Acta Physiol Plant (2015) 37:153
DOI 10.1007/s11738-015-1897-5

ORIGINAL ARTICLE

Priming-induced antioxidative responses in two wheat cultivars under saline stress

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Received: 14 November 2014 / Revised: 14 April 2015 / Accepted: 26 June 2015 / Published online: 19 July 2015

Abstract Wheat, a glycophyte grown in tropical and subtropical regions, is frequently being subjected to soil salinity ultimately affecting the plant growth and yield. Focus of the present study was to evaluate the ameliorative efficiency of different seed priming methods including hydropriming and halopriming [KCl and $CaCl_2$ (100 mM)] by observing change in the expression of antioxidant defense system and accumulation of phenolic as well as proline in the spring wheat Lu26s (salt tolerant) and Lasani-06 (salt sensitive), grown under salt stress of 100 mM NaCl. Results showed that salt stress provoked a marked decline in germination, growth and yield parameters as well as increased lipid peroxidation and hydrogen peroxide (H_2O_2) contents. However, higher accumulation of proline and low H_2O_2 contents were recorded in both cultivars under halopriming followed by hydropriming. Halopriming induced a significant increase in antioxidant enzyme activities (CAT, POD, APX) of salt-tolerant cultivar Lu26s, whereas such pattern of enhanced activities of antioxidant enzymes in cultivar Lasani-06 was also found but the content of these activities was less than control under saline regime. The cultivar Lu26s (salt tolerant) maintained lower Na^+ and higher K^+/Na^+ ratio in leaves than salt-sensitive cultivar Lasani-06. Reason behind the loss of grain yield under salinity was found due to the reduction in the grain spike^{-1} in cultivar Lasani-06, while, in cultivar Lu26s, it was due to decrease in the size of grains. Enhanced germination, low proline and Na^+ contents stimulated antioxidant activities as well as phenolic contents associated with improved salt tolerance in halo-primed plants. These results suggest that halopriming is an efficient approach for imparting tolerance in wheat against salinity stress.

Keywords Antioxidant enzymes · Germination · Hydropriming · Halopriming · Phenolic · Proline · Wheat

Communicated by W. Wang.

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Introduction

Salinity is an important land degradation problem that affects almost 800 million hectares of cultivated farmland, consequently poses detrimental impact on crop production across the globe (Rengasamy 2006). In arid and semi-arid environment, limited rainfall, high evapotranspiration and temperature are the main causes of salinity that result in poor germination and seedling establishment, inducing a notable reduction in germination rate and delay in the initiation of germination (Almansouri et al. 2001; Li et al. 2011). Water stress, nutritional instability and accumulation of sodium ion (Na^+) are regarded as the plant growth associated impacts of saline stress (Shannon 1997), causing adverse pleiotropic effects at physiological, biochemical and molecular level (Parida and Das 2005). Accumulation of the Na^+ in leaves restricts the plant growth by decreasing life of photosynthetic tissues (Munns 2002). The regulation of Na^+ and its active efflux in leaves is one of the significant adaptations for salinity tolerance which

includes osmotic adjustment and compartmentalization of toxic ions and scavenging of reactive oxygen species (ROS) by the antioxidant defense system (Sekmen and Takio 2007; Miller et al. 2010). Under optimum conditions, ROS production and destruction are the routine processes but under stress condition, antioxidant defense system protects the cell from the cytotoxic effects of ROS (Foyer and Noctor 2011).

Among different approaches to cope with salinity stress in plants, seed priming (pre-sowing seed treatment such as hydropriming, halopriming, osmopriming, hardening, osmohardening and hormonal priming) is an easy, low-cost and low-risk technique that has recently been used to accelerate synchronized seed germination, vigorous seedling establishment, stimulated vegetative growth and yield in many grass, vegetable and field crops, to overcome the adverse effects of salinity in agricultural lands (Iqbal and Ashraf 2006; Iqbal et al. 2006). The improved seed performance might be due to the osmotic adjustment, metabolic repair processes or stimulation of germination metabolites during priming trea tments (Haghpanah et al. 2009). It has been proposed that hydropriming is a cost-effective technique having beneficial role on the growth of maize, rice, soybean and chickpea (Ashraf and Foolad 2005). Priming phenomen has been known for more than two decades but is appreciated recently. It improves plant defense responses by boosting crop's resistance in biotic and abiotic stresses (Beckers and Conrath 2007). This approach has proven its effectiveness to improve crop establishment on saline soil (Afzal et al. 2005).

Wheat is the main crop that is cultivated in Pakistan both in irrigated and arid zones. It is one of the staple foods of the country. Its production (hectare^{-1}) is less than the ideal yield because of many reasons which also include salinization. However, halopriming (with $CaCl_2$ and KCl) is known to increase salt tolerance in different crops (Ahmadv et al. 2012) but these studies are limited to the germination studies only. Moreover, the biochemical and physiological basis of halopriming-induced salt tolerance are still unclear. Therefore, the present study was planned to investigate the effect of KCl and $CaCl_2$ priming on germination, chlorophyll contents, ions distribution and activities of antioxidant enzymes of two wheat cultivars differing in salt tolerance under salt stress.

Materials and methods

Plant material, growth and treatment conditions

Seeds of two wheat (*Triticum aestivum* L.) cultivars Lu26s (salt tolerant) and Lasani-06 (salt sensitive) were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. A two-factor factorial designed pot experiment was conducted with three replications consisting of four priming treatments and two salinity levels as factors. Total 75 healthy seeds were selected for each priming treatment (25 seeds per pot were sown, after emergence they were thinned by 7–8 plants pot^{-1}). Solution of KCl (100 mM), $CaCl_2$ (100 mM) and distilled water were used for seed priming. Before sowing, the seeds were soaked with KCl, $CaCl_2$ as well as in distilled water, respectively, for 12 h at room temperature (20 ± 2 °C). After priming, seeds were air dried on filter paper for 12 h at room temperature (20 ± 2 °C). All the haloprimed ($CaCl_2$ or KCl), hydroprimed (HP) and untreated seeds (UT) were grown in both control (0 mM NaCl) as well as in salinity stress (100 mM NaCl) (Iqbal et al. 2006). Plants were watered (with distill water and saline solution) as per need. All the pots were placed under natural light (sunlight) with air temperature ranging from 13 to 34 °C during the day and 10 to 25 °C during night, while, relative humidity was in range of 45 and 65 % at day and night, respectively.

