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EFFECT OF ABSCISIC ACID AND BENZYLADENINE ON GROWTH AND ION ACCUMULATION OF WHEAT UNDER SALINITY STRESS

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Abstract

A glass-house pot experiment was conducted to assess the role of Abscisic acid (ABA) and Benzyladenine (BA) on growth and ion accumulation of two spring wheat cultivars viz., cv. Inqulab-91 and cv. SAARC-1 under salinity stress. Seeds of both cultivars were treated prior to sowing with ABA and BA each at 10^{-5} M for 24h. Three weeks old plants of both the cultivars were exposed to 0 and 100 mol m^{-3} NaCl. Plants were harvested three weeks after salt treatment. Fresh and dry weight of shoot and root decreased with salt treatment, whereas ABA and BA treatments caused a significant ameliorative effect on both the cultivars. Under salt stress, plant height was more adversely affected in Inqulab-91 than in SAARC-1. Salt treatment increased the concentration of Na^+ and Cl^- in flag leaf of both the cultivars. Both ABA and BA treated plants showed significant decrease in Na^+ content but increased K^+ content in flag leaf of both the cultivars. ABA and BA treatment decreased plant height but increased number of grains per spike and grain yield. ABA and BA treatments further augmented the stimulatory effect of salt on proline accumulation. Higher proline accumulation was observed in SAARC-1 than in Inqulab-91 with ABA and BA treatment. Under salt stress the hormone mediated osmoregulation by increased proline production and the selectivity in uptake and accumulation of toxic ions like Na^+ and Cl^- has been discussed. BA was more effective to increase chlorophyll "a" & "b, flag leaf area, number of grains per spike and grain yield, under salt stress as well as in non saline soil than ABA.

Introduction

Soil salinity is a major constraint in food production because it limits crop yield and restricts use of lands for cultivation (Szaboles *et al*., 1994). Approximately 20% of agricultural land and 50% of cropland in the world are salt stressed (Nelson *et al*., 1999, Flowers *et al*., 1995). Water and soil management practices have improved agricultural production on soils affected by salinity, but this approach is costly. Whereas increasing salt tolerance of crops should be useful alternative solution (Munns *et al*., 2002). Different types of phytohormones are being extensively used to alleviate the adverse effect of salinity stress on crop growth where ABA, GA, BA have been the main focus of most of the plant scientists (Aldesaquy *et al*., 1998, Hisamatsu *et al*., 2000). Addition of cytokinins in the growth medium can improve the salt tolerance of the plants (Flower & Hajibagheri, 2002). Salinity damages in wheat and other species are commonly due to excessive sodium and chloride uptake by plant (Flowers *et al*., 1986). ABA reduces transpiration by closing stomata and thus leads to reduced ion uptake in plant (Yeo *et al*., 1985). Exogenous ABA application decreased Na^+ accumulation and increased K:Na

ratio in sorghum and rice shoot and leaves (Amzallag *et al*., 1990, Bohra *et al*., 1995). In a saline environment, treatment of plant with an appropriate concentration of ABA improved salinity resistance and increased growth in sorghum, pea and rice (Amzallag *et al*., 1990, Fedina *et al*., 1994, Bohra *et al*., 1995) Increase in growth with contemporary ABA treatment has been reported under saline condition and was mainly attributed to a reduction in Na^+ uptake with elevated ABA levels (Amzallag *et al*., 1990, Bohra *et al*., 1995). Cytokinins promote cell division, leaf expansion, accumulation of chlorophyll and delay leaf senescence and are involved in response to adverse environmental conditions (Hare *et al*., 1997, Brault *et al*., 1999). Application of synthetic cytokinin alleviated negative effect of NaCl on chlorophyll content in detached wheat leaves (Mumtaz *et al*., 1997). In wheat, the entry of Na^+ into the plant and the mechanism controlling Na^+ transport within the plant are not well understood (Schachtman *et al*., 1991).

The present study was aimed to evaluate the comparative effect of ABA and BA on the amelioration of salt stress on two cultivars of wheat differing in salt tolerance with particular emphasis on osmoregulation and accumulation of ions in flag leaf.

