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ROLE OF PHYTOHORMONES UNDER INDUCED DROUGHT STRESS IN WHEAT

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Abstract

The performance of plants (grown in pots) was studied for drought induced at critical stages of grain filling. Furthermore, the effect of abscisic acid (ABA) and benzyladenine (BA), were also studied on the physiology of plants during grain filling. Seeds of two wheat varieties cv Margalla-99 (cv1) and cv Manthar-2003 (cv2) were sown in pots. Stress treatments were imposed immediately after anthesis. Drought stress resulted in maximum decrease in IAA and GA content but proline and ABA content of leaves showed maximum increase at hard dough stage in cv1. With decrease in soil moisture content under induced drought stress, the percentage decrease in IAA and GA and increase in proline and ABA was greater in leaves and spikes of potted plants. All parameters showed greater decrease in cv2 than in cv1. Application of both ABA and BA, each at 10^{-6} M applied at anthesis stage, was involved in osmoregulation by the production of proline. The adverse effect of drought started at anthesis stage reaching maximum at hard dough stage. ABA was more effective at the later stages of grain filling whereas, BA was more effective at early stages.

Introduction

Wheat is the main staple food of the people of Pakistan. Approximately 70 to 90 % of the final grain yield is derived from photosynthates produced by the plant during grain filling. ABA can enhance the movement of photosynthetic assimilates to the developing seeds (Brenner & Cheikh, 1995). ABA applied to plants was found to reduce transpiration and improves water use efficiency (Trouverie *et al*., 2003). Present study was conducted to simulate the field situation by inducing drought stress at successive stages of grain filling in order to determine the critical phase of grain filling sensitive to drought stress. In view of the positive role of two hormones, abscisic acid (ABA) and benzyl adenine (BA) under abiotic stresses, effect of these hormones exogenously applied were evaluated on drought tolerance.

Materials and Methods

Wheat (*Triticum aestivum* L.) varieties cv Margalla-99 (cv1) and Manthar-2003 (cv2) were used during the experiment which differ in the period of anthesis. Seeds were obtained from National Agricultural Research Centre Islamabad and were sown in earthen pots, 24×30cm^2 filled with soil and sand mixture (3:1). Recommended irrigation practices were followed throughout the course of study. Stress treatments were imposed immediately after anthesis (80 days after sowing) for a period of 10 days. When plants reached at flowering stage, drought was imposed to plants by withholding water supply for a period of 10 days, at that time the soil moisture was 30% and measurements were performed subsequently at each successive stage of grain filling viz., anthesis (10 days after anthesis (d.a.a)], watery ripe (20 d.a.a), milky (30d.a.a), soft dough (40d.a.a) and hard dough stages (50d.a.a).

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Non-stressed plants continued to receive daily irrigation. With holding of water started independently for each cultivar as they reached flowering. In one group of plants after 10 days of the drought, plants were irrigated to saturation to start a second 10 days cycle of progressive drought, whereas other group of plants received irrigation. At anthesis, plants were sprayed with aqueous solution of growth regulators (10^{-6} M). Growth regulators were sprayed at anthesis on the leaves @ 50 ml per pot using insecticide plastic sprayer.

Proline estimation of flag leaves was done at five different developmental phases of grain filling following the method of Bates *et al*., (1973).

The flag leaves of wheat samples were collected at 5 different developmental phases, as described earlier for the extraction of endogenous level of GA, IAA and ABA. The extraction and purification was made following the method of Kettner & Doerffling (1995) for IAA, GA and ABA. The analysis was done in HPLC equipped with variable UV detector and C-18 column (39×300 mm). These growth hormones were identified on the basis of retention time and peak area of the standards. Methanol, acetic acid and water (30:1:70) were used as a mobile phase for IAA and GA and for ABA 0.1% acetic acid and methanol (30-70% methanol, linear gradient over 30 min.). The flow rate was adjusted at 0.5 ml/min for IAA and GA and 0.8% for ABA. The wavelength used for the detection of IAA was 280 nm (Sarwar *et al*., 1992), whereas for GA and ABA analysis it was adjusted at 254 nm (Li *et al*., 1994).

