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Full Length Article

Effect of Different Seed Priming Treatments on Growth and Nutrients Uptake in Wheat (*Triticum aestivum*) under Salt Stress

Abida Kausar[1*], Madiha Ilyas[2], Shahzad Maqsood Ahmad Basra[3], Zaib-Un-Nisa[4] and Sara Zafar[5]
[1,4]*Department of Botany, Government College Women University Faisalabad, 38000, Pakistan*
[2]*Department of Home Economics, GC Women University Faisalabad, 38000, Pakistan*
[3]*Department of Agronomy, University of Agriculture Faisalabad, 38000, Pakistan*
[5]*GC University, Faisalabad, 38000, Pakistan*
[*]For correspondence: abida.bot.gcw@gmail.com

Abstract

Wheat (*Triticum aestivum* L.) is highly significant among the cereals being cultivated more than any other marketable crop in the world. The high temperature, drought and soil salinity are major abiotic factors in reducing its productivity by altering certain biochemical and physiological responses. Different technologies are being utilized to enhance the tolerance of plants to grow in salt-hit areas. However, seed priming with different temperatures and chemicals is a cost effective technology which may improve seed performance and seedling establishment of field crops in saline soils. Therefore, seeds of two wheat varieties (Punjab 2011 and FSD-08) were primed with distilled water, chilling (5°C), heating (60°C) and sodium chloride (150 m*M*) then primed and non-primed seeds were grown at 150 m*M* NaCl for 42 days under Hoagland's nutrient solution (½ strength) in pots containing sand with completely randomized design (CRD). Results showed under saline conditions (150 m*M*) the shoot length (15 and 13%), root length (22.8 and 17.2%), shoot fresh weight (18 and 18%), root fresh weight (27 and 34%), shoot dry weight (12.8 and 11.4%), root dry weight (30 and 31%), shoot calcium (53.8 and 57.1%), root calcium (37.2 and 41.6%), shoot phosphorus (40 and 29.2%), root phosphorus (34.7 and 33.2%) shoot potassium (33.3 and 31.7%), root potassium (65 and 52.1%) and shoot nitrogen (22.9 and 27.8%), root nitrogen (16.1 and 21.8%) were decreased in Punjab 20 11 and FSD-08 respectively. However, less decrease were observed in plants raised from seeds primed with 150 m*M* NaCl grown under saline (150 m*M*) and non-saline (0 m*M*) environments as compared to other priming treatments in both FSD-08 and Punjab 2011 wheat varieties. The sodium contents and total free amino acids were increased in salt stress in both varieties of wheat. Punjab 2011 showed better behavior as compared to FSD-08 both in saline and non-saline medium. © 2019 Friends Science Publishers

Keywords: Wheat; Priming; Growth; Salinity; Ions analysis

Introduction

Wheat (*Triticum aestivum* L.) has dynamic significance among the food crops and due to its high food values it is cultivated all over the world (Kausar and Gull, 2014; Khokhar *et al.,* 2017). In Pakistan, it is cultivated on an area of about 9.0 million hectares with annual productivity of around 24.0 million tons (Anonymous, 2014). The wheat crop yield in Pakistan is far below than other wheat producing countries because of improper management of different biotic and abiotic factors (Ashraf *et al.,* 2008; Suzuki *et al*., 2014; Kausar *et al.,* 2016; Yang *et al*., 2017). Among abiotic factors salinity is a serious issue which severely reduces crop yield and world widely more than 800 million hectares of land is salt affected to various degrees (Turki *et al*., 2012; Zafar *et al*., 2015; Blum, 2017).

Salt stress adversely affects the yield and growth by affecting developmental processes in the plants through altering physiological and biochemical activities (Qin *et al.*, 2010; Lee *et al.*, 2016; Naqve et al., 2018). High salt concentrations in soil perturb the seed germination and its emergence then ultimately it hinders the establishment of vigor crop stand (Siringam *et al*., 2012; Shirazi *et al*., 2015).