Materials and Methods

The seed material of two spring wheat (*Triticum aestivum* L.) cultivars i.e., Inqulab-91 and SAARC-1 were obtained from National Agriculture Research Centre (NARC) Islamabad, Pakistan. The experiment was conducted in a glasshouse during the winter/spring of 2003-04 with average day/night temperature $30^oC \pm (8^oC)$ and $13 \pm (5^oC)$ respectively and photo period ranging from 10-13.5 h. The experiment was repeated in two consecutive years. The plastic pots of 30×40 cm were filled with 10kg soil collected from the field of NARC (15 cm from the upper soil layer). Soil was dried, ground, sieved (<2mm) and analyzed for the physico-chemical characteristics (Table 2). Soil particle size distribution was determined by Hydrometer method (Moodie *et al*., 1959) and textural class according to USDA system. Phosphorus and Potassium was determined by the method of Black (1965). Organic matter was determined by the method of Walkely & Black (1934) and $CaCO_3$ by Puri's (1966). Electrical conductivity and pH of soil was determined following the method of Richards (1954) and Jackson (1965).

Seeds of wheat were treated for 24h in aqueous solution of plant growth regulators ABA, BA (10^{-5} M) and distilled water in case of control in black painted well aerated flask. After hormone treatment, seeds were washed with tap water followed by washing with distilled water. Then 10 seeds were sown in respective soil pots. After two weeks of germination, plants were thinned to five. Salinity was developed by using NaCl salt as calculated by Hand Book 60 (Richards 1954). The NaCl solution was applied to two week old plants with five equal splits in regular five days.

Experiment was conducted in two factor Completely Randomized Design with three replication with six treatments and two varieties V1 (Inqulab-91) and V2 (SAARC-1) (Table 1). Recommended fertilizer was applied to the pots @ 120, 90 and 60 kg ha^{-1} N, P_2O_5 and K_2O on soil weight basis, in the form of urea, Di Ammonium Phosphate and Sulphate of Potash. All the P and K and half N were applied at the time of sowing while the half N was applied at tillering stage.

Table 1. Treatments made

Treatments	Symbols
Seed treatment with distilled water in non saline soil.	T1
Seed treatment with Abscisic acid in non saline soil.	T2
Seed treatment with Benzyladenine in non saline soil.	T3
Seed treatment with distilled water + 100 mol m^{-3} NaCl.	T4
Seed treatment with Abscisic acid + 100 mol m^{-3} NaCl.	T5
Seed treatment with Benzyladenine + 100 mol m^{-3} NaCl.	T6

Table 2. Physico-chemical characteristics of soil.

Parameters studied	Values
pH (1:1)	7.8
ECe (1:1)	0.34 dSm^{-1}
Textural class	Sandy Loam
$CaCo_3$	1.45 (%)
Organic matter	0.85 (%)
AB-DTPA P	3.47 ppm
Exchangeable K	94 ppm
$Ca^{+2} + Mg^{+2}$	9 meL^{-1}
Na^{+}	1.20 meL^{-1}
Chlorides (Cl^{-})	3.45 meL^{-1}
Saturation Percentage	39 (%)

The data for different growth parameters were recorded three weeks after the application of salinity (at the tillering stage). Six plants per treatment were randomly harvested and their root and shoot were separated. All the plant samples were washed with distilled water and their fresh weights were recorded. Chlorophyll a and b were determined from fully expanded young leaves by the method of Arnon (1949). The shoot and root were oven-dried at 65^{o}C to constant dry weight. Flag leaf area was determined by measuring the length and width of flag leaf at grain milky stage. The area was calculated by the formula LA= Leaf Length × leaf Breadth × Factor, the factor was determined by passing about 20 leaves from leaf portable leaf area meter (Li-Cor, model LI-3000A) UK. Free proline content of flag leaf was determined by the method of Bates *et al*., (1973). Na^{+} and K^{+} contents were determined from dried flag leaves by wet digested in Nitric acid–Perchloric acid following the method of Baker & Amacher (1982). The Na^{+} and K^{+} were determined by flame photometry (Sherwood model 410, Japan) Ca was determined with Atomic Absorption spectrophotometer (Shimadzu 6200AA Japan). Chloride was determined by the method of Chapman (1961). The crop was harvested at its physiological maturity. The Agronomic data were recorded for number of grains per spike, grain yield per pot, plant height and 1000 grain weight. Statistical analyses of data were done by the procedure of Gomez (1984).

Results and Discussion

Significant interactions were observed between cultivars and treatments for shoot fresh and dry weights, concentrations of Na^{+}, K^{+}/Na^{+} ratio and number of grains per spike (Table 3, 4, 5).

