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SEED PRIMING IMPROVES SALINITY TOLERANCE OF WHEAT VARIETIES

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Abstract

To evaluate the response of wheat varieties to seed priming and salinity, an experiment was conducted in completely randomized design (CRD) with three replications at Institute of Biotechnology and Genetic Engineering (IBGE), KPK Agricultural University, Peshawar, Pakistan. The performance of 6 wheat varieties (Tatara-96, Ghaznavi-98, Fakhri Sarhad, Bakhtawar-92, Pirsabaq-2004 and Auqab-2000) at two seed conditions (primed with 30 mM NaCl and unprimed) under four salinity levels (0, 40, 80 and 120 mM) was studied. Statistical analysis of the data revealed that salinity, seed priming and varieties had significantly ($P \leq 0.05$) affected shoot fresh weight plant^{-1} shoot dry weight plant^{-1} , shoot Na^+ contents (mg g^{-1} dry weight), shoot K^+ contents (mg g^{-1} dry weight) and shoot K^+/Na^+ ratio. Maximum shoot fresh weight plant^{-1} (7.71 g), shoot dry weight plant^{-1} (1.68 g), shoot K^+ contents (1.39 mg g^{-1} dry weight) and shoot K^+/Na^+ ratio (1.45) were recorded from Bakhtawar-92 as compared with other varieties. Highest shoot Na^+ contents (1.43 mg g^{-1} dry weight) were recorded from Auqab-2000 when compared with other varieties. All parameters were enhanced with seed priming except shoot Na^+ contents, which reduced significantly ($p \leq 0.05$) with seed priming.

Introduction

Agriculture is the major sector of Pakistan's economy. Two third population of the country depends on agriculture for their livelihood. Naqvi *et al*., (1992) and Mellor (1988) had proposed a growth rate of more than five percent for alleviating poverty in Pakistan. Major problems of low crop yield in Pakistan are water logging and salinity in irrigated regions particularly in the Indus Plains. About 30% of irrigation water is derived from ground water. Out of over 562,000 private and 16,000 public tube wells in Pakistan, 70 % are pumping brackish water (PCST., 2003). Wheat (*Triticum aestivum* L.) is the major staple food of Pakistan. Wheat is grown for meeting the food demand of over growing population of the country. But its per hectare yield is below than its yield potential, which might be due to several reasons like poor nutrients management, shortage of good quality water, poor weeds & pest management, drought, salinity and water logging. Salinity is a serious threat for wheat production in Pakistan. Reclamation methods of salt affected soils include proper drainage system, irrigation water application, residues incorporation, leaching, plantation of tolerant crops, and chemical amendments (gypsum addition) to sodic or saline-sodic soils (Lamond & Whitney, 1992). Among the reclamation techniques, the low cost and successful tool to increase productivity from available resources is plantation of tolerant crops. As the other methods to combat salinity hazards are expansive and difficult to be carried out. The most promising solution for overcoming the soil salinity problem is the use of salt tolerant species that exhibit higher yield in saline soils (Shafi et al., 2010; Ashraf & Leary, 1996). In addition to that priming agents i.e., $CaCl_2$, KCl, and NaCl are effective in alleviating the adverse effects of salt stress on wheat plants (Iqbal *et al*., 2006). Pre-sowing seeds treatment with inorganic salts (halopriming) is very easy, low risk and low cost teshnique to alleviate the salinity hazards of agricultural lands. The halopriming technique is very effective for improving germination and crop establishment under salt stressed conditions .Generelly seed priming increases the uniformity and rate of seed emergence of crops (Bakht et al., 2010; Sivritepe *et al*., 2003). Keeping in view the adverse effects of salinity on agricultural production and to encourage the use of salt tolerant species the present study was initiated to investigate the role of seed priming in seed vigor under saline conditions and to screen out different wheat varieties for salinity tolerance.

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Materials and Methods

The experiment was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan during winter 2008 in completely randomized design (CRD) with three replications. Seeds of different wheat varieties namely Tatara-96 (T-96), Ghaznavi-98 (G-98), Fakhri Sarhad (F.Sd), Bakhtawar-92 (B-92), Pirsabaq-2004 (P-2004) and Auqab-2000 (A-2004) were first primed with saline water (30 mM NaCl) for 12 hours at 25 ^{0}C in case of priming. Primed (P) and unprimed (UP) seeds were grown in pots containing sand salinized with the desired NaCl levels (0, 40, 80 and 120 mM). Initially twenty seeds per pot were sown and after complete emergence thinning was done and ten plants per pot were maintained. Half strength Hoagland solution (Hoagland and Arnon, 1950) salinized with the said NaCl levels was applied to the pots periodically. Plants were harvested 55 days after sowing. The shoots of five randomly selected plants per treatment were weighed and then their mean value was calculated to obtain shoot fresh weight. Plants harvested for shoot fresh weight in each treatment were dried in an oven at 80^{O}C for 48 hours and again weighed after complete drying for recording shoot dry weight. The Na^+ and K^+ concentration of shoot were determined by flame photometer according to the methods of US Salinity Staff (1954) and then their K^+/Na^+ ratio was calculated.

