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RESPONSE OF BARLEY GENOTYPES TO SALINITY STRESS AS ALLEVIATED BY SEED PRIMING

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Abstract

The present study entitled "Response of barley genotypes to salinity stresses as alleviated by seed priming was conducted at the Greenhouses of the Institute of Biotechnology and Genetic Engineering, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan. The experiment was conducted in completely randomized design (CRD) with three replications. The response of twelve barely genotypes (Haider-93, Soorab-96, Arabic Asward, NRB-37, Frontier-87, Jau-83, Balochistan-Local, NRB-31, KPK-Local, Sanober-96, Awarn-2002 and AZ-2006) at two seed conditions (seed priming with 30 mM NaCl and no seed priming) under four salinity levels (0 mM, 50 mM, 100 mM and 150 mM) was studied. The results revealed that seed priming and salinity had significantly ($P \leq 0.05$) affected all parameters under study. However, the effect of seed priming was non significant on shoot length (cm) and root length (cm). Maximum shoot length (40.79 cm), shoot fresh weight plant^{-1} (10.15 g) and shoot dry weight plant^{-1} (1.81 g), was recorded in Balochistan-Local. Haider-93 produced highest root length (20.31 cm). Frontier-87 exhibited maximum shoot Na^+/K^+ ratio (1.49) and maximum shoot proline content of 144.31 µg g^{-1} fresh weight was recorded from KPK-Local. Seed priming had distinctly enhanced all the above mentioned parameters except shoot Na^+/K^+ ratio. It is concluded that salinity stresses had affected all parameters under study however; seed priming with NaCl had alleviated the adverse effects of salinity in barely genotypes.

Introduction

Agricultural crops are threatened by various biotic and abiotic stresses. Amongst the abiotic stresses, soil salinity is one of the severe environmental hazards. (Hamid *et al*., 2008), decreasing the crop productivity (Ali *et al*., 2007; Afzal *et al*., 2008) especially in the arid and semi-arid area. The problem of soil salinity is widespread through out the world. Saline soils are formed when annual rainfall is less than evapotranspiration in arid and semi arid region. Due to low rain fall and high evapo-transpiration rate, the salts accumulate in the soil profile. In addition to that, human activities like poor management of water and soil, and over irrigation of poorly drained soils are also responsible for soil salinity.

In Pakistan, barley is mainly grown for grain, green fodder and straw for small ruminants during winter (Khan *et al*., 1999). It is grazed continuously by the livestock in the driest seasons with no grain harvest. Barley is well adapted to rain fed areas of Pakistan (Rees *et al*., 1989), where it has potential to out yields wheat by 20% (Khan *et al*., 1993). It is mostly grown on irrigated land while in rainfed areas it occupies one third of land. Among all crops the salt tolerance of barley is the highest (Mass & Hoffman, 1977) and differences in salinity tolerance in barley and wheat species (Mano & Takeda, 1998; Forster *et al*., 2000; Shafi *et al*., 2010) have been reported. Improved plant growth in saline areas lead to generate salt tolerance in crops. Salt tolerance not only varies among species but also amongst different genotypes within species (Maas & Hoffman, 1977; Storey & Wyn Jones, 1978; Marschner *et al.*, 1981; Shafi *et al*., 2011). However, efforts to breed for salt tolerance become limited due to lack of understanding of the complexity of the tolerance mechanism (Shannon, 1985; Epstein, 1987) and its relation with the environment (Greenway *et al*., 1983). Salt tolerance includes the expression of a number of genes, and each expression may depend upon its relation with other genes, external salt concentrations growth stage and other environmental conditions.

Keeping in view the hazardous effects of salinity on crops productivity and to promote the use of salt tolerant species, the present study was planned to investigate the response of barley genotypes for growth performance and tolerance to salinity. This study has also investigated the impact of seed priming on alleviating salinity stresses in barley genotypes.