Results and Discussion

Drought resulted in two folds increase in proline content of leaves of potted plants than that of control whereas in spikes accumulation of proline was less than that of leaves in all the treatments (Table 1). Application of ABA showed significant effects at all the developmental stages in Margalla-99 in both leaves and spikes. Differences between the two varieties lie in early significant increase in ABA induced proline production under drought stress in cv1 (anthesis stage in cv1 versus milky stage in cv2). Similar pattern of proline accumulation was exhibited in spikes of two varieties. Noteworthy, BA treatment did not show any significant increase in proline over drought at any stage of measurement till hard dough stage in spike of cv2. This was in contrast to the significant increase in proline production over drought at soft dough stage in cv1. Increased proline content under drought stress is an adaptive mechanism of drought tolerance by decreasing osmotic potential (Verma *et al*., 1992). ABA increased the endogenous content of proline (Nayyar & Walia, 2003). Cytokinins may partially ameliorate the negative effects of water stress, by stimulating osmotic adjustment (Yadav *et al*., 1997). Similarly application of BAP induced accumulation of proline under water stress condition (Pospisilova, 2003). The drought tolerant cultivars accumulated more proline and showed high osmotic adjustment capacity (Carceller *et al*., 1999).

A significant correlation between enhanced tolerance and proline accumulation in plants has been found (Ashraf & Foolad, 2006). Results presented (Tables 2 & 3) from potted experiment revealed that in leaves drought treatment decreased the IAA and GA content by 41% and 28% respectively than that of control. ABA showed 55% and 24% increase respectively over that of drought. Increase in IAA and GA content due to BA treatment was 35% and 14% over that of drought but 21% and 18% less than that of control. The effect of BA was less as compared to ABA. In spikes, IAA content increased significantly till watery ripe stage in control thereafter declined. The percentage of decrease was greater after plants were exposed to drought. Drought decreased 27% and 13% IAA and GA content respectively at watery ripe stage than that of control. With the

application of ABA, the IAA and GA content of spikes was decreased to 24% and 10% less than that of control at watery ripe stage. BA application showed IAA and GA content 16% and 12% less than that of control at watery ripe stage. ABA treatment under drought stress has resulted in the amelioration of adverse effect of drought for proline production, IAA and GA content such that the value did not differ significantly from that of control (untreated) whereas, BA showed significant decrease over control but non significant increase over drought.

Drought stress significantly decreased IAA and GA concentration in leaves than that of control (Xie *et al*., 2003). It may be due to decreased IAA and GA synthesis (Xie *et al*., 2004) or increase in the destruction of IAA and GA by increasing the activity of oxidase (Davenport *et al*., 1980).

In spikes both IAA and GA content increased significantly till watery ripe stage in all treatments thereafter declined till hard dough stage. Increased IAA and GA in the grains at early grain filling stage were found to be associated with the increase of the grain filling rate suggesting that both hormones may regulate the grain filling of wheat at early stage (Yang *et al*., 2001). When the plants were sprayed with ABA and BA the IAA content increased over that of drought alone (Yang *et al*., 2002). Similarly application of benzyl adenine stimulated accumulation of IAA and GA in grains under drought as compared to drought alone but the magnitude of increase was less than that of ABA (Younis *et al*., 2003). The CK may increase the amount of IAA by reducing IAA conjugation or by increasing its biosynthesis (Srivastava, 2002). ABA partially overcame the drought induced decrease in IAA concentration. When the plants were sprayed with ABA and BA, IAA content increased (Yang *et al*., 2002) over that of drought alone.

ABA content of leaves (Table 6) was found to be maximum in case of ABA treated plants (156% greater than that of control). BA treatment decreased ABA content such that it was 25% less that of drought and 39% than that of ABA treatment but ABA content was 56% greater than that of control. In spikes, there were linear increases in endogenous ABA content up to milky stage, thereafter decline in ABA content occurred. Drought increased the ABA content by 159% over that of control at milky stage. With the application of ABA, endogenous ABA content of spikes was increased. The application of BA had the opposite effect. BA application resulted in 58% less ABA than that of drought and 8% more than that of control. The value was 62% less than that of ABA treatment. ABA showed significant increase whereas BA showed significant decrease over that of drought alone in both leaves and spikes of cv1 Margalla-99, whereas in Manthar-2003 the effect of ABA was non significant over drought at later stages of grain filling.

Abscisic acid accumulation in leaves of plants under water deficit was greater than that of control (Zhang *et al*., 2004). Yang *et al*., (2004) proposed that changes in ABA concentration in the grains followed a similar pattern to the grain filling rate. Rate of grain filling was significantly and positively correlated with the concentration of ABA. Under mild drought treatment, an enhanced ABA accumulation in the grains was closely associated with an increased grain filling rate. Increased ABA concentration in the grain might result from autosynthesis within the grain (King, 1982) and partly by the translocation from leaves and roots (Ober *et al*., 1991). Ahmadi & Baker (1999) suggested that water stress might increase the ABA level of flag leaves which provide assimilates *via* the phloem to the grains. According to Cowan *et al*., (1999), CK reduced the amount of ABA in plant by inhibiting the biosynthesis of ABA. CK might exert an effect on ABA metabolism by influencing the oxidation of xanthoxal to ABA and its further conversion to PA and DPA. Pospisilova *et al*., (2005) proposed that ABA accumulation was inhibited by BA application in bean and maize plants.

