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Full Length Article

Improving Salt Tolerance in Barley by Osmopriming and Biopriming

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Abstract

Salt stress hinders the plant growth and productivity by inducing changes in physiological and biochemical processes. This 2-years study was conducted to determine the effect of seed priming treatments on tolerance against salinity in barley. Seed priming treatments of two barley genotypes (Haider-93 and Frontier-87) involved hydropriming, osmopriming (1.5% $CaCl_2$) and biopriming (*Enterobacter* spp. strain FD17); dry seeds were taken as control. Seeds were sown in pots (30 cm diameter and 45 cm depth) containing soil. After stand establishment, salinity treatment *i.e.*, control (50 m*M* NaCl), moderate salinity (100 m*M* NaCl) and severe salinity (150 m*M* NaCl) were imposed. Salt stress hampered growth, yield, chlorophyll content, water relations and cell membrane stability (CMS) whereby increased osmolytes, leaf malondialdehyde (MDA) and Na accumulation in tested barley genotypes. However, seed priming techniques improved the plant height, leaf area, grain yield, harvest index, chlorophyll *a* and *b* contents, accumulation of total soluble phenolics and proteins, proline and glycine betaine, K and relative water contents, water, osmotic and pressure potentials and CMS, while decreased leaf MDA and Na contents under each level of salinity. Seed priming induced improvement in yield and related attributes under salinity was ordered as osmopriming > biopriming > hydropriming. Genotype Haider-93 performed better under each level of salinity than Frontier-87. In conclusion, seed priming induced salinity tolerance in barley was associated with enhanced osmolytes accumulation, improved water relations and decreased lipid peroxidation and Na accumulation. © 2018 Friends Science Publishers

Keywords: Barley; Osmoregulation; Oxidative damage; Salinity tolerance; Seed priming

Introduction

Salt stress hampers the growth and yield of crop plants by causing the osmotic and ionic stress (Hussain *et al*., 2018). However, there exists a great variation in the ability of plants to tolerate salt stress within species and even cultivars (Izadi *et al*., 2014). Barley is a moderately salt tolerant crop, however, its growth and productivity are severely hampered by salinity (Mahmood, 2011). Salt stress decreases net photosynthesis by causing a reduction in leaf appearance, chlorophyll content and stomatal conductance which ultimately results in reduced growth, number and weight of grains, and grain yield of barley (Harris *et al*., 2010; Mahlooji *et al*., 2018). Moreover, it causes over accumulation of Na^+ and Cl^- in plant cells, and aggravates the production of reactive oxygen species (ROS) which causes the lipid peroxidation, membrane instability and damage the organic molecules (Anjum *et al*., 2016; Tabassum *et al*., 2017; Hussain *et al*., 2018).

The plants usually adopt different physiological and biochemical processes to acquire tolerance to salt stress (Farooq *et al*., 2015). Plants maintain the balance between ionic and osmotic stress by regulation of uptake, translocation and subsequently sequestration of salt ions, and production and accumulation of osmolytes under salt stress (Flowers and Colmer, 2008). In response to salt stress, the activity of antioxidants and expression of heat shock proteins is increased in plants which enhance the stress tolerance (Faralli *et al*., 2015). Moreover, plants produce and accumulate compatible solutes/osmolytes such as proline and glycine betaine in greater quantities to cope with osmotic stress through improved tissue water status (Tabassum *et al*., 2017), scavenge free radicals, and enhance protection and stabilization of cellular membranes and macromolecules from ROS under salinity (Hoekstra *et al*., 2001; Anjum *et al*., 2016; Hussain *et al*., 2018).

Seed priming is a controlled hydration technique which allows the germination metabolism to occur without occurrence of actual germination. Seed priming effectively improves the salinity tolerance in cereals (Jafar *et al*., 2012; Tabassum *et al*., 2017). It improves the stress tolerance by enhancing the production and accumulation of osmolytes, stress proteins and activity of antioxidants while decreasing the ROS activity and lipid peroxidation under stressed conditions (Afzal *et al*., 2008; Chen and Arora, 2013; Tabassum *et al*., 2018). Seed priming with inorganic or

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organic salts and plant growth promoting bacteria (PGPB) might be quite effective for improvement in plant growth under salinity (Jafar *et al*., 2012; Tabassum *et al*., 2017, 2018). In osmopriming, the use of calcium salt has been effective in improving the tolerance against abiotic stresses (Farooq *et al*., 2017a, b; Kaczmarek *et al*., 2017; Tabassum *et al*., 2018). Calcium acts as secondary messenger in signaling pathways and improves the stress tolerance and plant growth by modulating the gene expression for stress related genes (Sarwat *et al*., 2013). Moreover, Ca^{2+} improves the production and accumulation of osmolytes, activity of antioxidants and cell membrane stability, and reduces the ROS activity and lipid peroxidation (Coria *et al*., 1998; Farooq *et al*., 2017a; Sakhonwasee and Phingkasan, 2017).

Plant growth promoting endophytic bacteria induce stress tolerance in plants by modulating the morphological and physiological processes in plants (Mahmood *et al*., 2016). These bacteria produce certain growth promoting hormones such as gibberellic acid, auxin and cytokinins while suppress the production of ethylene by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase which degrades the precursor of ethylene (ACC) into α-ketobutyrate and ammonia under stressed conditions (Glick *et al*., 2007; Santoyo *et al*., 2016). The PGPB improve stress tolerance by enhancing the production of osmolytes, antioxidant activity, carbohydrate metabolism and nutrient uptake and decreasing the ROS activity in plants (Dimkpa *et al*., 2009; Chakraborty *et al*., 2011). Moreover, they also produce osmolytes in response to stresses and thus enhance the stress tolerance (Dimkpa *et al*., 2009). Tabassum *et al*. (2018) also reported enhanced drought tolerance in barley by endophyte *enterobacter* spp. strain FD17.