Experiment was conducted in earthen pots lined with polythene layer, containing 10 kg of air dried soil with pH 7.5, electric conductivity 685 μS cm^{-1}, total organic matter 0.6 %, total soil phosphorus 5.30 mg kg^{-1} and total nitrogen of 500 mg kg^{-1} soil. Each pot received N, P, and K fertilizer in quantity of 10, 6 and 6 g m^{-2}, respectively, as per requirement/recommended dose. Before sowing, pots were irrigated with 2.5 dm^3 of water (control) or saline solution of NaCl (100 mM) according to the treatment plan. The respective electrical conductivity (EC) of 100 mM of NaCl solutions was 12.5 dS m^{-1}.

Germination percentage

Calculation of germination percentage of seeds from each treatment was recorded after every 5 days with the help of the following formula:

$$\text{Germination \% age} = \text{Number of seeds germinated}/\text{total number of seeds sown} \times 100$$

Days to 50 % germination

Days to 50 % germination (E50) were calculated following the formula developed by Coolbear et al. (1984) with slight modifications:

$$E_{50} = t_i + \left[(N/2 - n_i)(t_i - t_i)/n_j - n_i\right]$$

where N represents the final number of seeds emerged, and n_i and n_j represent the cumulative number of seeds emerged counted at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

Mean emergence time

Mean emergence time (MET) was worked out by following Ellis and Roberts (1981):

$$\text{MET} = (\Sigma D_n / \Sigma_n)$$

where n represents the number of seeds emerged on day D, and D represents the number of days from the onset of seed germination.

Coefficient of uniformity of emergence

Coefficient of uniformity of emergence (CUE) was calculated by following Bewley and Black (1985):

$$\text{CUE} = \Sigma n / \Sigma \left[(t' - t)^2 \times n \right]$$

In the above equation, t represents the time in days, starting from the day of sowing, and n denotes the number of seeds that have completed emergence on day t, and t' represents the MET.

Emergence index

Emergence index (EI) was calculated using following formula:

$$\begin{aligned}\text{EI} = {} & (\text{number of seeds germinated/days of first count}) \\ & + \ldots \\ & + (\text{number of seeds germinated/days of final count})\end{aligned}$$

Germination energy

Germination energy (GE) of the seeds was calculated on the fourth day after sowing (DAS) of seeds following Ruan et al. (2002b). The percentage of germinating seeds at fourth DAS is relative to the total number of seeds tested.

Plant growth, yield and biomass

Growth traits were measured at flowering stage in terms of plant height, shoot and root dry weight. The samples were put in the forced hot air draft oven at 70 ± 5 °C for 5 days to measure shoot and root dry weight (g) after recording shoot/root lengths (cm) and fresh weight (g) of plant. For the determination of leaf area (cm^2), length and maximum width of each leaf were measured and leaf area was calculated using the formula of Carleton and Foote (1965). At maturity (final harvest), the remaining plants (4–5 plants pot^{-1}) from each pot were harvested and data regarding the number of spike, grains $spike^{-1}$, grains $plant^{-1}$ and 100-grain weight were recorded.

Chlorophyll contents, antioxidant enzyme activities and total phenolic

Middle section of leaf without midrib was used for all biochemical analysis. Total chlorophyll contents at flowering stage were determined according to the method of Arnon (1949). The extent of the salt-induced oxidative damage was evaluated through changes in lipid peroxidation by measuring the malondialdehyde (MDA) formation (Cakmak and Horst 1991) in the leaves of wheat cultivars. Briefly, 1 g of fresh leaves was ground in 20 mL of 0.1 % TCA (Trichloroacetic acid) solution and centrifuged for 10 min at 12,000g. One mL of the supernatant was mixed with 4 mL of TCA containing 5 % TBA (Thiobarbituric acid), heated for 30 min at 95 °C and cooled on ice. The mixer was centrifuged at 12,000g for 10 min and absorbance of the supernatant was taken at 532 and 660 nm.

The activity of POD was measured by the method of Maehly and Chance (1954) where the activity of APX was assessed following the method of Nakano and Asada (1981) with minor modifications. An aliquot of 200 µL enzyme extract was added to a mixture containing 800 µL of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid and 0.1 mM H_2O_2. Optical density (OD) of the mixture was recorded for one min (as decrease in absorbance at 290 nm). The total phenolic was measured by adding 0.5 mL of enzyme extract in 2.5 mL of Folin–Ciocalteu reagent (10 % v/v) and 2 mL of Na_2CO_3 (7.5 %). The mixture was heated at 45 °C for 40 min and the absorbance was measured at 765 nm. The gallic acid was used as standard and expressed as mg of Gallic acid equivalent/g extract. Proline contents were measured according to Bates et al. (1973). For the determination of sodium and potassium ions, leaves were dried and ground. About 0.1 g of the ground leaf was digested with H_2SO_4 and H_2O_2, and then sodium and potassium contents were analyzed through flame photometer.

Statistical analysis

All values reported in this study are mean of at least three replicates. A two-way analysis of variance (ANOVA) was performed using a statistical package, SPSS version 16.0 (SPSS, Chicago, IL). Duncan's test was done to determine the significant difference among treatments.

Results

Seed germination parameters

Data regarding different germination parameters showed that seed pre-sowing treatment with $CaCl_2$ or KCl

significantly increased final germination, germination energy, coefficient of uniformity of emergence and germination index, while, significant decrease was recorded in the mean emergence time and days to 50 % emergence as compared to hydroprimed and unprimed seeds under 100 mM NaCl (Fig. 1). It was also observed that seed presowing treatment with $CaCl_2$ primed seeds performed well followed by KCl primed seeds. Among the wheat cultivars, values of germination parameters of Lu26s were higher than cultivar Lasani-06 (Fig. 1).