Hence to have good and vigorous crop stand maximum seed germination is necessary. The available literature suggested different techniques to have maximum seed germination and seed priming is one of them, through which better crop stand can be achieved which is helpful to have economical crop yield from salt-affected soils (Ashraf *et al*., 2003; Jafar *et al.,* 2012; Yang *et al*., 2017). The findings of different researchers also supported priming/ preconditioning of seeds to have higher germination and crop stand to combat in the saline environments (Farooq *et al.*, 2010; Anwar *et al*., 2011). For seed priming different temperatures or chemicals are being used to enhance the seed germination for obtaining good crop growth under

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saline environmental (Ashraf *et al*., 2003).

The seed priming influences physiological, biochemical, molecular and cellular signaling events involved in seed germination and good seedling establishment of crops (Basra *et al.*, 2005; Verma *et al*., 2013). So keeping in view the importance of the wheat crop world widely the present investigation was carried out to find out the effective seed priming treatments with varying temperatures, sodium chloride (150 m*M* NaCl) and in distilled water.

Materials and Methods

The experiments were conducted at Department of Botany, GC Women University, Faisalabad to assess alleviation of salt stress be different priming seed treatments using 150 m*M* NaCl. Seed of two wheat varieties *i.e*., Punjab 2011 (Punjab-11) and FSD-08 were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad. The surface sterilized seed with 10% sodium hypochlorite solution for seven minutes were of both varieties were kept at 5 °C for chilling (C) or 60 °C for heating (thermo priming) for 48 hour at fifty percent relative humidity. The relative humidity was maintained by humidifier. The seeds of both varieties were also soaked for eight hours in 150 m*M* NaCl (halopriming) solution (-0.672 MPa osmotic potential), or distilled water (hydropriming). The osmotic potential was determined by vapor pressure osmometer (Wescor 5520, USA). After NaCl pre-sowing treatments the seeds were rinse with water then dried in oven for fifty minutes at 40 °C to eliminate the surface moisture. The all primed seeds of both varieties were used within twenty hours of treatments. Then in wire house experiments primed seeds along with non-primed seeds of both varieties were grown in pots (diameter 30 cm and 35 cm deep) under natural conditions. Each pot was filled with 7 kg of washed river sand having particle size of 1.4–2 mm. Three liters 0 (control) or 150 m*M* sodium chloride in Hoagland, s nutrient solution were given in each week for 8 weeks. During this 250–300 cm^3 water was also applied daily to keep the sand moist and to maintain the salt level. After seven days of germination the thinning was done to four per pot. The pots were arranged in a completely randomized design (CRD) with four replicates. After 45 days of germination six plants from each pot were uprooted and washed with distilled water then fresh weights and lengths of roots and shoots were calculated. Then plants were oven dried at 70 °C till constant dry biomass. Dried ground material (0.05 g) was used for digestion by H_2O_2 (35%) and H_2SO_4 (Wolf, 1982). The solution filtered and volumes of extract were made up to fifty milli litter in flasks. The filtrate was used for ion analysis like calcium (Ca^{2+}), sodium (Na^+), potassium (K^+), nitrogen (N) and phosphorus (P). Sodium ($Na^{+)}$ and potassium (K^+) ions were calculated by using flame photometer (Jenway, PFP 7). Estimation of calcium (Ca^{2+}) ions was completed by titration method given in Hand Book-60 by U.S. Salinity Laboratory Staff (1954). Phosphorus (P) was estimated by spectrophotometer (U 2800 Hitachi, Japan) at 470 nm wave length using 2 mL of alquat and 2 mL of Bartons reagent as described by Jackson (1962). Chopped fresh leaves (0.5 g) were used for total free amino acids determination (Hamilton and Van Slyke, 1943). Extract was prepared by 0.2 *M* phosphate buffer (pH 7.0). Then use one milliliter extract and 10% of pyridine (1 mL) and aquous 2% (w/v) of ninhydrin (1 mL) in test tube. Heat the mixture in boiling water bath for 30 min. The sample was cooled and volume up to 50 mL with distilled water. Optical density of mixture was recorded at 570 nm on a spectrophotometer. Total free amino acids were determined by using standard curve developed with different concentrations of Leucine by using the formula given below:

$$\text{Total free amino acids } (\mu g\ g^{-1}\text{ fresh wt.}) = \frac{\text{Graph reading (Sample)} \times \text{Volume (sample)} \times \text{Dilution factor}}{\text{Fresh weight (Tissues)} \times 1000}$$

Nitrogen contents (N) were determined by the method of Micro–Kjeldhal (Bremner, 1965). 5 milli liter aliquot (used for ion analysis) was put in Kjeldhal flask and placed on ammonia distillation unit and 5 milli litter NaOH (40%) were added in all tubes. Total nitrogen was extracted in 5 mL Boric acid solution with mixed indicator (few drops) placed in a conical flask. The distillation was stopped by accumulation of 40 mL distillate. When distillate was cooled down then it titrated with standard H_2SO_4 (0.01 *N*) till end point (pink). A blank was also preceded in a same manner. Total N was determined using following formula:

$$\text{N percentage} = \frac{(V_2\text{-}V_1) \times N \times 0.014 \times 100}{W}$$

Where V_2 = Volume of standard H_2SO_4 used for titration sample soln., V1 = Volume of standard H_2SO_4 used for titration blank soln., N = Normality (H_2SO_4), W= Weight (sample in g).

Statistically data were analyzed by the technique of analysis of variance by using Costat computer program. The LSD (Least Significant Difference) test at 5% probability was used to calculate the differences among the mean values (Steel *et al.,* 1997).

Results

Salt stress reduced the lengths of shoots and roots in primed and non-primed treatments of both the wheat varieties (Fig. 1). Results showed that shoot lengths 15% and 13% and root lengths 22.8 and 17.2% were decreased by salt stress in both varieties *i.e*., FSD-08 and Punjab 2011, respectively (Fig. 1a and b). The highest shoot and root lengths were recorded by the plants arisen from primed seeds with NaCl under saline and non-saline conditions in both varieties of wheat among the other priming treatments. The decrease in shoot and root fresh weights of 18.8 and 18.2% and root fresh weights 27 and 24% were observed in both wheat varieties *i.e*., FSD-08 and Punjab 2011 and respectively (Fig. 2a and b) by the application of salinity. Similarly, shoot dry weights 12.8% and 11.4% and root dry weights 31.2% and 30.1% were decreased under saline conditions in FSD-08 and Punjab 2011 respectively. The maximum shoot and root fresh and dry weights among pre-sowing seed treatments were recorded by the plants raised from primed seeds with NaCl under saline and non-saline conditions in both varieties of wheat (Fig. 3a and b). A significant difference between treatments regarding shoot and root lengths and fresh and dry weights were observed (Table 1). It was clear from the results that the pre-sowing seeds treatments with NaCl (150 m*M*) depicted best values in data in saline medium as well as in non-saline environments followed by other priming treatments *i.e*., chilling, heating and distilled water. The variety Punjab 2011existing better results as compared to the FSD-08 both in salt stress and normal conditions. Result showed that salt stress caused a significant increase in sodium contents of shoots (36.6%) and roots (33.4%) overall of both wheat varieties (Fig. 4a and b). A non-significant difference among the treatments in accumulation of sodium contents were measured in plants raised primed and non-primed seeds in both wheat varieties in saline as well as in non-saline conditions (Table 2).