Previous reports have shown that seed priming with Ca^{2+} salts and PGPB improve the stress tolerance in plants (Akhtar *et al*., 2015; Hussain *et al*., 2016, 2017; Farooq *et al*., 2017a). However, to best of knowledge, no report is available on physiological and biochemical basis of salinity tolerance induced by osmopriming with calcium salt or biopriming with endophytic bacteria *enterobacter* spp. strain FD17 in barley. We hypothesized that osmopriming or biopriming may improve the salinity tolerance by enhancing the osmolytes accumulation and improving water relations while decreasing lipid peroxidation and Na accumulation in barley. This study was conducted to evaluate the potential of different seed priming techniques in improving tolerance of barley genotypes to salt stress.

Materials and Methods

The pot study was carried out for consecutive two years to assess the influence of seed priming in improving salt stress tolerance in the Green house, Faculty of Agriculture, University of Agriculture Faisalabad. Soil samples were collected before filling pots and analyzed for physic-chemical properties. Experimental soil was sandy loam having pH 8.0, electrical conductivity 1.07 dS m^{-1}, nitrogen 0.058%, phosphorus 6.67 ppm, potassium 178 ppm and soil organic matter 0.95%. Seed of two barley genotypes (Frontier-87 and Haider-93) were used in this study. The seed was subjected to seed priming with water (hydropriming), 1.5% solution of $CaCl_2$ (osmopriming) and *Enterobacter* spp. strain FD17 (biopriming). Dry seed was taken as control. The seed priming treatments were selected based on our previous studies (Tabassum *et al*., 2017, 2018). The microbial culture was prepared according to Naveed *et al*. (2014). Culture was prepared in 50 mL TSA broth and incubated at 28 ± 2°C for 48 h in shaking incubator at 180 rev min^{-1}. Optical density of broth was adjusted to 0.5 at 600 nm absorbance to obtain bacterial population of 10^8-10^9 colony forming units mL^{-1}.

Seeds were soaked in desired aerated solution (1:5 seed to solution ratio) for 12 h to carry out seed priming. Aquarium pump was used to provide aeration. Afterwards, seed was removed from solution, rinsed and dried under shade until original weight was achieved. Fifteen dry or primed seeds were sown in pots (30 cm diameter and 45 cm depth having 15 kg soil) on November 06, 2014 and November 11, 2015. After uniformity of emergence, thinning was done to maintain six plants per pot and salinity was induced at different levels *i.e*., control (50 m*M* NaCl), moderate salinity (100 m*M* NaCl) and severe salinity (150 m*M* NaCl) according to Mazhar *et al*. (2016). The experiment was conducted by using completely randomized design with factorial arrangement and four replications. Fertilizers were applied at the rate of 25-18-13 mg NPK per kg soil using urea (46% N), di-ammonium phosphate (18% N, 46% P_2O_5) and sulfate of potash (50% K_2O). Half of the N and whole of the P and K were applied at sowing while remaining half of N was applied at tillering stage. The crop was harvested at maturity on April 08, 2015 and April 13, 2016. The meteorological data during experimental periods (2014–2015 and 2015–2016) is presented in Table 1.

Measurements

Growth and yield attributes: Growth and yield attributes were measured from three selected plants from each pot. At maturity, plant height was measured with a ruler from base to tip of plants. Leaf area was recorded with leaf area meter after separating the leaves of one plant from each pot. Productive tillers per pot were counted from each pot. Grains were separated from spikes after harvesting the plants and grains were counted and weighed to record the number of grains per spike, 100 grain weight and the grain yield per pot. Above ground dry biomass was recorded to determine the biological yield. Harvest index was computed as the ratio of grain yield to biological yield and was expressed as percentage.

Biochemical Attributes

Flag leaves samples were collected at booting stage (75 DAS) from two plants per pot including the one used for determination of leaf area for biochemical analyses. Chlorophyll *a* and *b* contents were determined according to Arnon (1949) by soaking the fresh leaves in acetone overnight. Total soluble phenolics were determined by adding the Folin-Ciocalteu reagent and sodium carbonate in same acetone extract and expressed as gallic acid equivalents according to Ainsworth and Gillespie (2007). For determination of free leaf proline, fresh leaves were homogenized in acetic acid and sulfosalicylic acid. It was followed by addition of ninhydrin solution, incubation, cooling and addition of toluene. The toluene was aspirated, and proline was determined following Bates *et al.* (1973). Fresh leaves were ground in distilled water, potassium tri-iodide and HCl were added in filtrate and incubated at 4°C for 1 h to determine glycine betaine content. Then chilled water and 1,2-di-dichloroethane was added in mixture and glycine betaine content was determined according to Grieve and Grattan (1983).

Total soluble proteins were determined by extracting in phosphate buffer saline and calculated against standard curve of bovine serum albumin according to Bradford (1976). Leaf samples were homogenizing in thiobarbituric acid and MDA content was determined by following the Cakmak and Horst (1991). To determine CMS, fresh leaves samples were soaked in distilled water for 12 h at room temperature and electrical conductivity was measured. Afterwards, samples were heated in water bath in boiling water for 30 min, cooled and electrical conductivity of solution was measured again. The CMS was determined according to Blum and Ebercon (1981). Oven dried leaves samples were ground and digested in di-acid mixture on block digester to determine leaf Na and K contents. The filtrate was fed to flame photometer, and concentrations of Na and K were determined against standard curves according to Estefan *et al*. (2013).