Plant biomass and chlorophyll contents

All growth characters were found to be significantly affected by salinity. However, varied response of sensitivity to salinity was recorded in different treatments. The

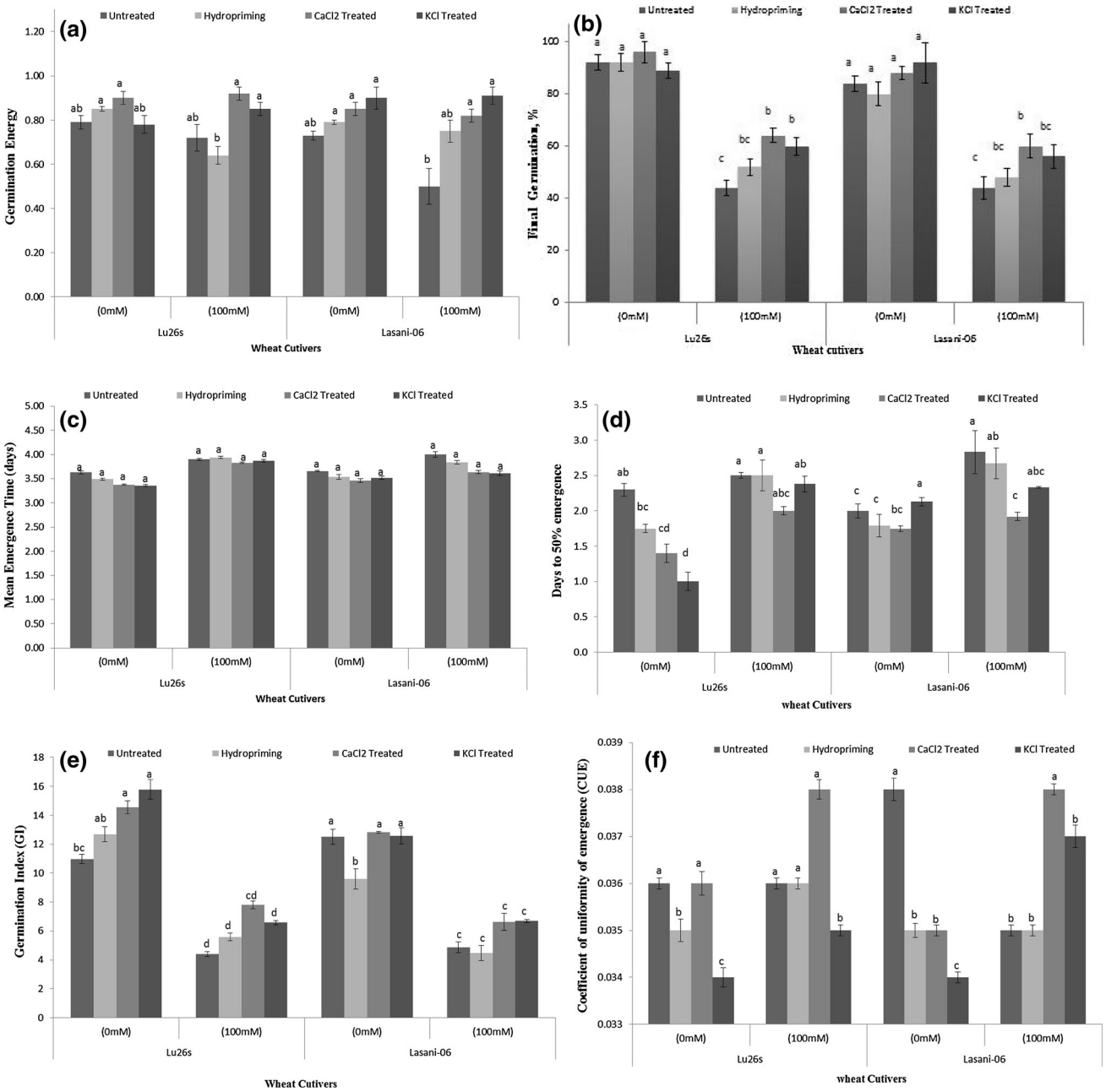


Fig. 1 Effect of different seed priming treatments on germination parameters of wheat cultivars under salinity stress. Data presented are means from three replicates with standard errors. Within each treatment, *different letters* indicate significant differences by Duncan's multiple range test at $P < 0.05$

seeds primed with $CaCl_2$ or KCl maintained significantly higher fresh and dry weight of root and shoot followed by hydroprimed seeds over unprimed control. Overall, the effect of halopriming was more pronounced in Lu26s (salt tolerant) than Lasani-06 (salt sensitive). Root/shoot ratios (in both cultivars) were higher in salt stress as compared to untreated control. It was found that shoot was more affected than root under salt stress (Table 1). Salt stress negatively affected the leaf area of both wheat cultivars. Indeed, under control conditions, the leaf area ranged from 281 to 320 cm^2 in Lu26s and 318 to 357 cm^2 in Lasani-06. The leaf area of salt-sensitive variety (Lasani-06) was more affected (57 % decrease) than the salt-resistant cultivar (34 % decrease) under salt stress (Table 1). Data regarding chlorophyll contents presented a significant variation among the cultivars (Table 1). In general, a decline trend was observed in both cultivars under salinity stress. Maximum reduction was recorded in Lasani-06; while, Lu26s retained high amount of chlorophyll, however, haloprimed treatments exhibited less reduction in chlorophyll contents under salinity. However, a significant decrease in chlorophyll contents was found in untreated and hydroprimed treatment (Table 1).

Yield attributes

Yield attributes such as the number of spikes, grains $plant^{-1}$, grains $spike^{-1}$ and 100-grains weight were reduced significantly under salinity stress (Table 2), though the change was higher in Lasani-06 than Lu26s. Among the priming treatments, yield components of the haloprimed seeds were significantly higher than those of hydroprimed and control treatments. Under saline stress, cultivar Lu26s decreased 100-grains weight by 65 and 35 % under unprimed and hydroprimed treatments, while 21 and 30 % reduction in 100-grains weight was recorded under KCl and $CaCl_2$ treatments, respectively. The decrease in all other described yield attributes was more pronounced in salt-sensitive cultivar (Lasani-06) as compared to Lu26s. However, among seed treatments, hydroprimed seeds were more affected than haloprimed seeds.