Table 1. Effect of drought stress on proline content (mg/g) of flag leaves and spikes of wheat samples of Margalla-99 (cv1) and Manthar-2003 (cv2).

Treatment	Proline content of leaves					Proline content of spikes				
	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage
	Margalla-99 (cv1)									
Control	0.070 M	0.15 LM	0.210 KL	0.243 JKL	0.310 IJK	0.117 M	0.180 LM	0.280 JKL	0.330 IJK	0.423 I
D	0.167LM	0.350 IJ	0.550 GH	0.827 DE	1.120 C	0.21 KLM	0.430 I	0.660 GH	0.927 E	1.427 C
ABA + D	0.370 IJ	0.750 EF	1.363 D	1.560 A	1.573 A	0.373 IJ	0.813 EF	1.343 CD	1.820 A	1.950 A
BA + D	0.2KLM	0.423 HI	0.683 FG	0.953 D	1.273 D	0.23 KLM	0.597 H	0.747 FG	1.260 D	1.573 B
	Manthar-2003 (cv2)									
Control	0.077 J	0.147 IJ	0.203 HIJ	0.250 HIJ	0.297GHIJ	0.113 L	0.177 KL	0.277 JKL	0.323 IJK	0.410 HIJ
D	0.17 IJ	0.253HIJ	0.477FGHI	0.64 DEFG	0.997BCD	0.217 KL	0.340 IJK	0.543 FGH	0.783 DE	1.240 BC
ABA + D	0.287GHIJ	0.55EFGH	1.220 CDE	1.333 AB	1.450 A	0.280 JKL	0.667 EF	1.173 C	1.647 A	1.767 A
BA + D	0.183 IJ	0.38GHIJ	0.49 FGHI	0.77 CDEF	1.050 BC	0.220 KL	0.460 GHI	0.600 FG	0.860 D	1.353 B

cv1 Leaves = LSD = 0.1397; cv1 Spikes = LSD = 0.1397
cv2 Leaves = LSD = 0.3628; cv2 Spikes = LSD = 0.1710

Table 2. Effect of drought stress on IAA content (ng/g) of flag leaves and spikes of wheat samples of Margalla-99 (cv1) and Manthar-2003 (cv2).

Treatment	IAA content of leaves					IAA content of spikes				
	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage
	Margalla-99 (cv1)									
Control	127.67 A	107 D	87.67 E	65.33 HIJ	41.33 MN	88 DE	134.33 A	102.67 C	75.67 FGH	52.33 LM
D	108.67 CD	78 FG	62.33 IJ	43.67 LM	24 O	66 HIJ	97.67 CD	65.33 IJK	44.67 MN	29 O
ABA + D	120.33 AB	86.33 EF	73.33 GH	57 JK	37.33 MN	76.67 FG	121 B	91 DE	59 JKL	38 NO
BA + D	116.67 BC	81.33 EFG	67.67 HI	52.33 KL	32.33 NO	73 GHI	113.33 B	85 EF	55.67 KL	36 NO
	Manthar-2003 (cv2)									
Control	126.67 A	105.67 B	85.67 CD	64.33 EFG	40.33 IJ	87 D	133.33 A	101 BC	74.67 EF	51 HI
D	92 C	58.67 FGH	40.33 IJ	19 LM	11.33 M	61.67 GH	84 DE	52.67 HI	31 KL	16.67 M
ABA + D	110.67 B	75 DE	54.33 GH	37 JK	25.33 KL	68.67 FG	110.33 B	73 F	45.33 IJ	31.33 KL
BA + D	105 B	68 EF	49.67 HI	32.33 JK	19.67 LM	61.33 GH	91.67 CD	60.67 GH	37.33 JK	23.33 LM

cv1 Leaves = LSD = 9.386; cv1 Spikes = LSD = 9.941
cv2 Leaves = LSD = 11.42; cv2 Spikes = LSD = 11.20

Table 3. Effect of drought stress on GA content (ng/g) of flag leaves and spikes of wheat samples of Margalla-99 (cv1) and Manthar-2003 (cv2).