Water Relation Attributes

Flag leaves were sampled at booting stage (75 DAS) from one selected plant from each pot for determination of water relation traits. Fresh leaves were weighed, soaked in distilled water for 4 h to determine saturated weight and dried to determine the dry weight. Relative water content was determined by using the method of Barrs and Weatherly (1962). Leaf water potential (ψ_p) was determined by using pressure chamber according to Scholander *et al*. (1964). Same leaf was frozen in freezer at 20°C for a week, thawed and cell sap was extracted to determine the osmotic potential (ψ_s) with an osmometer. Leaf pressure potential (ψ_p) was computed as a difference between ψ_w and ψ_s.

Statistical Analyses

The year effect was significant for studied parameters according to paired T test; therefore, data of both years was analyzed and presented separately. Analysis of data was carried out using Fisher's analysis of variance procedure and treatments' means were compared by using the least significant difference (LSD) test at 5% probability level (Steel *et al*., 1997).

Results

Plant Growth

Salinity hampered the plant growth of barley genotypes. Frontier-87 exhibited greater plant height and leaf area than Haider-93. However, seed priming improved the growth of both genotypes under normal and stressed conditions (Table 2). Under moderate salinity, biopriming of Frontier-87 and Haider-93 caused the greatest increase in plant height during first and second year, respectively. Leaf area was significantly improved by biopriming of Haider-93 and osmopriming of Frontier-87 during first and second year, respectively. However, under severe salinity, longest plants were recorded by osmopriming of Haider-93 and hydropriming of Frontier-87 during first and second year, respectively. Maximum increase in leaf area was caused by biopriming of Frontier-87 during both years (Table 2).

Yield and Yield Contributors

The yield and related traits of tested barley genotypes were declined by salt stress with increase in its severity. Haider-93 showed less reductions than Frontier-87. However, seed priming improved the yield and related traits of both genotypes (Table 3). Under moderate salinity, the highest productive tillers were produced by biopriming of Haider-93 and osmopriming of Frontier-87 during first and second year, respectively. Biopriming of Frontier-87 and Haider-93 recorded the greatest increase in number of grains per spike during first and second year, respectively. The 100 grain weight was improved by bio and osmopriming of Haider-93 during first and second year, respectively. Maximum increase in grain yield and harvest index was recorded by bio and osmopriming of Haider-93, respectively, during both years. Under severe salinity, number of grains per spike was enhanced the most by biopriming of Haider-93, while, number of productive tillers, 100 grain weight and grain yield was improved by osmopriming of Haider-93 during both years. However, osmopriming of Haider-93 produced similar number of grains during the second year. The greatest increase in harvest index was recorded by osmo and biopriming of Haider-93 during first and second year, respectively, while, biopriming of Haider-93 caused similar increase during the first year (Table 3).

Chlorophyll Contents

Salinity decreased the chlorophyll contents with more negative effects with increase in levels of salt stress. Greater reductions in chlorophyll *a* and *b* occurred in Frontier-87 and Haider-93, respectively.

Table 1: Weather data during the growing seasons of barley at the experimental site

Month	Total rainfall (mm)		Relative humidity (%)		Temperature (°C)						Sunshine (h)	
					Monthly maximum		Monthly minimum		Daily mean			
	2014–15	2015–16	2014–15	2015–16	2014–15	2015–16	2014–15	2015–16	2014–15	2015–16	2014–15	2015–16
November	10.0	8.8	61.7	61.5	26.3	27.1	11.5	12.1	18.9	19.6	7.6	6.6
December	0.0	0.0	75.0	62.6	18.5	21.8	5.9	7.2	12.2	14.5	4.7	7.0
January	12.2	13.1	75.3	74.4	16.6	17.3	6.9	7.7	11.7	12.5	5.0	3.5
February	20.5	7.8	66.0	58.1	22.0	23.3	11.1	9.3	16.5	16.3	5.6	8.5
March	67.9	66.7	64.0	59.7	24.5	26.8	13.6	15.6	19.1	21.2	4.9	6.6
April	32.8	5.6	43.9	34.2	33.2	34.3	20.7	20.2	27.0	27.2	9.1	8.3

All the values of mean temperature, relative humidity and sunshine shown in table are the monthly averages, while rainfall values are the total rainfall received during that month; Monthly maximum and monthly minimum are the highest and lowest temperature observed during that month at any day

Table 2: Effect of seed priming on the growth of barley genotypes under different levels of salinity

Treatments	2014–2015						2015–2016					
	Control		Moderate salinity		Severe salinity		Control		Moderate salinity		Severe salinity	
	H-93	F-87	H-93	F-87	H-93	F-87	H-93	F-87	H-93	F-87	H-93	F-87
Plant height (cm)												
Control	65.1 fg	77.8 c	58.9 j-m	62.6 ghi	52.4 n	58.6 lm	71.2 e	76.5 bc	69.9 e	65.2 f	44.4 i	58.4 g
Hydropriming	73.1 d	78.8 bc	61.8 hij	67.3 ef	57.6 m	59.5 j-m	72.6 de	79.2 ab	65.0 f	64.8 f	51.1 h	64.5 f
Osmopriming	81.0 ab	80.8 ab	61.4 i-l	64.8 fgh	63.7 ghi	61.1 i-l	75.2 cd	82.4 a	70.3 e	69.7 e	51.1 h	56.8 g
Biopriming	72.8 d	82.4 a	61.6 ijk	70.1 de	58.7 klm	63.1 ghi	70.7 e	77.9 bc	65.2 f	70.1 e	62.5 f	61.8 f
LSD value ($p \leq 0.05$)	3.03						3.47					
Leaf area (cm^2)												
Control	497 cd	580 b	439 fgh	427 gh	292 m	312 lm	495 efg	519 def	411.3 j	425.6 ij	324.8 m	314 m
Hydropriming	512 cd	585 b	412 hi	444 fgh	324 l	340 kl	538 cd	524 cde	451.8 hi	440.6 hij	321.7 m	341 lm
Osmopriming	507 cd	633 a	464 ef	454 efg	387 ij	363 jk	552 c	534 cd	492.8 fg	513.4 def	332.1 m	365 kl
Biopriming	521 c	645 a	482 de	432 fgh	331 kl	433 fgh	585 c	637 a	468.5 gh	439.5 hij	380.6 k	445 hi
LSD value ($p \leq 0.05$)	32.3						29.30					