Data were analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez and Gomez (1984). A computer software MSTATC was used to carry out the statistical analysis. The significance of difference among means was compared by using the LSD test at $p \leq 0.05$ (Steel and Torrie, 1980).

Results

Data concerning shoot fresh weight, shoot dry weight, shoot Na^+ contents, shoot K^+ contents and shoot K^+/Na^+ ratio of wheat varieties is presented in Table 1. Statistical analysis of the data showed significant ($p \leq 0.05$) effect of varieties on shoot fresh weight (g) plant^{-1} of wheat. All possible interactions except salinity x varieties x seed priming were significant ($p \leq 0.05$). Maximum shoot fresh weight plant^{-1} (7.71 g) was recorded from Bakhtawar-92 followed by Ghaznavi-98 (7.37 g) and Farhre-Sarhad (7.10 g) when compared with minimum shoot fresh weight plant^{-1} (6.75 g) from Auqab-2000. Shoot fresh weight was reduced consistently with application of additional increment of salinity. Lowest shoot fresh weight plant^{-1} (4.82 g) was observed in the treatment of 120 mM NaCl salinity level. Application of 80 and 40 mM salinity levels ranked 2nd and 3rd in term of shoot fresh weight plant^{-1} of 5.70 and 7.56 g when compared with highest shoot fresh weight of 10.59 g plant^{-1} from control. Seed priming showed maximum shoot fresh weight of 7.39 g plant^{-1} as compared with minimum shoot fresh weight of 6.95 g plant^{-1} from of no seed priming.

Table 1. Effect of salinity and seed priming on shoot fresh weight, shoot dry weight, shoot Na^+ contents, shoot K^+ contents and shoot K^+/Na^+ ratio of wheat varieties.

Varieties	Shoot fresh weight (g) plant^{-1}	Shoot dry weight (g) plant^{-1}	Shoot Na^+ contents (mg g^{-1} dry weight)	shoot K^+ contents (mg g^{-1} dry weight)	Shoot K^+/Na^+ ratio
T-96	7.22 c	1.54 c	1.24 d	1.24 c	1.21 c
G-98	7.37 b	1.60 b	1.14 e	1.34 b	1.35 b
F. S	7.10 d	1.49 d	1.27 c	1.22 d	1.17 d
B-92	7.71 a	1.68 a	1.08 f	1.39 a	1.45 a
P-2004	6.86 e	1.44 e	1.41 b	1.11 e	1.05 e
A-2000	6.75 f	1.40 f	1.43 a	1.08 f	1.01 f
			Salinity mM		
0	10.59 a	2.31 a	0.42 d	0.86 d	2.06 a
40	7.56 b	1.58 b	1.25 c	1.30 c	1.06 b
80	5.70 c	1.16 c	1.51 b	1.35 b	0.92 c
120	4.82 d	1.05 d	1.87 a	1.41 a	0.78 d
			Seed Priming		
P	7.39 a	1.58 a	1.21 a	1.27 a	1.29 a
UP	6.95 b	1.47 b	1.31 b	1.19 b	1.12 b
Interactions			Significance Level		
V x S	**	**	**	**	**
V x P	**	**	**	**	**
S x P	**	**	**	**	**
V x S x P	NS	NS	NS	NS	NS

V = Varieties, S = Salinity, P = Primed, UP = Unprimed, NS = Non significant

** = Significant at 0.01 level of probability

Mean values of the same category followed by different letters are significant at $p \leq 0.05$ level

The data on shoot dry weight (g) plant^{-1} revealed significant ($p \leq 0.05$) effect of salinity on shoot dry weight. Similarly the effects of varieties and seed priming were also significant ($p \leq 0.05$) on shoot dry weight (g) plant^{-1}. All possible interactions except salinity x seed priming x varieties were significant ($p \leq 0.05$). Inconsistent effect of varieties on shoot dry weight was observed. Bakhtawar-92 produced highest shoot dry weight plant^{-1} (1.68 g). Ghaznavi-98 ranked second (1.60 g shoot dry weight plant^{-1}) followed by Tatara-96 (1.54 g shoot dry weight plant^{-1}) when compared with lowest shoot dry weight plant^{-1} of 1.40 g from Auqab-2000. Minimum shoot dry weight plant^{-1} of 1.05 g was recorded from 120 mM salinity level followed by 1.16 g plant^{-1} and 1.58 g plant^{-1} respectively from the treatments of 80 mM and 40 mM NaCl when compared with maximum shoot dry weight plant^{-1} from control (2.31 g). Highest shoot dry weight plant^{-1} (1.58 g) was recorded from the treatment of seed priming as compared with 1.47 g plant^{-1} from the treatments of no seed priming.

