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Full Length Article

Improving the Drought Tolerance in Barley by Osmopriming and Biopriming

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Abstract

Determination of physiological and biochemical changes induced by drought stress can be helpful for induction of stress tolerance in plants. This 2-years study was conducted to determine the potential of different seed priming techniques in improving tolerance of barley against drought stress. Seeds of two barley genotypes (Haider-93 and Frontier-87) were primed with aerated water (hydropriming), and solutions of $CaCl_2$ (1.5%; osmopriming) or *Enterobacter* spp. strain FD17 (biopriming) for 12 h keeping seed to solution ratio of 1:5 (w/v) while non-primed (dry) seeds were taken as control. Primed and non-primed seeds were sown in soil-filled earthen pots. After uniformity of seedling establishment, soil moisture was maintained at 80 (well-watered), 60 (moderate drought) and 40% (severe drought) of water holding capacity. Drought stress decreased plant growth, yield, chlorophyll contents, water relations and grain nutrient contents, while increased osmolytes accumulation and lipid peroxidation in both genotypes. Seed priming, biopriming in particular, improved leaf area, grain yield, chlorophyll contents, accumulation of phenolics, proline, glycine betaine, total soluble proteins, relative water contents, water, osmotic and pressure potentials, cell membrane stability, and grain Zn, Mn and B contents, while decreased malondialdehyde contents in both genotypes under drought stress. Improvement in yield and related traits by seed priming under drought stress was in the order biopriming>osmopriming>hydropriming. However, seed priming induced tolerance was more prominent under moderate drought than severe drought stress. Performance of genotype Haider-93 was quite better than Frontier-87. In conclusion, seed priming induced increase in osmolytes accumulation enhanced drought tolerance in barley by improving water relations and decreasing lipid peroxidation. Thus, seed priming, mainly biopriming with *Enterobacter* spp. strain FD17, may be utilized to improve barley performance under drought stress.

Keywords: Barley; Drought tolerance; Lipid peroxidation; Osmolytes accumulation; Seed priming; Water relations

Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop grown for human consumption and animal feed (Alazmani, 2015). It grows well on both normal as well as marginal lands (Chapagain and Good, 2015). Although barley is quite tolerant to drought stress due to expression of barley abundant protein (*HVA1*) gene (Nguyen and Sticklen, 2013), its productivity is hampered by drought stress during the growth period (Samarah, 2005). Drought stress damages photosynthetic machinery, decreases photosynthetic rate, grain filling duration, grain number and weight, and ultimately hampers grain yield in barley regardless of its severity (Samarah, 2005; Ghotbi-Ravandi *et al*., 2014). Furthermore, plants exposed to drought stress face oxidative burst due to over production of reactive oxygen species (ROS), which causes exaggerated lipid peroxidation of biological membranes, denaturation of proteins and damage nucleic acid, eventually disturbing homeostasis (Farooq *et al*., 2009; Hussain *et al*., 2018).

Plants usually respond to drought stress via modifications in various morphological and physiological processes. For instance, plants accumulate compatible solutes in response to drought stress which are low molecular weight, highly soluble and non-toxic compounds (Farooq *et al*., 2009). Compatible solutes such as proline, glycine betaine, soluble proteins and phenolic compounds are accumulated in plants in response to drought stress, which improve tissue water status through osmotic adjustments, protect macromolecules, stabilize cellular membranes from lipid peroxidation and detoxify ROS (Anjum *et al*., 2017; Fahad *et al*., 2017). Accumulation of these compatible solutes in greater quantities may help plants to attain stress tolerance (Farooq *et al*., 2017a; Song *et al*., 2017).

Seed priming is a controlled seed hydration technique which allows the occurrence of germination metabolism without actual germination (Farooq *et al*., 2006). It has been the most pragmatic technique to improve the crop performance under sub-optimal

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conditions (Hussain *et al*., 2017; Tabassum *et al*., 2017). Seed priming increases the production and accumulation of osmolytes under stressed conditions by altering metabolic processes (Delavari *et al*., 2010). Solutions containing various inorganic and organic salts, plant growth regulating substances and plant growth promoting bacteria may be used in seed priming to control seed hydration (Jafar *et al*., 2012; Hussain *et al*., 2016; Mahmood *et al*., 2016; Farooq *et al*., 2017b). Osmopriming enhances the accumulation of transcription factors and metabolites by inducing osmotic stress that trigger the gene expression for osmolytes, heat shock proteins and antioxidants activity (Kibinza *et al*., 2011; Chen and Arora, 2013). Seed priming with $CaCl_2$ improved the growth and yield of cereals under drought stress at various growth stages (Farooq *et al*., 2015; Khan *et al*., 2015; Hussain *et al*., 2016). Kaczmarek *et al*. (2017) reported improved photosynthesis and seedling growth of barley by osmopriming with $CaCl_2$ under drought stress.