Means with similar letter, for a parameter during a year, don’t differ significantly at $p \leq 0.05$. H-93: Haider-93, F-87: Frontier-87

Table 3: Effect of seed priming on yield and related traits of barley genotypes under different levels of salinity

Treatments	2014–2015						2015–2016					
	Control		Moderate salinity		Severe salinity		Control		Moderate salinity		Severe salinity	
	H-93	F-87	H-93	F-87	H-93	F-87	H-93	F-87	H-93	F-87	H-93	F-87
Productive tillers per pot												
Control	10.29 de	10.31 de	9.42 fg	9.07 gh	7.67 kl	7.52 l	10.59 cd	10.62 cd	9.52 fg	9.53 fg	8.43 i	7.81 j
Hydropriming	10.78 cd	10.75 cd	9.88 ef	10.29 de	8.61 hij	8.12 jk	11.26 ab	11.08 abc	9.98 ef	10.80 bc	8.95 h	8.45 i
Osmopriming	10.45 cd	10.89 bc	10.53 cd	10.95 bc	9.25 g	8.31 ij	9.69 fg	11.23 ab	10.22 de	11.06 abc	9.49 g	8.72 hi
Biopriming	11.47 a	11.33 ab	11.46 a	10.51 cd	8.25 ij	8.73 hi	11.51 a	10.98 bc	10.79 bc	10.62 cd	8.67 hi	8.73 hi
LSD ($p \leq 0.05$)	0.50						0.49					
Grains per spike												
Control	34.5 c	32.9 d	30.3 fg	27.5 ij	24.6 lm	23.9 m	32.8 d	31.0 ef	28.6 gh	27.0 h	21.5 l	21.4 l
Hydropriming	35.8 bc	32.9 d	30.8 ef	30.9 ef	27.2 ij	26.1 jk	33.6 cd	32.3 de	31.0 ef	25.8 ij	22.6 kl	23.3 k
Osmopriming	37.7 a	35.9 bc	32.1 de	31.4 ef	25.4 kl	28.0 hi	35.5 b	34.6 bc	31.0 ef	28.6 gh	25.5 ij	22.9 kl
Biopriming	38.1 a	36.9 ab	31.8 de	32.2 de	29.0 gh	26.2 jk	37.4 a	33.0 d	32.2 de	29.8 fg	25.5 ij	25.2 j
LSD ($p \leq 0.05$)	1.44						1.55					
100-grain weight (g)												
Control	3.21 cd	3.06 ef	2.82 h	2.67 i	2.38 j	2.19 k	3.46 c	3.50 c	2.92 fg	3.01 ef	2.47 ijk	2.33 kl
Hydropriming	3.42 b	3.02 efg	2.97 fg	2.68 i	2.83 h	2.44 j	3.60 c	3.88 b	2.77 gh	3.18 d	2.45 jk	2.23 l
Osmopriming	3.49 b	3.69 a	3.12 de	3.08 ef	2.90 gh	2.49 j	3.80 b	4.35 a	3.26 d	3.18 d	2.72 h	2.55 ij
Biopriming	3.81 a	3.45 b	3.28 c	2.96 fg	2.67 i	2.65 i	3.92 b	4.19 a	3.15 de	3.16 de	2.63 hi	2.55 ij
LSD ($p \leq 0.05$)	0.13						0.17					
Grain yield (g pot^{-1})												
Control	8.53 e	8.65 e	7.05 g	6.83 g	5.13 jk	4.28 l	8.34 e	8.00 ef	6.47 ij	6.19 j	4.09 m	3.63 n
Hydropriming	9.62 c	8.68 e	8.05 f	7.18 g	5.88 hi	5.01 k	8.85 d	9.33 c	6.75 hi	6.74 hi	4.27 lm	3.97 mn
Osmopriming	9.72 c	10.46 b	8.82 de	8.93 de	6.09 h	5.33 jk	9.28 c	10.62 a	7.36 g	6.88 hi	4.85 k	4.23 m
Biopriming	11.73 a	9.75 c	9.28 cd	8.63 e	5.88 hi	5.55 ij	10.62 a	9.85 b	7.91 f	7.09 gh	4.67 kl	4.24 m
LSD ($p \leq 0.05$)	0.48						0.42					
Harvest index (%)												
Control	34.7 cd	32.5 ef	30.3 gh	28.9 hi	27.9 ij	25.8 k	35.1 bc	32.2 ef	30.2 ghi	28.9 hij	26.2 kl	25.4 l
Hydropriming	38.4 ab	30.3 gh	32.5 ef	27.0 jk	29.2 hi	27.9 ij	35.7 bc	36.6 b	33.0 de	27.2 jk	25.2 l	26.6 kl
Osmopriming	37.4 b	37.7 ab	34.0 cde	31.5 fg	30.2 gh	27.8 ij	36.6 b	38.5 a	34.4 cd	30.6 fgh	28.3 j	27.3 jk
Biopriming	39.6 a	35.4 c	33.5 de	32.4 ef	30.2 gh	27.4 ijk	38.8 a	36.2 b	33.1 de	31.7 efg	28.8 ij	26.1 kl
LSD ($p \leq 0.05$)	1.95						1.71					

Means with similar letter, for a parameter during a year, don’t differ significantly at $p \leq 0.05$. H-93: Haider-93, F-87: Frontier-87

However, seed priming improved the chlorophyll contents in both tested genotypes under salinity (Fig. 1). Chlorophyll *a* content was enhanced by biopriming of Frontier-87 and hydropriming of Haider-93 under moderate salinity during first and second year, respectively. However, highest increase in chlorophyll *b* was recorded by biopriming of Haider-93 during both years. Under severe salinity, osmopriming of Haider-93 and biopriming of Frontier-87 showed maximum increase in chlorophyll *a* content. Chlorophyll *b* content was enhanced by biopriming of Haider-93 during both years, while, osmopriming of Haider-93 and Frontier-87 showed the similar trend (Fig. 1).