Table 3. Interactions of treatments and varieties for shoot fresh weight (SFW), Shoot dry weight (SDW), Root fresh weight (RFW) and Root dry weight (RDW) at vegetative growth stage.

Treatments	SFW (g)		SDW (g)		RFW (g)		RDW (g)	
	V1	V2	V1	V2	V1	V2	V1	V2
T1	5.88 cd	5.2 ef	1.22 c	1.22 c	1.03	1.23	0.42	0.60
T2	8.13 a	6.73b	2.64a	2.64a	1.50	1.98	0.80	1.17
T3	7.62 a	6.26 bc	2.18 b	2.18 b	1.35	1.67	0.76	1.07
T4	3.25 h	4.03 g	0.76 d	0.76 d	0.62	0.85	0.18	0.37
T5	5.05 ef	5.97 cd	0.97 cd	0.97 cd	0.95	1.25	0.33	0.73
T6	4.81 f	5.05 de	0.90 c	0.90 c	0.82	0.95	0.32	0.69
Mean	5.79	5.61	1.50	1.47	1.04	1.32	0.47	0.77
LSD (0.01)	0.448		0.451		NS		NS	
CV %	4.88		13.30		18.4		14.21	

V1 = Inqulab-91
V2 = SAARC-1

Table 4. Interactions of treatments and varieties for Chlorophyll "a" (Chl "a"), Chlorophyll "b" (Chl "b"), Flag leaf area (cm^2) and Plant height (Pl. height).

Treatments	Chl "a"		Chl "b"		Leaf area (cm^2)		Pl. height (inches)	
	V1	V2	V1	V2	V1	V2	V1	V2
T1	3.69	3.65	0.30	0.33	20.30	19.41	34.33	28.17
T2	3.73	3.69	0.30	0.35	19.21	18.62	29.63	24.66
T3	4.67	4.47	0.48	0.44	22.65	21.74	31.20	26.77
T4	2.19	2.71	0.18	0.22	18.41	17.80	28.44	24.60
T5	2.38	2.72	0.20	0.23	17.12	17.20	26.03	21.65
T6	3.83	3.94	0.36	0.39	19.49	19.76	27.80	23.55
Mean	3.42	3.53	0.31	0.33	19.53	19.09	29.74	25.07
LSD (0.01)	NS		NS		NS		NS	
CV %	7.79		8.17		6.74		5.35	

V1 = Inqulab-91
V2 = SAARC-1

Table 5. Interactions of treatments and varieties for Sodium (Na^+ %), Potassium (K^+ %), Chlorides (Cl^- %) and Calcium (Ca^{++} %) Concentration in flag leaf.

Treatments	Na^+ (%)		K^+ (%)		Cl^- (%)		Ca^{++} (%)	
	V1	V2	V1	V2	V1	V2	V1	V2
T1	0.18 e	0.20 e	4.72	5.38	0.77	0.91	0.52 c	0.64 ab
T2	0.10 e	0.12 e	5.50	6.01	0.62	0.72	0.62 b	0.67 ab
T3	0.13 e	0.17 e	5.85	6.08	0.73	0.80	0.67 ab	0.70 ab
T4	6.37 b	6.81 a	1.85	2.14	4.67	5.14	0.28 e	0.30 de
T5	5.08 d	5.59 c	2.50	2.80	3.63	4.14	0.32 de	0.35 de
T6	5.66 c	5.88 c	2.34	2.52	3.90	4.38	0.37 de	0.38 d
Mean	2.92	3.13	3.80	4.16	2.39	2.68	0.46	0.51
LSD(0.01)	0.402		NS		NS		0.072	
CV %	5.78		4.52		8.41		5.35	

V1 = Inqulab-91
V2 = SAARC-1

Shoot fresh and dry weights were decreased by salt treatment but both ABA and BA had partially ameliorated the inhibitory effect of salt on shoot fresh and dry weight, among which ABA was more effective. The cv SAARC-1 had higher shoot fresh and dry weight than Inqulab-91 under salt treatment. Higher root fresh & dry weights were recorded with ABA seed pre-treatment in both the cultivars. The response of SAARC-1 was higher than that of Inqulab-91 with ABA and BA seed pre-treatment over the control. Marschner (1986) and Flowers (1991), reported that inhibition in growth is believed to be due to the osmotic effect, specific ion toxicity and nutritional imbalances. Mansoor (1994) reported that salinity decreased in the growth of shoot and root of two wheat varieties.