Materials and Methods

The study entitled "Response of barley genotypes to salinity stresses as alleviated by seed priming" was carried out at the greenhouses of the Institute of Biotechnology and Genetic Engineering Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan during winter 2007-08. The experiment was conducted in sand culture as root media with twelve barely genotypes (Haider-93, Soorab-96, Arabic Asward, NRB-37, Frontier-87, Jau-83, Balochistan-Local, NRB-31, KPK-Local, Sanober-96, Awarn-2002 and AZ-2006) at two seed conditions (seed priming with 30 mM NaCl and no seed priming) under four salinity levels (0 mM, 50 mM, 100 mM and 150 mM). The experiment was conducted in completely randomized design (CRD) with three replications. In case of priming, seeds of barley genotypes were first primed with saline water (30 mM) for 12 hours at 25^0C. Sand was sterilized and washed 3 times with water before using in pots. Each pot was filled with dry sterilized sand. Twenty primed or unprimed seeds as per treatment were grown in each pot containing sand salinized with the desired salinity (NaCl) levels (0, 50, 100 and 150 mM). After complete emergence ten plants per pot were maintained through thinning. Hoagland solution (Hoagland & Arnon, 1950) salinized with required NaCl levels was applied to pots periodically. The Hoagland solution was monitored periodically for salinity levels. The position of the replication in the greenhouse was changed periodically so that all the experimental units may be exposed uniformly to micro variation of the green house. Plants were harvested 50 days after sowing.

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Data were recorded on shoot length (cm), root length (cm),), shoot fresh weight (g plant^{-1}), shoot dry weight (g plant^{-1}), shoot Na^+/K^+ ratio and shoot proline content (µg g^{-1} fresh weight). Shoot length (cm) and root length (cm) of three randomly selected plants in each treatment was measured with a ruler and then their averaged was worked out. After that the shoots were separated from the roots and weighed with an electronic balance and then their average was worked out to obtain shoot fresh weight. The same samples were further dried in an oven at 80^{0}C for 48 hours and at complete drying weighed and averaged to record shoot dry weight. These dried samples were further used for estimation of shoot Na^+ and K^+ content according to the methods of US Salinity Staff (1954). Shoot Na^+/K^+ ratio was calculated by dividing the value of Na^+ by K^+ Proline in shoot of each treatment was estimated according to the standard procedure of Bates *et al.* (1973).

Statistical analysis: The data collected during the experiment were statistically analyzed using analysis of variance (ANOVA) appropriate for CR design and upon obtaining significant differences; Least Significant Difference (LSD) test was applied (Steel & Torrie, 1980).

Results

Shoot length (cm): Statistical analysis of the data revealed significant ($p<0.05$) effect of various salinity levels and genotypes on shoot length of barely crop (Table 1). The main effect of seed priming, and all the possible interactions were non significant ($p>0.05$). Mean values of the data showed highest shoot length from the treatment of Balochistan-Local (40.79 cm) which was statistically at par with Haider-93 (40.16 cm) followed by KPK local with shoot length of 38.56 cm. Lowest shoot length was recorded in Frontier-87 (30.03 cm), Jau-83 (30.54 cm) and Sanober-96 (31.18 cm) which were statistically at par. Primed seed has non-significantly enhanced shoot length (35.01 cm) than un-primed seed (34.54 cm). Shoot length was reduced by 11.76%, 15.56% and 21.57% at salinity levels of 50, 100 and 150 mM respectively when compared with control.

Table 1. Plant growth, Na^+/K^+ ratio and proline content of barley genotypes as affected by seed priming and salinity.