Osmolytes Accumulation

Accumulation of osmolytes was enhanced by salt stress in both barley genotypes. Osmolytes accumulation was increased with salinity levels (severe salinity > moderate salinity > control). The genotype Haider-93 accumulated more osmolytes than Frontier-87. Seed priming elevated osmolytes accumulation in both genotypes under salinity (Fig. 2 and 3). Under moderate stress, osmopriming of Haider-93 recorded the greatest total soluble phenolics and proteins during the first year, and glycine betaine content during both years; while, biopriming of Haider-93 enhanced the total soluble phenolics and proteins during the second year. Nonetheless, biopriming of Frontier-87 produced similar total soluble proteins during the second year. Free proline content was increased by biopriming of Frontier-87 and hydropriming of Haider-93 during first and second year, respectively. Under severe salinity, osmopriming of Frontier-87 and Haider-93 exalted phenolics content; while, bio and hydropriming of Haider-93 enhanced total soluble proteins during first and second year, respectively. However, maximum increase in proline and glycine betaine content was recorded by osmopriming of Haider-93 during first and second year, respectively, and biopriming of Haider-93 during second and first year, respectively (Fig. 2–3).

Lipid Peroxidation

Accumulation of MDA in leaves was exaggerated while CMS was decreased by salinity with deleterious effects increasing with its severity. However, Haider-93 accumulated less leaf MDA and had better CMS than Frontier-87. Seed priming treatments decreased the lipid peroxidation and improved the CMS in tested genotypes under salinity (Fig. 4). Osmo and biopriming of Haider-93 caused the greatest reduction in leaf MDA accumulation and improvement in CMS under moderate salinity. Nonetheless, under severe salinity, osmopriming of Haider-93 reduced the leaf MDA most during both years, while, biopriming of Haider-93 caused similar decrease during the second year. Maximum improvement in CMS was recorded by bio and osmopriming of Haider-93 as well as hydro and biopriming of Frontier-87 during the first year; while, hydropriming of Frontier-87 during the second year (Fig. 4).

Mineral Contents

There was an increase in Na and decrease in K content with increase in salinity levels (severe salinity > moderate salinity > control). Haider-93 accumulated less Na and more K than Frontier-87 under salinity. Seed priming effectively decreased the Na and increased Na accumulation in barley genotypes under salt stress (Fig. 5). Biopriming of Frontier-87 and osmopriming of Haider-93 caused the highest decrease in Na content; while, hydro and osmopriming of Haider-93 enhanced the K content during first and second year, respectively, under moderate salinity. Under severe salinity, osmopriming of Haider-93 recorded the greatest reduction in Na content during both years and increase in K content during the first year. However, during hesecond year, the maximum increase in K content was caused by hydropriming of Haider-93 (Fig. 5).

Water Relations

The water relation traits of barley genotypes were negatively affected by salt stress. The deleterious effects of salinity increased with severity. The negative effects of salinity on water relation traits were more prominent on Frotier-87 than Haider-93. Seed priming improved the relative water content, and water, osmotic and pressure potential of tested genotypes under salinity (Fig. 6 and 7). Under moderate salinity, highest leaf relative water content was recorded by biopriming of Frontier-87 as well as osmopriming of Haider-93 during the first year, while, bio and osmopriming of Haider-93 as well as osmopriming of Frontier-87 during the second year. Hydropriming of Haider-93 caused the highest improvement in water potential during the first year; whereas, osmopriming of Haider-93 improved the water potential during the second year, osmotic potential during both years and pressure potential during the first year. Under severe salinity, osmopriming of Haider-93 improved relative water content the most during both years and water potential during the first year. However, biopriming of Haider-93 recorded maximum increase in water potential during the second year and osmotic potential during both years. Pressure potential was enhanced the most by hydropriming of Haider-93 during the first year (Fig. 6 and 7).

Discussion

Salt stress hampered the barley growth and yield formation (Table 2 and 3). However, seed priming improved the performance of tested barley genotypes as indicated by enhanced growth (Table 2), chlorophyll *a* and *b* contents (Fig. 1), water relations (Fig. 6 and 7), and accumulation of phenolics, total soluble proteins, free proline and glycine betaine (Fig. 2 and 3), while decreased lipid peroxidation (Fig. 4) and Na accumulation (Fig. 5) which resulted in improved yield and related traits (Table 3) under salinity.