Chlorophyll "a" contents were higher in SAARC-1 than Inqulab-91. Chlorophyll "a" and "b" contents were decreased by salt stress (Table 4). ABA was not effective to increase chlorophyll "a" content, while BA showed stimulatory effect over control. Maximum chlorophyll "a" and "b" were observed in Inqulab-91 with BA treatment under non saline soil conditions. Krishnamarthy *et al.,* (1987) reported that a decrease in chlorophyll content has been observed in salinity sensitive rice, however tolerant lines show a slight increase in the presence of hormone.

Flag leaf transport assimilates to spike and developing grains. Flag leaf area was decreased by salinity stress; however BA seed pre-treatment proved to be effective to overcome the inhibitory effect of salt on flag leaf area, while ABA had no marked effect on the salt induced decrease in flag leaf area (Table 4). Maximum flag leaf area was observed in Inqulab-91 with BA seed pre-treatment under non saline condition. Similarly leaf area reduction under saline conditions was recorded by Neuman *et al*., (1988).

Plant height was decreased by salt stress. (Table 4). ABA and BA had no marked effect either in saline or non-saline conditions. Zaidan *et al*., (1990) showed that 100 mM NaCl in the growth medium caused reduction in the length of epidermal cell and in the rate of apparent cell productions.

Data from Table 5 showed that salt stress increased the concentration of Na^+ and Cl^- in the flag leaf of wheat, while it decreased the concentrations of K^+. Abscisic acid and Benzyladenine treatment decreased the concentration of Na^+ but increased the concentration of K^+ under salt stress. The magnitude of decrease in Na^+ content under salt stress was greater in ABA treatment than BA (Table 5).

Interactions of treatments and varieties were significant at LSD 0.01=0.402 and cv was 5.78 %. Both ABA and BA decreased the concentration of Na^+ and Cl^- in flag leaf of wheat under salt stress. cv-SAARC-1 accumulate higher Na^+ and Cl^- content than cv-Inqulab-91. The magnitude of decrease in Cl^- content under salt stress was greater in ABA treatment than BA (Table 5). According to Flower *et al*., (1995), ABA reduced transpiration by closing stomata and thus leads to reduction in ion uptake in plants, Ashraf & O' Leary, (1996), reported that uptake and accumulation of toxic ions such as Na^+ and Cl^- in crop species including wheat are enhanced under saline condition.

Increase in growth with contemporary ABA treatment has been reported in saline conditions and was mainly attributed to reduction in Na^+ with elevated ABA levels (Amzallag *et al*., 1990, Bohra *et al*., 1995). ABA and BA increased the concentrations of K^+ over control at salt stress. Higher K^+ concentration at salt treatment was observed in SAARC-1 with ABA seed pre-treatment (Table 5). ABA decreased Na^+ and increased K^+ concentrations. K^+/Na^+ ratio was significantly positively correlated with treatments and cultivars at LSD 0.01= 10.48. Higher K^+/Na^+ ratio in NaCl treatment was observed with

ABA and BA respectively (Table 6). Higher ratio of K/Na in flag leaves can be considered good indicators for salinity tolerance. ABA and BA increased the concentration of Ca^{+2} over control at salt stress. BA treatment observed higher concentration of Ca^{+2}. No marked effect was observed between the two cultivars in case of Ca^{+2}. The Ca^{+2}/Na^{+} ratio was significantly positively correlated with treatments and cultivars at LSD 0.01= 1.67. SAARC-1 had high K^{+}/Na^{+} and Ca^{+2}/Na^{+} ratios under salt treatment (Table 6). Previous studies emphasized the important role of $K^{+}/Na^{+}$and Ca^{+2}/Na^{+} for salinity tolerance, (Gorham, 1993. Sharma, 1994).

Proline contents were relatively higher at $100molm^{-3}$ than non-saline soils (Table 6). Higher proline content was observed in cv-SAARC-1 with NaCl treatment. Both ABA and BA treatment further augmented the salt induced increase in the proline content of flag leaf. The proline content was higher in SAARC-1 than in Inqulab-91. Flower & Yeo, (1989) reported that accumulation of proline and some other organic solutes associated with stress may serve as a compatible solute in order to maintain the osmotic balance between the cytoplasm and vacuole. Santos-Diaz & Alego (1994) also reported that proline was many fold greater in resistance than susceptible cultivars.