Effects of seed priming and salinity on oxidative stress

A significant increase in H_2O_2 accumulation was observed in the leaves of both wheat cultivars after salt exposure as compared to control plants, but to greater extent in Lasani-06 as compared to Lu26s cultivar. Lasani-06 showed highest H_2O_2 level in untreated and hydroprimed plants; while, significant decrease was recorded in haloprimed plants (Fig. 2a, b). Salt-stressed leaves had more MDA contents in both wheat cultivars under all priming treatments as compared to control plants. MDA contents were significantly higher in salt-sensitive cultivar (Lasani-06) as compared to salt-resistant cultivar (Lu26s). Priming with KCl or $CaCl_2$ significantly reduced the MDA contents under salt stress as compared to hydroprimed and untreated plants (Fig. 2c, d).

Antioxidant enzyme activities

A significant difference in CAT activity (Fig. 2e, f) and APX activity (Fig. 2i, j) was observed among two wheat cultivars under salt stress. In general, salt-resistant cultivar Lu26s showed higher contents of CAT and APX enzyme activities over all priming treatments. However, CAT and APX activities were significantly decreased in salt-sensitive cultivar (Lasani-06) under all priming treatments. This decrease was more pronounced in hydroprimed and untreated control as compared to haloprimed plants. Salinity stress induced a greater level of POD activity in leaves of Lu26s cultivar as compared to control and more increase was pronounced in haloprimed treatment (Fig. 2g, h). However, POD activity significantly decreased in salt-sensitive cultivar. Moreover, significantly lower value of POD activity was recorded in all priming treatments.

Proline and phenolic contents, and K^+ and Na^+ accumulation

Results showed that leaf proline and phenolic contents were significantly higher under salt stress in both cultivars. However, haloprimed treatment in salt-resistant cultivar Lu26s and sensitive cultivar Lasani-06 induced a significant increase in proline (Fig. 2k, l) and phenolic (Fig. 2m, n) contents under salinity stress. Further, leaf chemical analysis of wheat cultivars indicated that Na^+ contents ameliorated under salinity stress (Table 3). The cultivar Lasani-06 had relatively higher Na^+ contents than Lu26s as compared to control. Relatively less concentration of Na^+ was found in haloprimed seed plants in both cultivars, while reduced K^+ contents were recorded in all priming treatments under salinity stress in cultivar Lasani-06. Cultivar Lu26s maintained high concentration of K^+ in all priming treatments under salinity stress, whereas relatively reduced K^+/Na^+ ratio was observed in Lasani-06 as compared to Lu26s (Table 3).

Discussion

The plant growth and development are adversely affected attributes of the plant under salinity stress which result in ultimate loss of yield (Ashraf and Harris 2004). Pre-sowing treatment with inorganic salt resulted in amelioration of

Table 1 Effect of seed priming on growth of wheat cultivars affected by seed priming under control (0 mM NaCl) and salinity stress (100 mM NaCl)

Wheat cultivars	Priming treatment	T. Chl (mg g^{-1} F wt)	S.F.W (g)	S.D.W (g)	R.F.W (g)	Root/shoot ratio	R.D.W (g)	L.A (cm^2)
Lu26s	0 mM NaCl							
	Untreated	$2.98 \pm 0.18^{a,b,c}$	43.00 ± 1.58^{d}	4.94 ± 0.23^{e}	7.12 ± 0.1^{f}	$0.163 \pm 0.0^{c,d,e}$	$1.18 \pm 0.0^{b,c}$	281.67 ± 6.01^{e}
	Hydropriming	$2.90 \pm 0.17^{a,b,c}$	$45.20 \pm 1.04^{c,d}$	$5.13 \pm 0.35^{d,e}$	7.59 ± 0.20^{e}	$0.170 \pm 0.01^{a,b,c,d}$	$1.10 \pm 0.06^{c,d}$	$293.00 \pm 6.51^{d,e}$
	$CaCl_2$ treated	3.30 ± 0.26^{a}	$51.14 \pm 1.70^{a,b}$	6.95 ± 0.2^{a}	10.14 ± 0.5^{b}	0.197 ± 0.01^{b}	1.80 ± 0.06^{a}	$300.00 \pm 9.29^{c,d,e}$
	KCl treated	$3.10 \pm 0.21^{a,b}$	$49.95 \pm 2.44^{a,b}$	$6.65 \pm 0.23^{a,b}$	9.42 ± 0.29^{a}	$0.190 \pm 0.01^{b,c}$	1.88 ± 0.08^{a}	$320.00 \pm 12.17^{b,c}$
	100 mM NaCl							
	Untreated	$2.10 \pm 0.15^{d,e,f}$	$15.00 \pm 0.74^{g,h}$	3.05 ± 0.27^{g}	1.94 ± 0.08^{k}	$0.140 \pm 0.02^{d,f}$	0.58 ± 0.01^{f}	157.00 ± 2.08^{j}
	Hydropriming	$2.34 \pm 0.20^{c,d,f}$	18.68 ± 0.65^{h}	3.78 ± 0.14^{f}	3.20 ± 0.15^{h}	$0.173 \pm 0.00^{a,b,c}$	0.60 ± 0.03^{f}	170.00 ± 2.89^{j}
	$CaCl_2$ treated	$2.53 \pm 0.25^{b,c,d,f}$	23.29 ± 0.67^{e}	$5.08 \pm 0.31^{d,e}$	5.53 ± 0.16^{g}	0.240 ± 0.01^{a}	$1.30 \pm 0.06^{d,e}$	240.00 ± 7.64^{f}
	KCl treated	$2.61 \pm 0.1\ 7^{b,c,e}$	$18.34 \pm 0.40^{f,g}$	4.87 ± 0.18^{e}	$3.14 \pm 0.09^{h,i}$	$0.173 \pm 0.01^{a,b,c}$	0.98 ± 0.05^{e}	210.00 ± 8.96^{i}
Lasani-06	0 mM NaCl							
	Untreated	$2.67 \pm 0.07^{b,c,e}$	$47.00 \pm 2.5^{b,c,d}$	$5.29 \pm 0.17^{d,e}$	6.87 ± 0.09^{f}	$0.147 \pm 0.01^{d,e,f}$	$1.15 \pm 0.03^{d,e}$	$318.67 \pm 5.55^{b,c,d}$
	Hydropriming	$2.60 \pm 0.1^{b,c,e}$	$49.60 \pm 1.99^{a,b,c}$	$5.67 \pm 0.19^{c,d}$	$7.26 \pm 0.08^{e,f}$	$0.147 \pm 0.00^{d,e,f}$	$1.24 \pm 0.03^{c,d}$	$325.00 \pm 8.96^{b,c}$
	$CaCl_2$ treated	$2.78 \pm 0.11^{a,b,c}$	52.13 ± 2.71^{a}	$6.15 \pm 0.21^{b,c}$	8.77 ± 0.33^{c}	$0.170 \pm 0.01^{a,b,c,d}$	$1.80 \pm 0.10^{b,c}$	357.00 ± 13.08^{a}
	KCl treated	$2.85 \pm 0.10^{a,b,c}$	$50.33 \pm 0.88^{a,b}$	$6.13 \pm 0.15^{b,c}$	8.15 ± 0.04^{d}	$0.163 \pm 0.00^{c,d,e}$	$1.85 \pm 0.03^{b,c}$	$332.74 \pm 5.06^{a,b}$
	100 mM NaCl							
	Untreated	1.58 ± 0.32^{f}	7.33 ± 0.24^{k}	1.98 ± 0.1^{h}	1.03 ± 0.09^{l}	$0.143 \pm 0.01^{d,e,f}$	0.71 ± 0.03^{h}	110.00 ± 4.04^{j}
	Hydropriming	1.64 ± 0.19^{f}	8.27 ± 0.1^{k}	2.73 ± 0.19^{g}	1.32 ± 0.15^{l}	0.123 ± 0.01^{f}	0.80 ± 0.03^{g}	145.00 ± 16.26^{k}
	$CaCl_2$ treated	$1.94 \pm 0.22^{d,f}$	$13.94 \pm 0.46^{g,h}$	3.80 ± 0.21^{f}	2.60 ± 0.09^{i}	$0.193 \pm 0.01^{b,c}$	1.30 ± 0.07^{f}	$220.00 \pm 8.62^{g,h}$
	KCl treated	$2.10 \pm 0.15^{d,e,f}$	$10.60 \pm 0.60^{k,h}$	$3.26 \pm 0.20^{f,g}$	1.94 ± 0.04^{k}	$0.183 \pm 0.01^{b,c}$	$1.13 \pm 0.07^{f,g}$	$226.00 \pm 6.08^{g,h}$