Genotypes	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Shoot Na^+/K^+ ratio	Shoot proline content (µg g^{-1} fresh weight)
Haider-93	40.16a	20.31a	9.68b	1.77b	0.84i	138.75b
Soorab-96	36.56c	19.28e	9.06c	1.69c	0.94h	117.29d
Arabic Asward	35.80cd	19.53d	9.04c	1.65d	0.99g	107.71e
NRB-37	34.59de	19.00f	8.76d	1.61e	1.05f	101.80f
Frontier-87	30.03g	18.11j	7.03i	1.35k	1.49a	79.51j
Jau-83	30.54g	17.96k	6.85i	1.39j	1.43b	71.20k
Balochistan-Local	40.79a	20.08b	10.15a	1.81a	0.79j	123.69c
NRB-31	33.48ef	18.78g	8.50e	1.56f	1.11e	94.97g
KPK-Local	38.56b	19.90c	9.95a	1.74b	0.87i	144.31a
Sanober-96	31.18g	17.86k	7.29h	1.42i	1.34c	79.28j
Awarn-2002	32.75f	18.51h	7.87g	1.47h	1.19d	90.42h
AZ-2006	32.82f	18.36i	8.16f	1.53g	1.20d	84.03i
Salinity (mM)						
0	39.62a	22.63a	11.20a	2.13a	0.12d	58.63d
50	34.96b	20.68b	9.07b	1.69b	0.90c	92.69c
100	33.45c	18.41c	7.77c	1.43c	1.25b	113.90b
150	31.07d	14.16d	6.08b	1.08d	2.15a	145.76a
LSD	0.874	0.085	0.144	0.016	0.023	1.683
Seed priming						
Un-priming	34.54	18.94	8.15b	1.51b	1.26a	96.36b
Priming	35.01	19.00	8.91a	1.66a	0.95b	109.14a
Interactions			**Significance level**			
G x S	ns	Ns	s	s	s	s
G x P	ns	Ns	s	s	s	s
S x P	ns	Ns	s	s	s	s
G x S x P	ns	Ns	ns	ns	ns	ns

s and ns represent significant and non significant difference at $p<0.05$
Means of same category followed by same letters are not significantly different at $p\leq0.05$ using LSD test

Root length (cm): Mean values of the data showed significant ($p<0.05$) effect of various salinity levels and genotypes on root length of barley crop (Table 1). The effect of seed priming and all possible interactions were non-significant ($p>0.05$). Mean values of the data revealed largest root length from Haider-93 (20.31 cm) followed by Balochistan-Local and KPK-Local with root lengths of 20.08 and 19.90 cm respectively. Smallest root length was observed in Sanober-96 (17.86 cm) and Jau-83 (17.96 cm) which were statistically non-significant. Primed seeds had non-significantly ($p>0.05$) increased root length (19.00 cm) than un-primed seeds (18.94 cm). Mean values of the data indicated gradual reduction in root length with the addition of each increment of salinity.

Root length was 8.60%, 18.64% and 37.41% reduced at salinity level of 50, 100 and 150 mM respectively when compared with control.

Shoot fresh weight (g plant^{-1}): Table 1. revealed significant ($p<0.05$) effect of various salinity levels, genotypes and seed priming on shoot fresh weight. All possible interactions except seed priming x salinity x genotypes was significant ($p>0.05$). Highest shoot fresh weight was recorded in Balochistan-Local (10.15g plant^{-1}). KPK-Local and Haider-93 ranked 2nd and 3rd with shoot fresh weight of 9.95 and 9.68(g plant^{-1}) respectively. Lowest shoot fresh weight was produced by Frontier-87 (7.03g plant^{-1}). The treatment of seed priming proved superior and enhanced shoot fresh weight by 9.33% in primed seed (8.91g plant^{-1}) than un-primed seed (8.15g plant^{-1}). Application of additional increment of salinity has gradually reduced shoot fresh weight. Salinity levels of 50, 100 and 150 mM has significantly decreased shoot fresh weight by 19.04%, 30.66 and 45.70% respectively when compared with control.

Shoot dry weight (g plant^{-1}): Data on shoot dry weight of twelve barley genotypes exposed to various levels of salinity and seed priming revealed significant ($p<0.05$) effect on shoot dry weight (Table 1). All possible interactions except seed priming x salinity x genotypes were significant ($p<0.05$). Maximum shoot dry weight was produced from Balochistan-Local (1.81 g plant^{-1}). Haider-93 and KPK-Local ranked 2nd and 3rd with shoot dry weight of 1.77 and 1.74 g plant^{-1} respectively. Minimum shoot dry weight was recorded in Frontier-87 (1.35 g plant^{-1}). Shoot dry weight was enhanced by 9.68% in primed seed (1.66 g plant^{-1}) than un-primed seed (1.51 g plant^{-1}). Shoot dry weight was reduced by 20.32%, 32.98% and 49.01% at salinity level of 50, 100 and 150 mM respectively when compared with control.