Data exhibited that decrease in shoot calcium 53.8 and 57.1% were determined in wheat varieties *i.e*., Punjab 2011 and FSD-08 respectively in saline environments. Similarly, 37.2 and 41.6% decreases values of root contents were calculated in Punjab 2011 and FSD-08 respectively in saline medium. The maximum value of shoot and root calcium were recorded in Punjab 2011 in both saline and non-saline environments as compared to FSD-08 from the plants which seeds primed with NaCl among all other priming treatments (Fig. 5a and b).

Similarly, the decrease in potassium were measured of shoots 33.3 and 31.7% and in root 65.1 and 52.1% in Punjab 2011 and FSD-08 respectively in saline medium as compared to non-saline medium. The maximum potassium contents were observed in plants primed with NaCl and minimum were in plants primed with distilled water both in saline and non-saline conditions (Fig. 6a and b). The Punjab 2011 showed better results as compared to FSD-08 wheat variety.

The salt stress decreased the shoot nitrogen (22.9 and 27.8%) and root nitrogen (16.1 and 21.8%) contents in both wheat varieties Punjab 2011 and FSD-08 respectively in plants grown from primed with NaCl in saline environments. The maximum values of nitrogen were calculated in plants arise from seeds primed with NaCl in saline conditions from both varieties and minimum value were recorded from plants primed with distilled water (Fig. 7a and b). Difference between varieties and treatments were non-significant (Table 3).

The salt stress decreased the shoot phosphorus 40 and 29.2% and root phosphorus 34.7 and 33.2% contents in both wheat varieties Punjab 2011 and FSD-08 respectively in plants grown from seeds primed with NaCl. The maximum values of phosphorus were calculated in plants arise from seeds primed with NaCl in saline and non-saline conditions from both varieties and minimum value were recorded from plants grown from seeds primed with distilled water (Fig. 8a and b).

The total free amino acids were also decreased by the application salt stress in both the varieties. The maximum decrease was observed in plants primed with distilled water

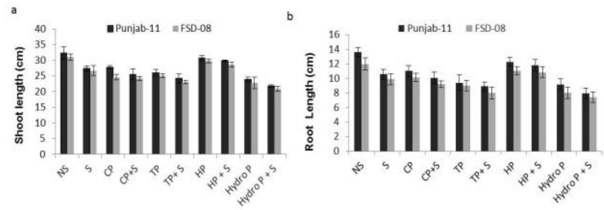

Fig. 1: Effect of different pre-sowing seed treatments on lengths of shoots (**a**) and roots (**b**) of two wheat varieties under 150 m*M* NaCl stress

(NS = Non saline, S = Saline, CP = chilling at 5 °C , TP = Thermo priming at 60 °C, HP = Halopriming with 150 m*M* NaCl, Hydro P = Hydropriming with distilled water)

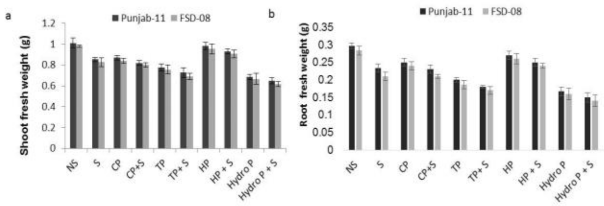

Fig. 2: Effect of different pre-sowing seed treatments on fresh weights of shoots (**a**) and roots (**b**) of two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

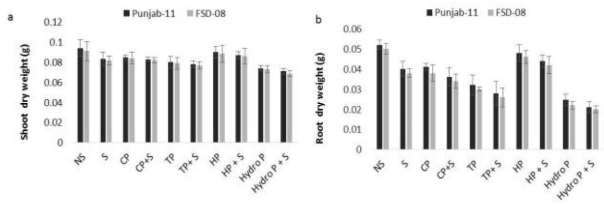

Fig. 3: Effect of different pre-sowing seed treatments on dry weights of shoots (**a**) and roots (**b**) on two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