Data presented are means from three replicates with standard errors

Within each treatment, different letters indicate significant differences by Duncan's multiple range test at $P < 0.05$

T. Chl total chlorophyll, *S.F.W* shoot fresh weight, *R.F.W* root fresh weight, *S.D.W* shoot dry weight, *R.D.W* root dry weight and *L.A* leaf area

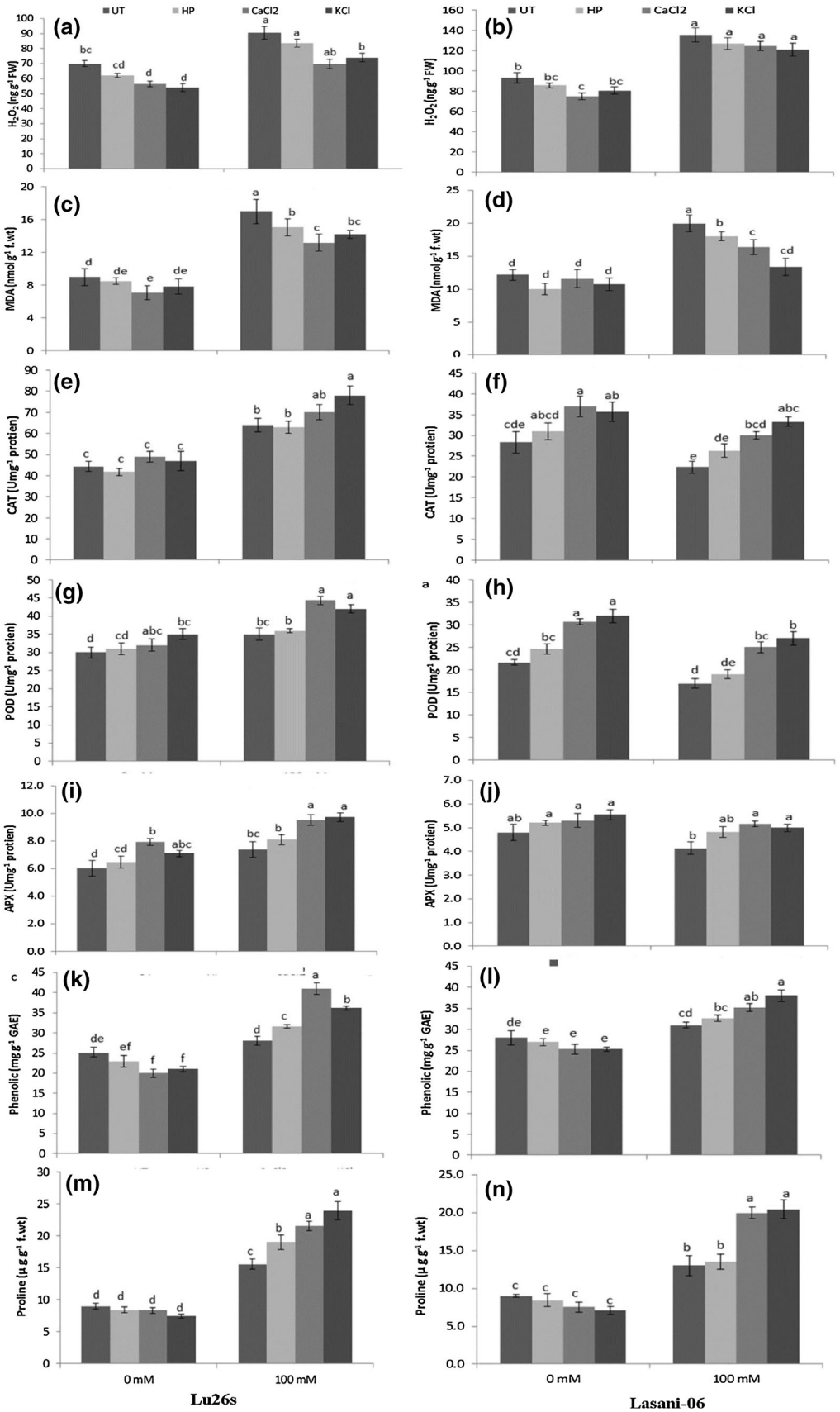
Table 2 Yield attributes of wheat cultivars affected by seed priming under control (0 mM NaCl) and salinity stress (100 mM NaCl)