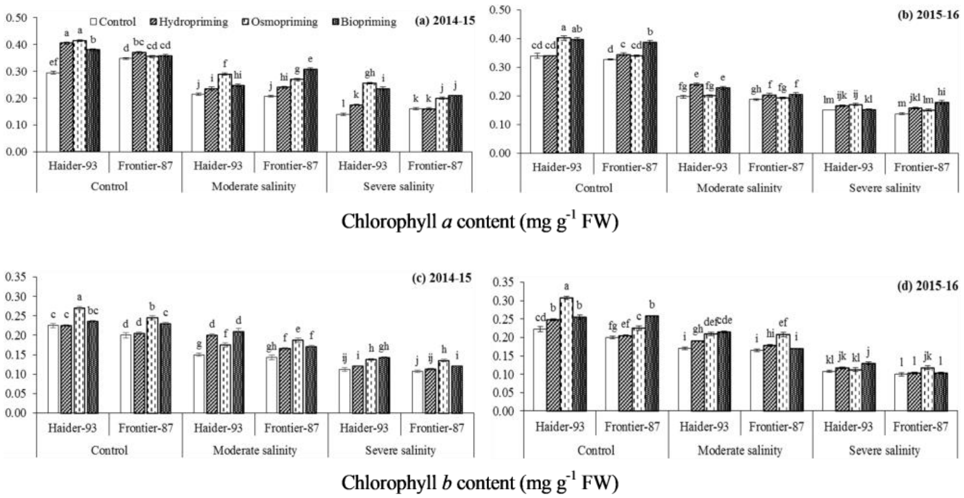

Fig. 1: Influence of seed priming on a) chlorophyll *a* (2014-15), b) chlorophyll *a* (2015-16), c) chlorophyll *b* (2014-15) and d) chlorophyll *b* (2015-16) contents of barley under salinity. Each bar is mean ± SE of four replications. Bars with similar letter, for a parameter during a year, don't differ significantly at $p \leq 0.05$

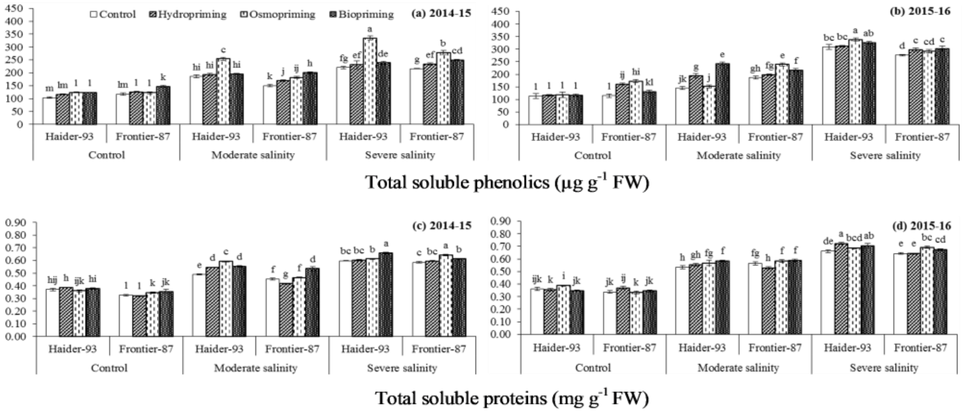

Fig. 2: Influence of seed priming on (a and b) total soluble phenloics and (c and d) total soluble proteins contents of barley under salinity. Each bar is mean ± SE of four replications. Bars with similar letter, for a parameter during a year, don't differ significantly at $p \leq 0.05$

Seed priming enhances stress tolerance by increased accumulation of osmolytes, stress proteins and antioxidants activity through up regulation of related genes under stressed conditions (Chen and Arora, 2013). The enhanced accumulation of osmolytes improves the tissue water status through osmotic adjustment (Tabassum *et al*., 2017, 2018) and decreases lipid peroxidation by scavenging the ROS (Anjum *et al*., 2017). In present study, the improved stress tolerance by osmopriming was associated with enhanced accumulation of osmolytes (Fig. 2 and 3) which improved water relations (Fig. 6 and 7) and enhanced the chlorophyll contents (Fig. 1) by better protection from ROS which was visible from better CMS and decreased leaf MDA content (Fig. 4) ultimately resulting in improved growth, yield and harvest index (Tables 2 and 3) under salinity. Enhanced salinity tolerance by osmopriming might be due to Ca^{2+} which is involved in regulation of calmodulin like proteins, and trigger various growth mechanisms and protect plants from stresses (Sarwat *et al*., 2013). Moreover, Ca^{2+} acts as a secondary messenger which enhances gene expression for osmolytes and enhances stress tolerance (White and Broadley, 2003).

In present study, seed biopriming with endophytic bacteria *Enterobacter* sp. strain FD17 enhanced stress tolerance of barley genotypes, which was manifested by better growth and yield under salinity (Table 2 and 3). The improved performance of barley by biopriming under salinity is attributed to enhanced accumulation of osmolytes (Fig. 2 and 3) which resulted in improved tissue water status (Fig. 6 and 7), CMS (Fig. 4) and chlorophyll contents (Fig. 1) whereby decreased the lipid peroxidation (Fig. 4). Endophytic bacteria enhance gene expression for

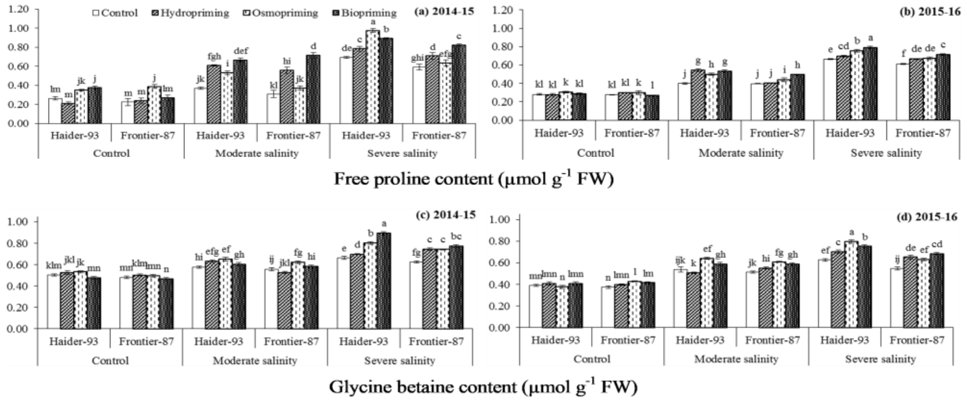

Fig. 3: Influence of seed priming on (a and b) free proline and (c and d) glycine betaine contents of barley under salinity. Each bar is mean ± SE of four replications. Bars with similar letter, for a parameter during a year, don't differ significantly at $p \leq 0.05$