Plant growth promoting bacteria induce several natural processes to maintain the growth and physiological functions of plants under stressed environments (Yang *et al*., 2008). These bacteria improve plant growth by enhancing the production of endogenous growth promoting hormones *viz*. auxin, gibberellic acid and cytokinins and decreasing the levels of ethylene by producing ACC deaminase under normal and stressed conditions (Santoyo *et al*., 2016). Moreover, they enhance the production and accumulation of compatible solutes or osmolytes in plants which decrease the oxidative stress by reducing lipid peroxidation and ROS activity (Vardharajula *et al*., 2011). The bacteria also produce osmolytes that act synergistically with plant produced osmolytes under stressed conditions and improve plant growth (Dimkpa *et al*., 2009). Akhtar *et al*. (2015) reported improved maize performance by endophytic bacteria *Enterobacter* spp. strain FD17 under stressed conditions.

Several reports indicate that osmopriming with $CaCl_2$ and seed priming with plant growth promoting bacteria improve drought tolerance of different crop plants. However, to best of our knowledge, the physiological and biochemical basis of drought tolerance induced by osmopriming with $CaCl_2$ or endophytic bacteria *Enterobacter* spp. strain FD17 in barley has never been explored. We hypothesized that osmopriming with $CaCl_2$ or biopriming with endophytic bacteria *Enterobacter* spp. strain FD17 will improve the drought tolerance in barley by improving the solute accumulation and water relations while decreasing the lipid peroxidation. Therefore, to test this hypothesis a pot study was conducted involving two genotypes, three seed priming treatments and drought stress at three severity levels.

Materials and Methods

Experimental Details

This 2-years pot study was conducted to evaluate the potential of seed priming in improving drought tolerance at various levels and unravel the biochemical/physiological basis of stress tolerance. The study was done in greenhouse at Faculty of Agriculture, University of Agriculture Faisalabad. Experimental soil was sandy loam with pH (8.0 and 7.9), electrical conductivity (1.07 and 1.11 dS m^{-1}), nitrogen (0.06 and 0.057%), phosphorus (6.90 and 6.57 ppm), potassium (176 and 180 ppm) and soil organic matter (0.97 and 0.93%) during both years (2014–15 and 2015–16), respectively. Seed of two barley genotypes, i.e., Haider-93 and Frontier-87 were hydroprimed (water), osmoprimed (1.5% (w/v) solution of $CaCl_2$) or bioprimed (*Enterobacter* sp. strain FD17). The *Enterobacter* spp. strain FD17 was selected because of its ability to colonize and improve the plant performance under normal and stressed conditions (Akhtar *et al*., 2015). The culture for biopriming was prepared following Naveed *et al*. (2014). The inoculant was prepared in 50 mL trypticase soy agar or tryptone soya agar (TSA) broth in 100 mL Erlenmeyer flasks and incubated at 28±2°C for 48 h in the orbital shaking incubator at 180 rev min^{-1}. The optical density of the broth was adjusted to 0.5 measured at 600 nm using spectrophotometer to obtain a uniform population of bacteria (10^8–10^9 colony-forming units (CFU) mL^{-1}) in the broth. In all the cases, the seed priming was done by soaking the seed for 12 h in aerated water and/or solution keeping seed to solution ratio of 1:5 (w/v). Aeration was provided by aquarium pump. After removing from the relevant solution, seeds were thoroughly rinsed with tap water and dried in forced air under shade till original weight.

The non-primed (control) and primed seeds were sown in pots (15 seeds per pot of 30 cm diameter and 45 cm depth containing 15 kg soil) on 1st November during 2014 and 2015. After seedling establishment, six plants were maintained per pot, and drought stress was imposed at different levels *viz*. well-watered, moderate drought and severe drought maintained at 80, 60 and 40% water holding capacity. The desired levels of moisture were attained by determining the required amount of water, then weighing the pots after application of calculated amount of water and designating it as target weight. Drought was imposed by attaining the target pot weight with the application of water on every alternate day. The experiment was laid out in 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), diammonium phosphate (18% N, 46% P_2O_5) and sulfate of potash (50% K_2O). The N was applied in two splits (sowing and tillering), while P and K were applied once at sowing. Crop was harvested on April 5, 2015 and April 2, 2016 at harvest maturity. The weather data during the period of experiment during both years is given in Table 1.

Observations and Measurements

Morphological and yield traits: Three plants were randomly selected from each replication for the

determination of morphological traits. Height of selected plants was measured at maturity from soil surface to tip and averaged. Leaves of one plant from each replication were detached and their area was measured using digital leaf area meter (JVC TK-5310). At maturity, number of productive tillers was counted from selected plants. Plants were harvested and weighed to record biological yield. Grains were separated from spikes, numbers of grains per spike were counted and 100 grain weight was recorded by using electronic weighing balance (sensitivity 0.0001 g). The grains separated from three plants were weighed to determine the grain yield and expressed on per plant basis. Harvest index was calculated as the ratio of grain yield to total biological yield and expressed in percentage.