Wheat cultivars	Priming treatment	Number of spike	Spike per grains	Grain per plant	100-seed weight
Lu26s	0 mM NaCl				
	Untreated	$16.47 \pm 0.80^{a,b,c,d}$	$40.46 \pm 0.62^{a,b}$	$117.00 \pm 2.19^{a,b}$	4.40 ± 0.15^{b}
	Hydropriming	$16.50 \pm 0.85^{a,b,c,d}$	41.28 ± 1.16^{a}	$113.33 \pm 2.60^{a,b}$	4.42 ± 0.11^{b}
	$CaCl_2$ treated	18.33 ± 1.24^{a}	44.00 ± 1.73^{a}	$113.67 \pm 4.48^{a,b}$	5.07 ± 0.16^{a}
	KCl treated	$17.20 \pm 0.95^{a,b}$	44.10 ± 1.35^{a}	111.67 ± 2.31^{a}	4.66 ± 0.18^{b}
	100 mM NaCl				
	Untreated	8.98 ± 0.90^{g}	$29.68 \pm 1.29^{f,g}$	$43.67 \pm 2.08^{j,k,l}$	1.53 ± 0.12^{e}
	Hydropriming	12.03 ± 0.95^{g}	$35.81 \pm 1.45^{d,f}$	$42.52 \pm 2.12^{h,i}$	2.87 ± 0.11^{d}
	$CaCl_2$ treated	$15.03 \pm 1.23^{c,d,f}$	$36.88 \pm 2.02^{b,c}$	68.05 ± 2.57^{g}	3.97 ± 0.14^{c}
	KCl treated	$14.68 \pm 0.74^{a,d,f}$	$32.33 \pm 1.69^{b,c}$	$66.33 \pm 2.91^{g,f}$	3.22 ± 0.06^{d}
Lasani-06	0 mM NaCl				
	Untreated	$14.17 \pm 0.73^{d,f}$	$40.73 \pm 0.93^{a,b}$	95.33 ± 2.03^{f}	2.47 ± 0.08^{e}
	Hydropriming	$14.67 \pm 0.88^{d,f}$	41.67 ± 0.88^{a}	97.33 ± 0.88^{f}	2.65 ± 0.09^{e}
	$CaCl_2$ treated	$17.00 \pm 0.58^{a,b,c}$	41.48 ± 1.74^{a}	$109.33 \pm 3.48^{b,c}$	$2.90 \pm 0.12^{d,e}$
	KCl treated	$15.33 \pm 0.88^{b,c,d,f}$	43.53 ± 0.94^{a}	$104.00 \pm 2.08^{c,d}$	2.77 ± 0.16^{e}
	100 mM NaCl				
	Untreated	8.33 ± 0.44^{h}	10.00 ± 1.53^{k}	30.67 ± 1.45^{l}	1.00 ± 0.05^{h}
	Hydropriming	11.67 ± 0.60^{g}	$21.00 \pm 1.39^{h,k}$	44.67 ± 0.88^{l}	1.16 ± 0.09^{h}
	$CaCl_2$ treated	$15.40 \pm 0.78^{b,c,d,f}$	30.82 ± 1.14^{f}	$54.60 \pm 1.80^{j,k}$	1.87 ± 0.07^{g}
	KCl treated	$14.30 \pm 0.20^{f,g}$	$26.00 \pm 0.58^{g,h}$	$48.33 \pm 0.88^{k,l}$	1.40 ± 0.26^{h}

Data presented are means from three replicates with standard errors. Within each treatment, different lowercase letters indicate significant differences by Duncan's multiple range test at $P < 0.05$

salinity stress as studied in different plants (Cayuela et al. 1996; Sivritepe et al. 2003). Present study conducted under salinity stress elaborates that seed emergence rate, germination index and energy are the crucial contributors of seed vigor. Higher seed emergence rate is the primary factor that ensures overall seedling performance. Data regarding germination parameters demonstrated that salinity and seed priming significantly influenced the wheat germination as previously found by Kaya et al. (2006). This may be due to the metabolic activities in primed seed during germination that had started much earlier than the appearance of radicle and plumule; thus, primed seeds have better efficiency of water absorption from growth media compared to unprimed seed (Hopper et al. 1979). Seed priming in fact initiates the metabolic processes (Bewley and Black 1985) such as cell division, which reduces seed germination time (Sivritepe et al. 2003). Indeed, priming is an effective technique that increases seed vigor and improves germination and seedling growth (Jumsoon et al. 1996) which is also confirmed by our findings.