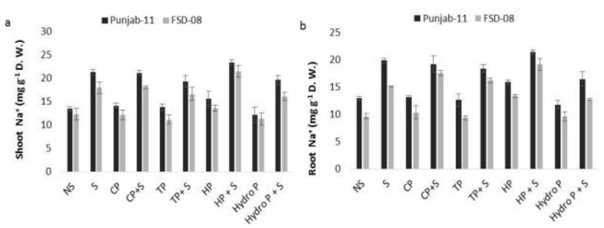

Fig. 4: Effect of different pre-sowing seed treatments on sodium (Na^+) contents of shoots (**a**) and roots (**b**) of two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

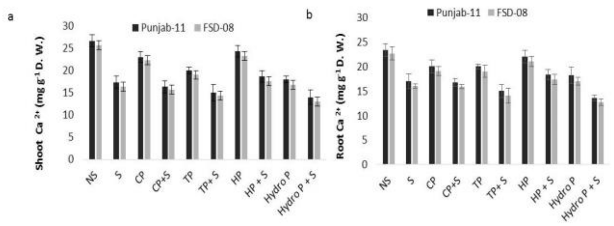

Fig. 5: Effect of different pre-sowing seed treatments on calcium (Ca^{2+}) contents of shoots (**a**) and roots (**b**) of two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

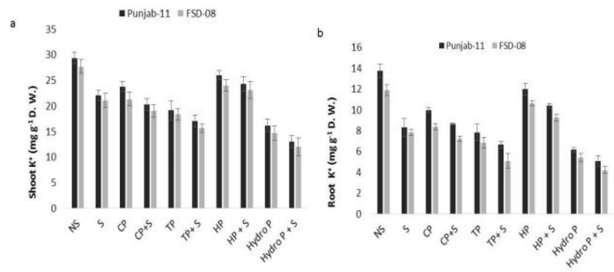

Fig. 6: Effect of different pre-sowing seed treatments on potassium (K^+) contents of shoots (**a**) and roots (**b**) of two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

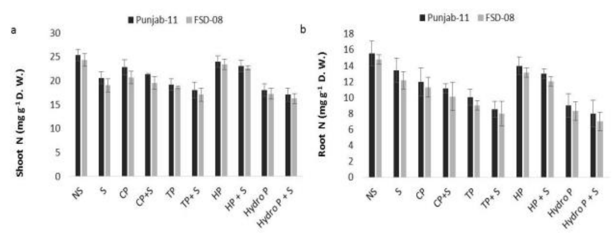

Fig. 7: Effect of different pre-sowing seed treatments on nitrogen (N) of shoots (**a**) and roots (**b**) of two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

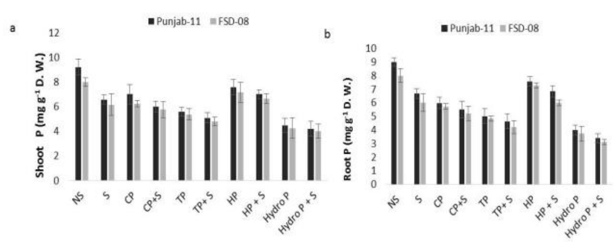

Fig. 8: Effect of different pre-sowing seed treatments on phosphorus (P) of shoots (**a**) and roots (**b**) of two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

and minimum decrease was noted in plants primed with NaCl as compared to other priming treatments (Fig. 9) in both varieties of wheat. The variety and treatment difference was non-significant (Table 4). The variety Punjab-11 showed better performance as compared to FSD-08 both in saline and non-saline conditions.