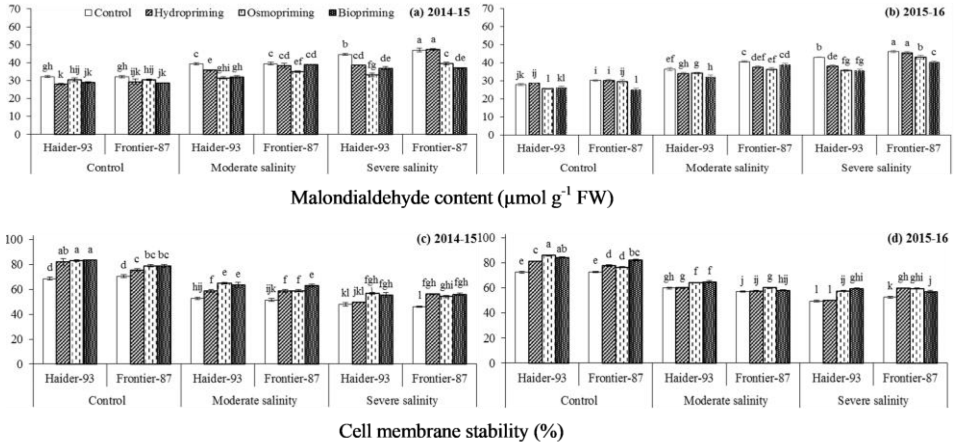

Fig. 4: Influence of seed priming on (a and b) malondialdehyde content and (c and d) cell membrane stability of barley under salinity. Each bar is mean ± SE of four replications. Bars with similar letter, for a parameter during a year, don't differ significantly at $p \leq 0.05$

accumulation of osmolytes by rapid and greater accumulation of transcription factors in plants under stressed conditions (Theocharis *et al*., 2012; Miotto-Vilanova *et al*., 2016). Moreover, endophytic bacteria also produce osmolytes under stress which act synergistically with plant produced osmolytes (Dimkpa *et al*., 2009). The increased accumulation of osmolytes due to enhanced gene expression decreases lipid peroxidation while improves CMS and tissue water status of plants under stressed conditions (Dimkpa *et al*., 2009; Sarwar *et al*., 2017). Endophytic bacteria enhanced root growth by increasing auxin while decreasing the ethylene production (Santoyo *et al*., 2016; Vurukonda *et al*., 2016) resulting in improved water uptake and tissue water status in plants thus improving stress tolerance.

Seed priming treatments decreased the lipid peroxidation and had better CMS (Fig. 4) which is attributed to enhanced accumulation of phenolics, total soluble proteins, and proline and glycine betaine (Fig. 2 and 3) in barley genotypes under salinity. Phenolic compounds protect the membranes by scavenging the ROS and sometimes outcompete the enzymatic antioxidants (Sharma *et al*., 2012). Soluble proteins hydrate and repair the membranes and protect from damage caused by ROS (Wahid *et al*., 2007). Likewise, proline and glycine betaine increase under stresses and protect the membranes by and scavenging and quenching the ROS (Niu *et al*., 2016; Anjum *et al*., 2016, 2017). In present study, seed primed plants exhibited less Na and more K content than unprimed plants under salinity (Fig. 5). In osmoprimed plants, less Na

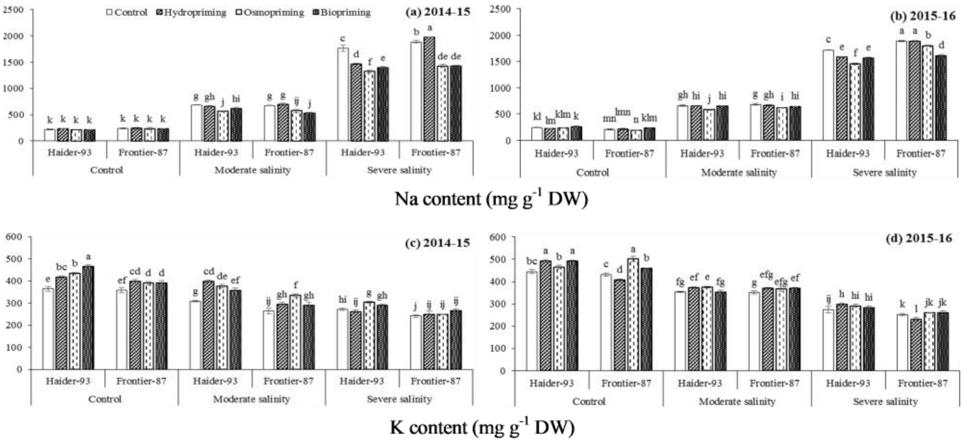

Fig. 5: Influence of seed priming on (a and b) Na content and (c and d) K content of barley under salinity. Each bar is mean ± SE of four replications. Bars don't sharing same letter differ significantly at $p \leq 0.05$. Bars with similar letter, for a parameter during a year, don't differ significantly at $p \leq 0.05$