Treatment	GA content of leaves					GA content of spikes				
	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage
	Margalla-99 (cv1)									
Control	290.67 A	147.67 E	100 H	74 J	54.67 LM	291.33 B	322.33 A	176 E	93 I	59 K
D	244 D	114 G	84 I	57.33 L	39 O	257.67 D	278.67 BC	113.67 H	61.67 K	35.33 M
ABA + D	265.67 B	132.33 F	92.33 HI	69.33 JK	48.33 MN	270.33 CD	290.67 B	145.33 F	80.67 IJ	49.67 KL
BA + D	256.67 C	128.67 F	89 I	63 KL	44.67 NO	265 B	282.67 BC	129.67 G	77.67 J	44.67 LM
	Manthar-2003 (cv2)									
Control	289 A	145.33 E	98.67 GH	72.67 JK	53.33 L	290.33 B	321 A	174.67 G	92.33 I	58.33 JK
D	209.33 D	89 HI	68 K	34.67 NO	17.67 P	207.33 F	256.33 DE	100.67 I	42.33 KL	23 M
ABA + D	244 B	114 F	80.33 IJ	50.33 LM	35.67 NO	244.33 E	281 BC	131.33 H	64.67 J	44 KL
BA + D	222.33 C	105.33 FG	71.33 JK	40.67 MN	24.33 OP	219 F	265.67 CD	118.67 H	51.33 JK	33.67 LM

cv1 Leaves = LSD = 8.566; cv1 Spikes = LSD = 13.09
cv2 Leaves= LSD = 11.41; cv2 Spikes = LSD = 16.90

Table 4. Effect of drought stress on ABA content (ng/g) of flag leaves and spikes of wheat samples of Margalla-99 (cv1) and Manthar-2003 (cv2).

Treatment	ABA content of leaves					ABA content of spikes				
	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage
	Margalla-99 (cv1)									
Control	75 FG	61 H	44 JK	32 LM	25 M	53 K	90 J	139 G	83 J	35 L
D	125 B	108 C	92 D	73 G	52 IJ	105 I	220 D	360 B	180 E	80 J
ABA + D	153 A	123 B	103 C	87 DE	64 H	125 H	260 C	399 A	222 D	112 I
BA + D	82 EF	73 G	59 HI	51 IJ	39 KL	62 K	111 I	152 F	106 I	50 K
	Manthar-2003 (cv2)									
Control	61 FG	42 H	33 HI	29 IJ	22 J	49 K	85 HI	140 D	80 I	33 L
D	102 B	82 C	69 DEF	59 G	35 HI	79 I	162 C	201 B	111 F	58 K
ABA + D	120 A	95 B	73 CD	63 EFG	41 H	90 GH	198 B	250 A	130 E	70 J
BA + D	72 DE	56 G	42 H	37 HI	28 IJ	56 K	97 G	122 E	91 GH	50 K

cv1 Leaves = LSD = 8.466; cv1 Spikes = LSD = 11.74
cv2 Leaves = LSD = 9.118; cv2 Spikes = LSD = 8.524

Table 5. Effect of drought stress on number of infertile spikelets per spike of wheat samples of cv1 and cv2.

Treatment	*cv1	**cv2
Control	2.69 C	2.39 C
D	9A	11.2 A
ABA + D	4.27 C	5.23 B
BA + D	6.47 B	7 B

*LSD = 2.150; **LSD value = 2.313

All such means which share common letters, are statistically non significant at <0.01

Aqueous solution of ABA (10^{-6}M) was provided as foliar spray at anthesis stage (80 DAS). Drought stress was induced immediately after anthesis stage, watery ripe stage (10 DAA), milky stage (20 DAA), soft dough stage (30 DAA), and hard dough stage (40 DAA).

Table 6. Effect of drought stress on changes in dry weight (mg/ grain) of grains of wheat samples of cv1.

Treatment	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage	Treatment mean**
Control	10 L	30 GHI	50 C	70 B	79 A	47.8
D	10 L	20 JK	30 GHI	38 DEFG	42 CDE	28 C
ABA + D	12 KL	25 HIJ	35 EFG	42 CDE	49 C	32.6 B
BA + D	9 L	22 IJ	32 FGH	40 DEF	45 CD	29.6 BC
***Stages mean	10.3 E	24.3 D	36.8 C	47.5 B	53.8 A	

LSD (Stages x Treatments) = 8.262; **LSD value = 3.695; ***LSD = 4.131

All such means which share common letters, are statistically non significant at < 0.01

Aqueous solution of ABA (10^{-6}M) was provided as foliar spray at anthesis stage (80 DAS). Drought stress was induced immediately after anthesis stage, watery ripe stage (10 DAA), milky stage (20 DAA), soft dough stage (30 DAA) and hard dough stage (40 DAA).