Shoot Na^+/K^+ ratio: Mean values of the data showed significant ($p<0.05$) effect of salinity levels, genotypes and seed priming on shoot Na^+/K^+ ratio (Table 1). Interaction of genotypes x seed priming, genotypes x salinity and seed priming x salinity were significant ($p<0.05$). Interaction of seed priming x salinity x genotypes was non significant ($p>0.05$). Highest shoot Na^+/K^+ ratio was recorded in Frontier-87 (1.49). This is followed by Jau-83 and Sanober-96 with shoot Na^+/K^+ ratio of 1.43 and 1.34 respectively. Lowest shoot Na^+/K^+ ratio was observed in Balochistan-local (0.79). The treatment of seed priming has reduced shoot Na^+/K^+ ratio by 24.46% in primed seed (0.95) than un-primed seed (1.26). Mean values of the data revealed significant increase in shoot Na^+/K^+ ratio with the application of additional increment of salinity. Shoot Na^+/K^+ ratio was enhanced with the application of 50, 100 and 150mM salinity levels respectively when compared with control.

Shoot proline content (µg g^{-1} fresh weight): Table 1 revealed significant ($p\leq0.05$) effect of salinity, seed priming and genotypes on shoot proline content (µg g^{-1} fresh weight). All possible interactions except salinity x seed priming x genotypes were significant ($p\leq0.05$). Highest shoot proline content (144.31µg g^{-1} fresh weight) were recorded from KPK-Local followed by Haider-93 and Baluchistan-Local with shoot proline content of 138.75 and 123.69 µg g^{-1} fresh weight respectively. Lowest shoot proline content was recorded in Jau-83 (71.20 µg g^{-1} fresh weight). Primed seed has increased shoot proline content by 13.26% than un-primed seeds. Statistical analysis of the data revealed steady raise in shoot proline content with application of additional increment of salinity. Maximum shoot proline content of 145.76 µg g^{-1} fresh weight was recorded from the treatment applied with 150 mM NaCl followed by 100mM, 50mM with shoot proline content of 113.90 and 92.69 µg g^{-1} fresh weight respectively.

Discussion

Salinity is one of the major abiotic stresses that negatively affect crop production (Richards, 1954). Excess of salts in the soil alone, or in combination with water logging, has adverse consequences for crop production. The most widely accepted effects of salinity on plant growth include water stress, specific ion toxicity (Serrano *et al*., 1999) and ion imbalance.

Pre-sowing seed treatments have shown to enhance stand establishment in non-saline areas (Khan, 1992; Bakht *et al*., 2010; Bakht *et al*., 2011) and have potential in saline areas as well (Ashraf & Ruaf, 2001). Halopriming is a pre-sowing soaking of seeds in salt solutions, which enhances germination and seedling emergence uniformity under adverse environmental conditions (Bakht *et al*., 2010). NaCl priming could be used as an adaptation method to improve the salt tolerance of seeds (Bakht *et al*., 2011). NaCl pretreatment could have two effects: a stimulative effect related to salt acclimation, and a toxic effect due to salt stress. At the high NaCl pretreatment level (50 mM), the toxic effect would be increased and the stimulative effect would be nullified. Considerable improvement due to seed priming in solutions of different inorganic salts has been observed in germination and at latter growth stages of different crops, e.g., wheat (Mehta *et al*., 1979), *Sorghum bicolor* (Kadiri & Hussaini, 1999), *Vicia faba* (Salam, 1999) and maize (Bakht *et al*., 2011). Keeping in view the findings of these studies, it is evident that halopriming alters plant physiological and biochemical responses to salt stress (Cayuela *et al*., 1996).