Grain yield/pot and 1000-grain weight were also decreased under salt stress. BA and ABA increased grain yield over control (Table 7). Similar trend was observed in cultivars in case of grain yield/pot; the BA had higher grain yield than ABA. 1000-grain weight was increased with ABA and BA over control. Relatively higher 1000-grain weight was recorded with ABA treatment. Treatments were significant at LSD 0.01 (Table 8) for means of ions ($Na^{+,}$ K^{+}, Cl^{-}), proline contents and grain yield of wheat.

Interactions between varieties and treatments were significant for number of grains/spike. Higher number of grains/spike was recorded in Inqulab-91 with BA in non-saline soils while at $100molm^{-3}$ SAARC-1 performed better (Table 7).

It is inferred from the present investigation that ABA and BA were effective in ameliorating adverse effects of salt on both the cultivars, but wheat cv-SAARC-1 was better than cv-Inqulab-91 because it showed higher shoot-root dry weight, greater proline content and higher K^{+}/Na^{+} ratios, number of grains/spike and 1000 grain weight under induced salt stress as compared to cv-Inqulab-91. BA was more effective to increase chlorophyll "a" & "b, flag leaf area, number of grains per spike and grain yield, under salt stress as well as in non saline soil than ABA.

Table 6. Interactions of treatments and varieties of K^{+}/Na^{+}, Ca^{+2}/Na^{+} and Proline contents (µ moles g^{-1} f wt) in flag leaf of wheat.

Treatments	K^{+}/Na^{+}		Ca^{+2}/Na^{+}		Proline	
	V1	V2	V1	V2	V1	V2
T1	25.97 d	26.70 d	2.86 b	2.91 b	104 h	112 gh
T2	62.69 a	49.90 bc	8.12 a	5.65 b	108 gh	128 g
T3	56.48 ab	38.89 c	6.60 a	4.22 b	106 gh	114 gh
T4	0.29 e	0.31 e	0.043 c	0.043 c	1092 f	1220 c
T5	0.49 e	0.50 e	0.063 c	0.063 c	1159 d	1340 a
T6	0.41 e	0.43 e	0.070 c	0.073 c	1121 e	1290 b
Mean	25.42	19.62	2.93	1.95	615.10	700.8
LSD(0.01)	10.48		1.67		22.14	
CV %	20.37		30		84.73	

Table 7. Interactions of treatments and varieties for number of Grains/spike, Grain yield/pot and 1000 grain weight of wheat at physiological maturity.

Treatments	Number of grain/spike		Grain yield/pot (g)		1000 Grain wt:	
	V1	V2	V1	V2	V1	V2
T1	38.00 b	36.33 b	6.79	6.37	36.78	38.83
T2	40.00 ab	38.33 b	10.33	9.71	41.41	42.53
T3	44.00a	41.00 ab	12.46	12.08	42.40	42.50
T4	16.33 d	19.66 cd	2.73	3.10	33.06	33.46
T5	20.66 cd	22.66 c	5.36	5.87	35.80	38.73
T6	22.00 c	24.33 c	6.17	6.52	36.77	39.46
Mean	30.17	30.39	7.31	7.28	37.71	39.76
LSD(0.01)	4.85		NS		NS	
CV %	7.03		6.86		5.06	

V1 = Inqulab-91
V2 = SAARC-1

Table 8. DMRT for treatment means of accumulated ions ($Na^{+,}$ K^{+}, Cl^{-}), and proline contents of flag leaf and grain yield/pot of wheat.

Treatment	Na^{+} (%)	K^{+} (%)	Cl^{-} (%)	Proline (µ moles g^{-1} f wt)	Grain yield /pot (g)
T1	0.19 d	5.05 b	0.84 c	108 b	6.58 c
T2	0.19 d	5.76 a	0.67 c	118 b	10.02 b
T3	0.13 d	5.96 a	0.77 c	110 b	12.27 a
T4	6.59 a	1.99 d	4.90 a	1156 a	2.91 e
T5	5.34 c	2.66 c	3.90 b	1250 a	5.62 d
T6	5.77 b	2.43 c	4.14 b	1205 a	6.34 cd
LSD (0.01)	0.284	0.346	0.346	141.80	0.81

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