, and potassium fertilizers were applied. Pots were arranged in Randomized Complete Block Design (RCBD) under a rain shelter. A week after germination the plants were thinned to five per pot. The plants were watered as required. Drought was imposed by withholding water for a period of 9 days. During this period the volumetric water content declined from approx. 15 to about 7 %. The first drought treatment was applied at 50 % booting and the second at 50 % grainfilling on different plants. At the time of each sampling soil moisture content (SMC) was determined gravimetrically. Sampling was done 3 (SMC = 12-13.5 %), 6 (SMC = 8.1-9.5 %) and 9 days (SMC = 6.6-7.3 %) after the start of drought treatment. After 48 and 72 h of re-watering

(SMC = 15.3-16.7 %) whole plants and soils were sampled for studying the recovery.

Osmotic Potential

The osmotic potential of the cell sap was measured from the flag leaves with a freezing point osmometer according the method of Capell and Doerffling [27]. To obtain cell sap (50µl) the leaf material was enclosed in a 2ml plastic syringe and was stored at 20°C subsequently it was pressed to ooze out the sap from the thawed leaf. Readings were taken from freezing point osmometer (Gonotec GmbH model OSMOMAT 010) as mosmol/Kg and ware converted to -MPa.

Proline Content

Proline content of leaves was estimated at both stages using the method of Bates *et al.* [28].

Glycine Betaine Content

The amount of GB was estimated according to the method of Grieve and Grattan [29]. The plant tissue was finely ground and mechanically shaken with 20 ml deionized water for 24 h at 25 °C. The samples were then filtered and filtrates were diluted to 1:1 with 2 N H_2SO_4. Aliquots were cooled in ice water for 1 h. Cold KI–I_2 reagent (0.20 ml), prepared by dissolving 15.7 g of iodine and 20.0 g of KI in 100 ml water, was added and the reactants were gently stirred with a vortex mixture. The tubes were stored at 4 °C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0 °C. The supernatant was carefully aspirated with a fine glass tube. The periodide crystals formed were dissolved in 9 ml of 1, 2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as standard.

Sugar Content

Sugar content of leaves was estimated following the method of Dubois *et al.* [30]. Fresh plant material (0.5 g) was homogenized with 10 ml of distilled water in a clean mortar. It was centrifuged at 3000 rpm for 5 minutes. Then in 0.1ml supernatant 1ml 5 % v/v phenol was added. After 1h incubation at room temperature 5ml of concentrated H_2SO_4 was added .The absorbance of each sample was recorded at 420 nm. The concentration of unknown sample was calculated with reference to standard curve made of glucose.

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

Conclusions

Present investigation reveals that the inhibitory effects of water stress on plant at booting and grainfilling stages of wheat can be alleviated by exogenous application of ABA used as seed soaking particularly in relatively sensitive wheat genotypes. Effect of ABA appears stage specific and booting stage was found to be more responsive the possible reason may be that the effects of ABA seed soaking diminished with the developmental changes. Considerable variation in terms of osmotic adjustment under water stress exists in local germplasm, which maybe used in breeding programs. On physiological basis accession 011417 was found to be most tolerant to water stress and least responsive to exogenous ABA while accession 011320 was the most sensitive and most responsive to ABA. Adverse effects of water stress on physiological and biochemical attributes become more pronounced at grain filling as compared to booting. The contribution of proline and glycine betaine in osmotic adjustment was more as compared to sugar thus these can serve as better physiological markers than sugar for selecting water stress tolerant wheat genotypes.

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