The data regarding shoot Na^+ (mg g^{-1} dry weight) showed significant ($p \leq 0.05$) effect of varieties, salinity and seed priming on shoot Na^+ contents (mg g^{-1} dry weight) of wheat crop. Interactions among salinity x seed priming, salinity x varieties and seed priming x varieties were also significant ($p \leq 0.05$). Highest shoot Na^+ contents (1.43 mg g^{-1} dry weight) were recorded from Auqab-2000 followed by Pirsbaq-2004 and Tatara-96 with 1.41 and 1.24 mg g^{-1} dry weights, respectively when compared with 1.08 mg g^{-1} dry weight from Bakhtawar-92. Maximum shoot Na^+ contents (1.87 mg g^{1} dry weight) were recorded from 120 mM NaCl followed by 1.51 and 1.25 mg g^{1} dry weights from 80 mM and 40 mM, respectively when compared with minimum shoot Na^+ contents (0.42 mg g^{-1} dry weight) from control. Minimum shoot Na^+ contents of 1.21 mg g^{-1} dry weight plant^{-1} were recorded from the treatment of seed priming. Whereas, maximum Na^+ contents of 1.31 mg g^{-1} dry weight plant^{-1} were recorded from unprimed seeds.

Statistical analyses of the data revealed that varieties, salinity, and seed priming had significantly ($p \leq 0.05$) influenced shoot K^+ contents (mg g^{-1} dry weight) of wheat crop. Interactions among all possible combinations except salinity x seed priming x varieties were also significant ($p \leq 0.05$). Maximum shoot K^+ contents of 1.39 mg g^{-1} dry weight were observed in Bakhtawar-92. Ghaznavi-98 and Tatara-96 ranked 2nd and 3rd with shoot K^+ contents of 1.34 and 1.22 mg g^{-1} dry weight when compared with minimum of 1.08 mg g^{-1} dry weight shoot K^+ contents from Auqab-2000. Maximum shoot K^+ contents of 1.41 mg g^{-1} dry weight were recorded from salinity levels of 120 mM NaCl followed by 80, 40 mM with shoot K^+ contents of 1.35 and 1.30 mg g^{-1} dry weight. Whereas, minimum shoot K^+ contents of 0.86 mg g^{-1} dry weight were recorded from control. Highest shoot K^+ contents of 1.27 mg g^{-1} dry weight were recorded from the treatments of seed priming. Whereas, lowest shoot K^+ contents of 1.19 mg g^{-1} dry weight were recorded from the treatments of no seed priming.

Statistical analysis of the data showed significant ($p \leq 0.05$) effect of varieties, salinity and seed priming on shoot K^+/Na^+ ratio of wheat crop. Interactions among all possible combinations except salinity x varieties x seed priming were also significant ($p \leq 0.05$). Maximum shoot K^+/Na^+ ratio of 1.45 was recorded from Bakhtawar-92 followed by shoot K^+/Na^+ ratio of 1.35 and 1.17 from Ghaznavi-98 and Fakhre-Sarhad respectively. Minimum shoot K^+/Na^+ ratio of 1.01 was recorded from Auqab-2000. Mean values of the data showed maximum shoot K^+/Na^+ ratio of 2.06 from control followed by 40mM and 80 mM salinity with K^+/Na^+ ratio of 1.06 and 0.92 respectively as compared with minimum shoot K^+/Na^+ ratio of 0.78 from the treatment applied with 120 mM salinity. Highest shoot K^+/Na^+ ratio (1.29) was recorded from primed seeds whereas, lowest shoot K^+/Na^+ ratio (1.12) was recorded from unprimed seeds.

Discussion

Salt sensitivity of wheat is well known and documented like other glycophytes (Zhu, 2003). Wheat crop is responsive to salt stresses (Sharma *et al*., 2005). It is evident that varieties, seed priming and salinity levels had significantly affected shoot fresh weight (g) $plant^{-1}$ and shoot dry weight (g) $plant^{-1}$. Bakhtawar-92 was ranked first in term of shoot fresh and dry weights production when compared with other varieties. The effect of seed priming with NaCl was high in salt tolerant varieties and low in salt sensitive varieties. Reduction in shoot growth might be attributed to excessive accumulation of Na^+ with in the plant body and followed by reduction of enzymatic process and synthesis of protein (Shafi et al., 2009; Tester & Davenport, 2003). Bakht et al., 2011; Bhatti *et al*., (2004) concluded drastic effects on shoot fresh and dry weight with exposure to salinity. They further concluded that reduction was highest in salt sensitive lines and lowest in salt tolerant lines. Halopriming is increasing the superoxide dismustase activity of plants under saline conditions. This increase was shown to be narrowly related to the genetic background of wheat cultivars (tolerant/sensitive), level of salt stress and pre-sowing seed treatments. Therefore, it is likely that increased antioxidant enzymatic activity in various wheat cultivars due to halopriming is a key component in tolerance against salt stress (Bakht et al., 2011; Afzal *et al*., 2006).