On the other hand, increased saline stress inhibits the germination which may be due to toxic effects of Na^+ and Cl^- on germination (Khajeh-Hosseini et al. 2003), which ultimately limits the water absorption ability of seed (Dodd and Donovan 1999). Significant rise in mean germination time of unprimed seeds and early germination of primed seeds (with KCl or $CaCl_2$) under salinity stress is in line with the findings of Elouaer and Hannachi (2012) and Shehzad et al. (2012). Ruan et al. (2002a) also demonstrated the improved germination index in rice seed primed with KCl and $CaCl_2$. Greater efficiency of seed priming with KCl is possibly related to the osmotic advantage of K^+ that acts as co-factor in the activities of numerous enzymes (Taiz and Zeiger 2002). Potassium and calcium ions actually mitigate the harmful effects of Na^+ on plant metabolism by reducing the Na^+ uptake (Greenway and Munns 1980). In the present study, variation in growth and development of wheat cultivars in response to saline stress might be due to the variations in physiological and biochemical characteristics that have occurred in the process of salt tolerance, i.e., antioxidants enzymes, ion balance and accumulation of compatible solutes. Photosynthesis is another critical attribute for assessment because it is responsible for plant productivity under both normal and stress conditions (Natr and Lawlor 2005; Athar et al. 2008). Decrease in the contents of chlorophyll in studied plants may be due to the production of proteolytic enzyme, i.e., chlorophyllase, which reduces the chlorophyll and damages the photosynthetic machinery (Sabater and Rodrguez 1978) or this reduction may be attributed to the instability of the pigment protein complex. Saline stress in the present investigation also decreased the root, shoot dry and fresh


UT
HP
CaCl2
KCl
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
H2O2 (ng g-1 FW)
MDA (nmol g-1 f.wt)
CAT (Umg-1 protien)
POD (Umg-1 protien)
APX (Umg-1 protien)
Phenolic (mg g-1 GAE)
Proline (µ g g-1 f.wt)
0 mM
100 mM
Lu26s
Lasani-06

◀**Fig. 2** Changes of H_2O_2 (ng g^{-1} FW) (**a**, **b**), malonaldehyde (MDA, nmol g^{-1} FW) (**b**, **c**), catalase (CAT) (**e**, **f**), peroxidase (POD) (**g**, **h**), ascorbate peroxidase (APX) (**i**, **j**), phenolic (mg g^{-1} Gallic Acid Equivalent) (**k**, **l**) and proline (μg^{-1}f.wt) (**m**, **n**) in untreated control (UT), hydroprimed (HP), $CaCl_2$ and KCl primed wheat plants under different levels of salinity stress. Data presented are means from three replicates with standard errors. Within each treatment *different letters* indicate significant differences by Duncan's multiple range test at $P < 0.05$

In cultivar Lu26s, there might be no limitation of photo-assimilates accumulation due to this reason more grain numbers were recorded in Lu26s as compared to Lasani-06, but at grain-filling stage less availability of assimilates due to prolonged saline stress reduced the weight of grains. Our results may support the earlier findings (Rahnama et al. 2011; Husain et al. 2003; Houshm et al. 2005) who

Table 3 Concentration of Na^+, K^+ and K^+/Na^+ ratio in leaves of wheat cultivars affected by seed priming under control (0 mM NaCl) and salinity stress (100 mM NaCl)

Cultivars	Priming treatments	0 mM NaCl			100 mM NaCl		
		Na^+ (mg^{-1} g DW)	K^+ (mg^{-1} g DW)	K^+/Na^+ ratio	Na^+ (mg^{-1} g DW)	K^+ (mg^{-1} g DW)	K^+/Na^+ ratio
Lu26 s	Untreated	0.20 ± 0.03	2.14 ± 0.21	10.7 ± 1.87	1.38 ± 0.50	1.35 ± 0.22	1.0 ± 0.21
	Hydropriming	0.18 ± 0.02	2.16 ± 0.13	12.0 ± 2.65	1.36 ± 0.38	1.38 ± 0.09	1.0 ± 0.11
	$CaCl_2$ Treated	0.19 ± 0.01	2.15 ± 0.18	11.3 ± 1.53	1.31 ± 0.26	1.42 ± 0.16	1.1 ± 0.18
	KCl Treated	0.19 ± 0.02	2.19 ± 0.21	11.5 ± 1.11	1.33 ± 0.25	1.43 ± 0.12	1.1 ± 0.22
Lasani-06	Untreated	0.19 ± 0.04	2.1 ± 0.19	11.1 ± 1.36	2.13 ± 0.16	1.1 ± 0.14	0.5 ± 0.07
	Hydropriming	0.18 ± 0.03	2.12 ± 0.10	11.8 ± 0.90	2.1 ± 0.21	1.1 ± 0.18	0.5 ± 0.03
	$CaCl_2$ Treated	0.17 ± 0.02	2.18 ± 0.12	12.8 ± 0.97	1.98 ± 0.27	1.15 ± 0.22	0.6 ± 0.10
	KCl Treated	0.15 ± 0.02	2.15 ± 0.13	14.3 ± 1.70	1.92 ± 0.35	1.19 ± 0.09	0.6 ± 0.19

Data presented are means from three replicates with standard errors

weight of primed and unprimed seeds under salinity stress but magnitude of reduction was relatively less in primed seeds. Reduction in root and shoot fresh weight can be supported by the findings of Jaleel et al. (2007) who demonstrated that reduction in the shoot fresh and dry weight was attributed to decrease in photosynthesis due to less leaf area that resulted a reduction in plant biomass (Basra et al. 2003).

Results of the present study also showed higher root/shoot ratio under salinity stress. Saline stress often reduces shoot growth more than root growth (Läuchli 1990) and can reduce the number of florets per ear, increase sterility and affect the time of flowering and maturity in both wheat (Maas and Grieve 1990) and rice (Khatun et al. 1995). Thus, the biomass percentage allocated to roots, stems and leaves was found widely different and related to time of exposure, species/varieties and ontogenetic stages under saline conditions (Munns and Tester 2008). It was observed that decrease in grain yield in Lu26s cultivar was related to the decline in 100-grains weight but in Lasani-06 it was due to decrease in grain number $plant^{-1}$. The decrease in grain number $plant^{-1}$ in Lasani-06 cultivar may be due to the lack of photo-assimilates accumulation that reduces the number of grain $plant^{-1}$. This shows that availability of photo-assimilates is the rate limiting factor to grain number. Availability of the assimilates during saline stress determined the grain size (Poustini and Siosemardeh 2004). observed similar phenomena with wheat grains.