Table 7. Effect of drought stress on changes in dry weight (mg/grain) of grains of wheat samples of cv 2.

Treatment	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage	Treatment mean**
Control	10IJ	29.33 DEF	49 B	71 A	78 A	47.5
D	9 J	15 HIJ	22 FGH	26 EFG	28 DEFG	20 C
ABA + D	11 IJ	21 GH	29 DEF	34 CD	39 C	26.8 B
BA + D	10 IJ	17 HI	25 EFG	29 DEF	32 CDE	22.7 C
***Stages mean	10 E	20.6 D	31.25 C	40.1 B	44.3 A	

LSD (Stages x Treatments) = 7.957; **LSD value = 3.558; ***LSD = 3.978

All such means which share common letters, are statistically non significant at < 0.01

Aqueous solution of ABA (10^{-6}M) and BA (10^{-6}M) was provided as foliar spray at anthesis stage (80 DAS). Drought stress was induced immediately after anthesis stage, watery ripe stage (10 DAA), milky stage (20 DAA), soft dough stage (30 DAA) and hard dough stage (40 DAA).

Table 8. Effect of drought stress on changes in dry weight (mg/ grain) of grains of wheat samples of cv1.

Treatment	Anthesis stage	Watery ripe stage	Milky stage	Soft dough stage	Hard dough stage	Treatment mean**
Control	10 L	30 GHI	50 C	70 B	79 A	47.8
D	10 L	20 JK	30 GHI	38 DEFG	42 CDE	28 C
ABA + D	12 KL	25 HIJ	35 EFG	42 CDE	49 C	32.6 B
BA + D	9 L	22 IJ	32 FGH	40 DEF	45 CD	29.6 BC
***Stages mean	10.3 E	24.3 D	36.8 C	47.5 B	53.8 A	

LSD (Stages x Treatments) = 8.262; **LSD value = 3.695; ***LSD = 4.131

All such means which share common letters, are statistically non significant at < 0.01

Aqueous solution of ABA (10^{-6}M) was provided as foliar spray at anthesis stage (80 DAS). Drought stress was induced immediately after anthesis stage, watery ripe stage (10 DAA), milky stage (20 DAA), soft dough stage (30 DAA) and hard dough stage (40 DAA).

In potted plants ABA was found to be more effective at later stages of grain filling whereas BA was more effective at anthesis stage. Benzyladenine had a similar although weaker effect than abscisic acid. Both the growth regulators have protective effect against water deficit. These hormones were applied only once at anthesis stage but it appears that they have long lasting effect.

The results indicated that both the varieties of wheat differed with respect to their IAA, GA and ABA content, accumulation of proline in both flag leaves and spikes. The tolerant variety had higher level of IAA, GA, ABA and proline, indicating its greater phytohormone content and osmoregulation as compared with the susceptible variety. Milky stage appears to be the critical stage for maximum response exhibited by the applied growth regulators. The significant effect of moisture stress in both varieties starts mostly at milky stage. Blum *et al.*, (1999) also indicated that variety having higher osmoregulation showed greater grain yield than other one. It was reported that varieties having higher ABA content have better capacity to osmoregulate and hence perform better in terms of growth and yield under water stress (Quarrie *et al.*, 1999), indicating a close association between ABA and solute accumulation. This was corroborated by the present study where exogenous application of ABA to the plants experiencing water stress accelerated the accumulation of proline, and improved their water status. Margalla-99 (cv1) showed better performance under drought stress, even the response of ABA and BA was greater in Margalla-99 as compared to that of cv2 Manthar-2003.

Drought showed 234% more infertile spikelets than that of control in cv1. ABA treatment decreased infertility and showed 59% more infertile spikelets than control which was 52% less than that of drought whereas BA treated plants showed 141% more infertile spikelets than that of control which was 28% less than that of drought. Similar was the case with cv 2 but number of infertile spikelets was more in cv 2 than in cv 1. The grain yield depends upon fertile spikelets, number of grains per ear and grain weight. The decrease in production of spikelets and grain weight may be due to disturbed nutrients uptake efficiency and photosynthetic translocation under water stress (Iqbal *et al.*, 1999).

Maximum dry weight was achieved in control treatment. Grain dry weight from drought treated plants was 64% less than those harvested from control plants at hard dough stage. The dry weight of the grain was 17% more than that of drought whereas BA showed only 07% higher dry weight of the grains as compared with that of drought in hard dough stage. The cv2 (Table 6) contained less grain dry weight than that of cv1.

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