Our results revealed that salt stressed environment had markedly increased Na^+ contents in shoots of all varieties in comparison with control. The uptake of Na^+ was more pronounced in Auqab-2000 as compared to Bakhtawar-92. As a priming agent the effect of NaCl in the study was also prominent in alleviation of salt stress for all the varieties but its effect on Bakhtawar-92 was highest when compared with rest of varieties especially Auqab-2000. High concentration of Na^+ in saline soils disturbs the acquisition of K^+ and other essential elements for plant growth, causing the osmotic stress and other complications such as oxidative stress (Zhu et al., 2001). Na^+ inhibits many cytosolic enzymes, as K^+ is normally required as a co-factor (Flowers *et al*., 1977). K^+ uptake plays an important role in growth and development of plants (Ashley *et al*., 2006). Afzal *et al*., (2008) observed that priming-induced salinity tolerance was linked with enhanced seedling vigor, metabolism of reserves, enhanced K^+ and Ca^{2+} and reduced Na^+ accumulation in wheat plants. Our findings were also substantiated by the findings of other research workers. Shafi et al., 2011; Naseem *et al*., 2000 reported that Bakhtawar-92 had proved to be salt tolerant due to exclusion of Na^+ and Cl^- ions. Maximum K^+ concentrations and minimum Na^+ uptake may be one of the possible phenomena of increased salt tolerance in wheat by Si application (Tahir *et al*., 2006). Munns & James (2003) concluded that genotypes with lowest Na^+ contents resulted highest dry matter.

Potassium (K^+) concentration in plants is a good indicator of salinity tolerance. Lower uptake of K^+ by various varieties under saline conditions hampers overall production of these varieties. Tahir *et al*., (2006) reported that K^+ concentration had a vital role in improvement of plant water status and minimizing the toxic effects of Na^+. Our results revealed that various varieties, seed conditions and salinity levels had significantly affected shoot and K^+ contents. Bakhtawar-92 was ranked on the top having highest K^+ contents. Whereas, Auqab-2000 had lowest K^+ contents. With exposure to salt stress a marked increase in shoot K^+ contents was observed in tolerant varieties as compared to sensitive varieties. It is clear that key feature of salt tolerant plants is the ability of cells to maintain optimal K^+/Na^+ ratio (Tester & Davenport, 2003). High salinity tolerance of wheat plants were recorded from primed seeds which might be due to higher capacity for osmotic adjustments and maintenance of ionic homeostasis by enhancing K^+ and Ca^{2+} accumulation (Farhoudi & Sharifzadeh, 2006).

Various studies have revealed that growth performance of wheat crop growing under salt stressed environment mainly depends upon their ability of minimizing accumulation of Na^+ and to have maximum concentrations of K^+ and K^+/Na^+ ratio in their active growing leaves (Rashid *et al*., 1999). It is clear from our results that K^+/Na^+ ratio of various wheat varieties was markedly affected by salinity levels and seed priming. The K^+/Na^+ ratio decreased with salt stresses. Seed priming with NaCl had significantly increased K^+/Na^+ ratio of various wheat varieties. The rate of decrease with salt stress was less in salt tolerant varieties and vice versa. The impact of seed priming was high in salt tolerant varieties when compared with salt sensitive varieties. Zhu *et al*., (2001) observed that plants were able to tolerate moderate saline conditions with a higher ability to exclude Na^+ from their shoots or at least the leaf blades and concurrently maintain higher level of K^+. Similarly the K^+ contents in spikes and shoots is an index of osmotic adjustment (Gorham, 1997). Various seed treatments has markedly increased K^+/Na^+ ratio of various cultivars. The increase was highest in salt tolerant cultivars (Gurmani *et al*., 2009). High K^+ contents and K^+/Na^+ ratio and low Na^+ contents helped Bakhtawar-92 in maintaining high photosynthetic rates and stomatal conductance and hence maximum growth was resulted. The better performance of Bakhtawar-92 in salinity environment may be due to better regulation of K^+ contents uptake and restricted uptake and transport of Na^+ contents.

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