To understand the salt tolerance mechanism in plant, ions regulation is another important critical parameter. Thus, the assessment of ion accumulation, especially Na^+ and K^+ in different plant organs is essential to infer the salt tolerance mechanisms (Noreen et al. 2010). In the present investigation, high K^+ and K^+/Na^+ ratio and low Na^+ ion helped Lu26s to maintain high growth while among the seed treatments, haloprimed seed plants maintained high contents of K^+ and K^+/Na^+ ratio thus revealing that salt stress also imbalances homeostasis that leads to the ionic and osmotic stresses in plant cells (Athar et al. 2008). Higher concentration of Na^+ in Lasani-06 may be due to increased amounts of Na^+ in root, passive Na^+ diffusion through damaged membranes and decreased efficiency of exclusion mechanisms as described by Bajji et al. (2001).

Plants under stress also produce some defense mechanisms to protect themselves from the harmful effects of salinity-induced oxidative stress. ROS are involved in the initiation of a number of auto-oxidative chain reactions, including lipid peroxidation, DNA damage and protein degradation (Mittler 2002). In the present study, lipid peroxidation was assessed by measuring amount of MDA in the leaves of wheat cultivars, because salt stress is known to result in extensive lipid peroxidation, which is an effective indicator of salt-induced oxidative damage at the

cellular level (Islam et al. 2014a, b; Hernández and Almansa 2002). Alleviation of oxidative damages and maintaining integrity of the cellular membranes is a key mechanism in salinity tolerance (Stevens et al. 2006). In the present study, significant increase in MDA contents in salt-sensitive cultivar Lasani-06 than salt-tolerant cultivar Lu26s under salt stress indicates that Lu26s may have a better protection against oxidative damage. However, among priming treatments, $CaCl_2$ and KCl primed plants showed lower MDA contents than hydroprimed and untreated control under salinity stress. The same phenomenon was found by Masood et al. (2006) while studying the effect of salinity in Azolla cultivars. The lesser MDA contents in haloprimed plants may be due to Ca^+ and K^+ ions that prevent the electrolyte leakage and lipid peroxidation and play crucial role against salinity tolerance (Nedjimi and Daoud 2009; Wu et al. 2013). It is evident that priming can increase the activities of free radical scavenging enzymes, i.e., catalase (CAT), ascorbate (APX) and peroxidase (POD) in seeds (Ashraf and Ali 2008; Chiu et al. 1995; Chang and Sung 1998). Thus, seed priming in this study has increased the activity of scavenging enzymes and improved the seedling vigor as indicated by increased POD, APX and CAT activities in the leaves of salt-tolerant wheat cultivar. Improved protection in Lu26s as compared to Lasani-06 under halopriming may reflect a more efficient antioxidative system. Though, activity of antioxidant enzymes was reduced significantly in salt-sensitive cultivar but this decrease was most pronounced in hydroprimed and untreated control as compared to $CaCl_2$ or KCl primed plants. Catalase is a key enzyme in scavenging H_2O_2 by breaking it down to form H_2O and O_2 (Mittler 2002). A decrease in CAT activity after priming in salt-sensitive cultivar confirmed the findings of Srinivasan and Saxena (2001) who reported that CAT activity was not increased after priming in radish; however, the decrease/increase was closely related to the genetic background of cultivars (sensitive/tolerant), level of salt stress (NaCl concentration and duration) and type of pre-sowing seed treatments. Therefore, it is expected that enhanced antioxidant enzyme activity in wheat cultivars due to halopriming is a key component against tolerance to NaCl stress (Afzal et al. 2006) as observed in the present study. These results suggest that salt-tolerant cultivar Lu26 s may have a better protection against reactive oxygen species (ROS) by increasing the activity of antioxidant enzymes (APX, POD and CAT) under salt stress. Similar observations have also been reported for salt-tolerant and -sensitive cultivars of potato by Rahnama and Ebrahimzadeh (2005).

Phenolic being carbon-rich secondary metabolites, involves in the scavenging of free radicals and oxidative species, regulation of osmotic pressure, protection of membrane integrity, stabilization of enzymes/proteins, maintaining appropriate $NADP^+$/NADPH ratios and improvement of antioxidant activity of saline-stressed plants. Proline's ROS-scavenging ability helps in minimizing oxidative damage produced by salinity and protects membrane integrity (Fidalgo et al. 2004). In the present investigation, a substantial increase in proline levels might be attributed to the strategies adapted by plants to cope with salinity stress. Under salinity stress, plants produce jasmonic acid and its methylated derivatives ultimately result in the accumulation of phenolic contents in stressed plant (Pedranzani et al. 2007) to combat with reactive oxygen species. However, the production of phenolic contents in saline-stressed plant is species dependent as broccoli (López-Pérez et al. 2009) and Romanian lettuce (Kim et al. 2008) were unable to produce phenolic under saline stress, whereas phenolic production was found higher under salinity stress in maize (Hichema et al. 2009) and Spanish lettuce (Blasco et al. 2013). However, in the present investigation, a significant increase in phenolic contents was found in haloprimed wheat plants in both cultivars. This increase played functional role to protect the plant against salt-induced oxidative stress, resulting better growth and yield production in haloprimed plants than untreated control and hydroprimed plants.

Conclusions

Findings of present study suggest that halopriming ameliorates the salinity stress by upregulating the growth, photosynthesis, production of proline, phenolic contents and activities of antioxidant enzymes (POD, APX and CAT). However, it was found that the precise impact of salinity was genotype dependent. The increased tolerance of cultivar Lu26s was due to the lower concentration of Na^+ and higher K^+/Na^+ ratio in shoot, while KCl or $CaCl_2$ priming helped the plant to lower Na^+ in shoot and increase K^+/Na^+ ratio both in control and saline stress. However, a further study is needed to explore the underlying molecular and physiological mechanisms of seed priming on germination in relation to plant hormones and transcription. Moreover, the mechanism of halopriming was observed against a single level of salinity stress (NaCl 100 mM) in this preliminary investigation which needs to be evaluated against certain levels to make an authenticated statement for the expressions of wheat cultivars with halopriming with KCl or $CaCl_2$.

Author contribution statement This work was designed by F. Islam, T. Yasmeen and S. Ali. Experiments and data analyses were performed by F. Islam, B. Ali, M. A. Farooq and S. Ali. However, manuscript was written by F. Islam, T. Yasmeen, R. A. Gill and B. Ali.

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