Salinity stress has significantly effected shoot and root length of all genotypes. Enormity of decline was less in tolerant genotypes (Balochistan-local and Haider-93) as compared with sensitive genotypes (Frontier-87, Jau-83 and Sanober-96). Growth parameters like root length decreased with increasing salinity of the growth medium in barely varieties. (Naseer, 2001; Shafi *et al*., 2010) and wheat (Ahmad *et al*., 1980; Kumar *et al*., 1981; Sharma & Grag, 1985). It was observed that boosting levels of salinity has gradually decreased plant height which might be due to decreased physiological activities resulting from water and nutrients stress occurring under high salinity stress. The adverse effect of salinity on plants may lead to disturbances in plant metabolism, which consequently led to reduction of plant growth and productivity (Ahmad & Abdullah, 1979; Greenway et al., 1983; Sharma & Hall 1991; Shafi. *et al*., 2009). An increase in root length in primed seeds as compared to control might be the result of embryo cell wall extensibility. To offset the action of free radicals and increase the activity of membrane bound enzyme seed priming increase the free radical scavenging enzymes (peroxidase, catalase and superoxide dismutase) to improve plant viability and vitality under salinity stress (Chang & Sung, 1998; Shafi *et al*., 2009).

Seed priming and salinity levels have extensively affected shoot fresh and dry weight (g plant^{-1}) of barely genotypes. Balochistan-Local performed better by maintaining maximum shoot fresh and dry weight while Frontier-87 produced least quantity of shoot fresh and dry weight. Shoot weight decreased progressively with the rise of stress level compared with control in barely. Fortmeir & Swchuber (1995) also reported similar results. In general, shoot biomass was less affected in tolerant cultivars as compared with medium tolerant and sensitive respectively (Chen *et al*., 2005, Shafi *et al*., 2009). The increase in salinity levels resulted in the development of water and nutrient stresses. The toxic effect of sodium at high salt levels and physical damage to roots decreased their ability to absorb water and nutrient which caused marked reduction in photosynthesis, enzymatic process and protein synthesis (Tester & Davenport, 2003), which resulted in stunted growth and poor leaf area development. The decrease in the rate of photosynthesis due to leaf area might be responsible to decrease shoot fresh and in turn dry weight. It is evident from results, that primed seeds in comparison with dry seeds resulted in more crop growth rate (Basra *et al*., 2003) Salt-tolerant genotypes were slightly affected by salt stress, and it could be further least affected by seed priming with NaCl. In contrast, growth parameters (plant height, root length, dry weight) of the salt-sensitive genotypes decreased more drastically than the salt-tolerant under NaCl stress (Zheng *et al*., 2008) and those growth parameters were improved by seed priming. Therefore, it is concluded that seed priming could be more effective in improving the growth of the salt-tolerant barely than the salt sensitive. These results agree with the finding of Harris *et al*. (2001) and Basra *et al*. (2003). They reported greater plant weight following seed priming.

Salinity stress has caused significant increase in Na^+ concentration, and considerable decrease in K^+ concentration, resulting in drastic increase in the Na^+/K^+ ratio. Thus, maintenance of low ratio of Na^+/K^+ will be suitable for the metabolic processes occurring within plants and essential for plants to survive salt stress (Ashraf and Khanum, 1997). Na^+/K^+ may be used as possible criterion for selecting salt tolerant genotypes (Mao and Liu, 1990). A suitable Na^+/K^+ ratio is important for the adjustment of cell osmoregulation, turgor maintenance, stomatal function, activation of enzymes, protein synthesis, oxidants metabolism and photosynthesis (Shabala et al., 2003). One of the key features of plant salt tolerance is the ability of plant cells to maintain an optimal Na^+/K^+ ratio in the cytosol.

Proline plays an important role in reducing the injurious effects of salinity and an acceleration of the repairing processes following stresses. The content of proline was progressively increased in shoots of the barely seedlings as the NaCl level increased (El-Tayeb, 2005). Tolerant barely genotypes had more ability to adapt to salinity stress and higher accumulation of proline is involved in tolerance to osmotic stress, acting as a compatible osmolyte. The higher accumulation of proline in the tolerant genotypes of barley indicates strong correlation between proline and salt induced osmotic stress. Proline has already been reported to act as an osmoprotectant and associated with mechanism of salt tolerance under salinity stresses. (Yu Lei and Shaozheng, 2000). Besides osmolyte, proline also confers enzyme protection and has increased membrane stability.

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