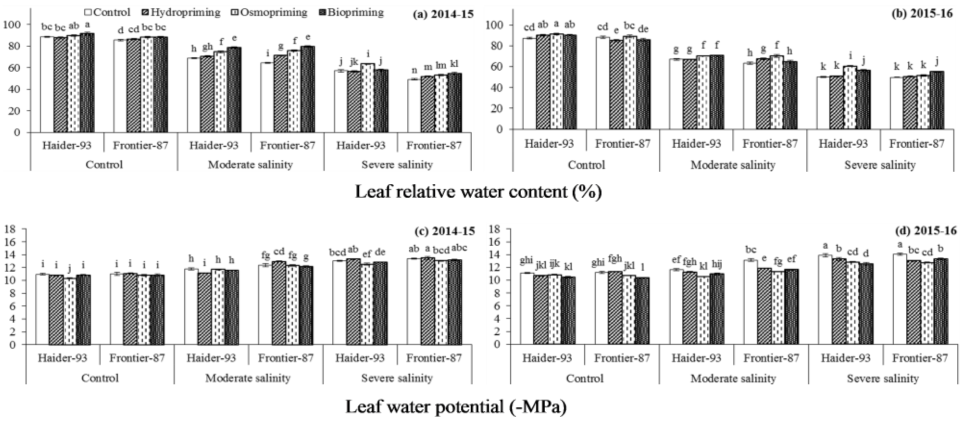

Fig. 6: Influence of seed priming on (a and b) leaf relative water content and (c and d) leaf water potential of barley under salinity. Each bar is mean ± SE of four replications. Bars with similar letter, for a parameter during a year, don't differ significantly at $p \leq 0.05$

and more K accumulation might be due to enhanced gene expression for salt overly sensitive 1 (*SOS* 1) antiporter and high affinity potassium transporter protein 1 (*HAK1*) (Souza *et al*., 2016). Furthermore, extracellular Ca^{2+} decreases influx of Na (Rathod and Anand, 2016) and efflux of K by non-selective cation channels (Shabala *et al*., 2006). However, in bioprimed plants, less Na accumulation might be due to excretion of exopolysaccharides by endophytic bacteria which bind Na and decrease its uptake under salinity (Ashraf *et al*., 2004). They enhance the uptake of K which increases K : Na ratio. Additionally, they decrease the apoplastic flow of Na^{+} into stele by greater covering of root zone with soil sheath (Dodd and Pérez-Alfocea, 2012).

Salinity decreased the growth and yield formation of both tested genotypes of barley (Table 2 and 3). However, Haider-93 performed better than Frontier-87 under each level of salt stress which indicated that Haider-93 was relatively tolerant (Table 2 and 3). Better growth and yield of Hiader-93 was associated with greater accumulation of phenolics, total soluble proteins, and proline and glycine betaine (Fig. 2 and 3) and less Na accumulation (Fig. 5) which resulted in higher chlorophyll contents (Fig. 1), CMS (Fig. 4), water relations (Fig. 6 and 7) and less leaf MDA content (Fig. 4) than Frontier-87. Plants accumulate osmolytes in greater amounts to cope with stressed conditions and confer stress tolerance (Song *et al*., 2017). Anjum *et al*. (2017) observed that tolerant genotypes accumulated more osmolytes than sensitive ones.

Yield and yield related attributes of tested genotypes of barley were hampered by salt stress and the negative effects of salinity increased with its levels (Table 3). Haider-93 recorded more grain yield and harvest index than

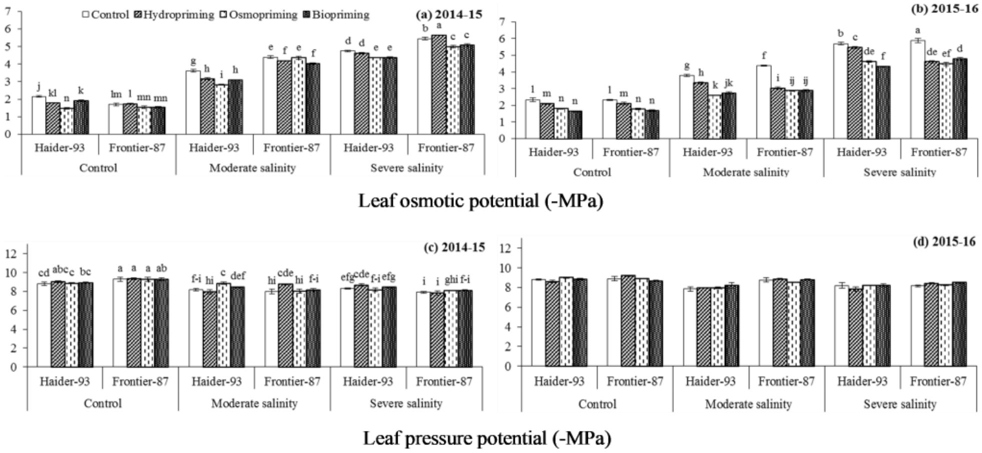

Fig. 7: Influence of seed priming on (a and b) leaf osmotic potential and (c and d) leaf pressure potential of barley under salinity. Each bar is mean ± SE of four replications. Bars with similar letter, for a parameter during a year, don't differ significantly at $p \leq 0.05$

Frontier-87 which is attributed to higher number of productive tillers, number of grains and grain weight under each level of stress (Table 3). However, seed priming further improved the yield and related traits of tested barley genotypes (Table 3). In present study, the improved water relations (Fig. 6 and 7), chlorophyll contents (Fig. 1) and CMS (Fig. 4) might have improved the photosynthesis, assimilate translocation and pollen viability (Arshad *et al.*, 2017) which might have improved the grain yield and harvest index of barley genotypes under salt stress (Table 3).

Conclusion

Salinity decreased the growth and yield of barley by perturbing water relations and decreasing chlorophyll contents while increasing leaf MDA and Na accumulation. However, seed priming improved the growth and yield of barley genotypes by enhancing accumulation of osmolytes, improving water relation trains and CMS whereby decreasing lipid peroxidation and Na accumulation under salinity. The order of improvement in yield and yield contributors under severe salinity was osmopriming > biopriming > hydropriming.

Acknowledgements

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