Table 1: Mean squares values of growth attributes from different priming treatments of two wheat varieties under normal and saline conditions

SOV.	D. F.	Shoot lengths	Root lengths	Shoots F.W	Roots F.W
Treatments (T)	9	64.8186***	15.5842***	0.08993***	0.01308***
Varieties (V)	1	25.1683***	13.0294***	0.01040^{ns}	0.00241**
V × T	9	0.7409^{ns}	0.1946^{ns}	0.00004^{ns}	0.00004^{ns}
Error	40	1.2597	0.4025	0.00695	0.00034
Total	59				

*, **, *** = significant at 0.05, 0.01, 0.001 levels, respectively.
ns = non-significant

Table 2: Mean squares values of shoot dry weights and sodium contents of leaf and roots from different priming treatments of two wheat varieties under normal and saline conditions

SOV.	D.F.	Shoots D.W.	Roots D. W	leaf Na^+	Roots Na^+
Treatments (T)	9	2.900**	6.151***	100.456***	57.338***
Varieties (V)	1	3.22^{ns}	6.407**	114.817***	387.604***
V × T	9	7.48^{ns}	4.000^{ns}	21.381***	15.498^{ns}
Error	40	7.20	2.21	5.075	15.365
Total	59				

*, **, *** =significant at 0.05, 0.01, 0.001 levels, respectively
ns = non-significant

Table 3: Mean square values of calcium and potassium of leaf and roots from different priming treatments of two wheat varieties under normal and saline conditions

SOV	D.F.	Calcium Shoots	Calcium roots	Potassium leaf	Potassium roots
Treatments (T)	9	62.2222^{ns}	71.1111^{ns}	19.4949^{ns}	19.9537^{ns}
Varieties (V)	1	60.0000^{ns}	26.6667^{ns}	15.5042^{ns}	37.6042^{ns}
V × T	9	85.9250^{ns}	37.7778^{ns}	31.8097^{ns}	27.4190^{ns}
Error	40	75.0000	61.6667	32.3500	23.5938
Total	59				

*, **, *** = significant at 0.05, 0.01, 0.001 levels, respectively
ns = non-significant

Discussion

The application of salt stress (150 m*M*) decreased growth of shoots and roots in both wheat varieties *i.e.*, FSD-08 and Punjab 2011(Fig. 1, 2, 3). The decrease in growth and overall plant productivity under salt stress is earlier reported in different crops (Dogan *et al*., 2010; Khan *et al*., 2014; Lee *et al*., 2016; Blum, 2017). However, plants raised from halo primed seeds showed less decrease in growth when grown under saline conditions as compared with other priming treatments in both varieties of wheat, particularly in Punjab 2011. It may be because of the fact that halo priming caused the hydration of proteins and cell membranes and early initiated various metabolic processes in plants (Harris *et al*., 1999; Elvazi, 2012; Ashraf *et al*., 2013; Yang *et al*., 2017). These processes arrest but not reverse when seeds are dried and restart when seeds again rehydrated in the soil. Therefore, it creates suitable conditions for the early germination of seeds and then establishment of crop (Nascimento, 2003; Sivritepe *et al*.,

Table 4: Mean square values of nitrogen and phosphorus contents of leaf and roots and total free amino acids from different priming treatments of two wheat varieties under normal and saline conditions

SOV	D.F.	Nitrogen of shoots	Nitrogen of roots	Phosphorus of shoots	Phosphorus of roots	Total free amino acids
Treatments (T)	9	27.0667^{ns}	9.7773^{**}	11.6930^{***}	1.88475^{**}	1.678^{**}
Varieties (V)	1	66.9927^{ns}	98.8167^{***}	0.4422^{ns}	6.33750^{**}	0.985^{ns}
V × T	9	67.2001^{ns}	14.7681^{ns}	12.7549^{***}	1.98796^{**}	0.876^{ns}
Error	40	24.5493	12.5563	1.0135	1.02292	0.345
Total	59					

*, **, *** = significant at 0.05, 0.01, 0.001 levels, respectively
ns = non-significant

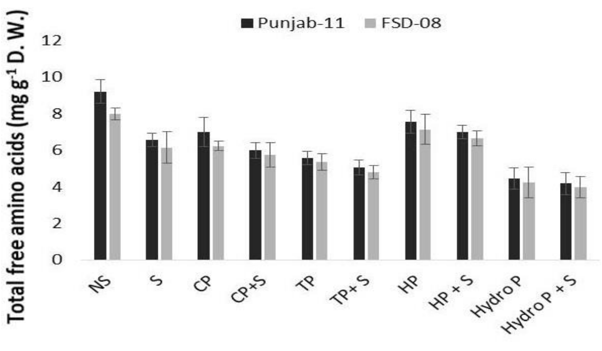

Fig. 9: Effect of different pre-sowing seed treatment on total free amino acids of two wheat varieties under 150 m*M* NaCl stress (same as in Fig. 1)

2003). The beneficial effects of seed priming were reported in wheat (Afzal *et al*., 2006, 2008; Eisa *et al.*, 2012; Fuller *et al.*, 2012), sugarcane (Ashraf *et al*., 2008; Farooq *et al.*, 2010; Wahid *et al.*, 2010), and in tomato (Cayuela *et al.*, 2001).

In the present investigations it has been observed that nutrients uptake like sodium, potassium, calcium, nitrogen and phosphorus were reduced under salt stress. The accumulations of total free amino acids were also reduced under salinity stress in both verities of wheat. Results revealed that plants grown from seeds primed by low and high temperature reduced growth as well as absorption and uptake of nutrients like sodium, potassium, calcium, nitrogen, and phosphorus (Soleimani *et al*., 2011).

Further, these pre-sowing treatments reduced the accumulation of total free amino acids in both wheat varieties. The negative effects of low and high temperature treatments could be due to osmotic imbalance as well as ionic toxicity which ultimately caused reduction in plant growth and its production (Firuzsalari *et al.*, 2012; Hela *et al.*, 2012). Similarly, the hydroprimed seeds showed very poor performance regarding biomass production as well as uptake and accumulation of nutrients both under saline and non-saline environments in the two wheat varieties. The pre-sowing seed treatment with distilled water may leach some essential nutrients as a result decrease growth (Ashraf *et al.*, 2003; Iqbal *et al*., 2006; Khan *et al*., 2011). However, seeds primed with chemicals like NaCl resulted in improved performance in biomass linked with uptake and accumulation of essential nutrients like potassium, calcium, nitrogen and phosphorus. Similar results were reported by other scientists in different crops (Verslues *et al*., 2006; Amjad *et al.*, 2007; Bakht *et al.*, 2010; Moghanibashi *et al.*, 2012). The accumulation of total free amino acids as has been suggested to increase the osmotic adjustment of plants in water stressed environments (Miri and Sayed, 2013). Further, priming of seeds with sodium chloride may alleviate the adverse effects of salt stress by the activation of some stress responsive genes and in turn initiate the synthesis of some heat shock proteins as well as accumulation of compounds which helped in osmotic adjustments during stress conditions (Ashraf *et al*., 2003; Vinocur and Altman, 2005; Munns and Tester, 2008; Blum, 2011). The primed and non-primed seeds of wheat variety Punjab 2011 performed better than FSD-08 under both saline and non-saline conditions. It is thus possible that pre-sowing seed treatments with NaCl may have stimulated some factors/genes which ultimately enhanced more growth of plants in Punjab 2011.

Conclusion

Salt stress decreased the overall plant biomass production, mineral accumulation and total free amino acids in plants grown from all primed and non-primed seeds of both wheat varieties. However, seed priming with NaCl was effective in alleviating the adverse effect of salt stress in growth, accumulation of minerals and total free amino acids of both wheat varieties. The variety Punjab 2011 showed better performance than FSD-08 in all primed and non-primed seeds both in saline and non-saline conditions and could be used in future for cultivation in saline environments to obtain better results as compared to other wheat genotypes. Further research work is needed to optimize the effectiveness of pre-sowing seed treatments of NaCl using more varieties of wheat in combination of other agents/factors.

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