Water Relation Traits

One plant was selected from each pot for the determination of water relations traits. Flag leaf samples were collected from the selected plants at booting stage (75 DAS) for the measurement of water relation traits. Relative water contents (RWC) were determined by weighing and soaking the fresh leaves in deionized water for 4 h to record saturated weight followed by drying. The RWC were calculated following the method of Barrs and Weatherly (1962). Leaf water potential (ψ_p) (-MPa) was measured with pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) following the method described by Scholander *et al*. (1964). The same leaf, used for determination of water potential, was frozen below -20°C for seven days. The frozen leaf material was thawed, cell sap was extracted and osmotic potential (ψ_s) (-MPa) was measured using an osmometer (Digital Osmometer, Wescor, Logan, UT, USA). Leaf pressure potential (ψ_p) (MPa) was calculated as a difference between ψ_w and ψ_s.

Biochemical and Mineral Analyses

Two plants, including the one used for determination of leaf area, were selected from each pot for biochemical analyses. Flag leaves were collected at booting stage (75 DAS) for biochemical analyses. Fresh leaf samples were soaked in acetone overnight and chlorophyll *a* and *b* were determined by following the method of Arnon (1949). For total soluble phenolics, Folin-Ciocalteu reagent and sodium carbonate was added in the same acetone extract used for chlorophyll determination. Total soluble phenolics were expressed as gallic acid equivalents (Ainsworth and Gillespie, 2007). Free leaf proline content was estimated by homogenizing the fresh leaf samples in sulfosalicylic acid and glacial acetic acid, Ninhydrin solution was added to the filtrate, incubated and cooled in ice bath, and toluene was added and vortexed. The chromophore containing toluene was aspirated from the aqueous phase and proline was determined according to the method of Bates *et al.* (1973). For the determination of glycine betaine, fresh leaf samples were ground in distilled water, filtered and potassium triiodide and HCl was added. The filtrate was incubated at 4°C for 1 h and chilled. Water and 1,2-dichloroethane was added in the cooled mixture. The mixture was vortexed and concentration of glycine betaine was calculated against the standard curve as described by Grieve and Grattan (1983).

Total soluble proteins were extracted in phosphate buffer saline and standard curve was prepared by using bovine serum albumin. The concentration of total soluble proteins was calculated against the standard curve as described by Bradford (1976). For malondialdehyde (MDA), leaf tissues were homogenized in thiobarbituric acid and MDA concentration was determined according to Cakmak and Horst (1991). Cell membrane stability (CMS) was determined by soaking the fresh leaf samples in distilled water for 12 h at room temperature. Electrical conductivity of solution was measured with a conductivity meter (Model DDS-11A; Shanghai Leici Inc., Shanghai, China). Samples were heated in boiling water for 30 min, cooled at room temperature, and electrical conductivity of solution was measured. The CMS was expressed in percentage following Blum and Ebercon (1981).

Seed samples were ground, soaked overnight in diacid mixture and digested on block digester. The digested samples were fed to an atomic absorption spectrometer and concentrations of Zn and Mn was determined according to Estefan *et al*. (2013). Boron was determined by dry ashing the ground seed material in muffle furnace at 550°C for 6 h (Chapman and Pratt, 1961). The ash was taken in 0.36N H_2SO_4 and B concentration was determined by using the azomethine-H colorimetric method (Bingham, 1982).

Statistical Analysis

The data were checked for normality before carrying out analyses. The data was found normal and therefore analysis was performed on non-transformed data. The year effect was significant for studied parameters according to paired T test; therefore, the data of both studied years was analyzed and presented separately. A 3-way analysis of variance (ANOVA) was used to test the differences among different treatments and their interactions. Least significant difference (LSD) test at 0.05 probability level was used to compare the means where ANOVA indicated significant differences (Steel *et al*., 1996). For the easier interpretation of data, significant 3-way interactions were presented.

Results

Plant Growth

Drought stress substantially decreased plant growth of tested barley genotypes during both years, while seed priming improved the growth of both genotypes (Table 2).

Table 1: Weather data during the growing seasons of barley at 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	9	62	62	26	27	12	12	19	20	8	7
December	0	0	75	63	18	22	6	7	12	15	5	7
January	12	13	75	74	17	18	7	8	12	13	5	1
February	21	8	66	58	22	23	11	9	16	16	6	9
March	68	67	64	60	24	27	14	16	19	21	5	7
April	33	6	33	34	21	34	27	20	27	27	9	8

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

Table 2: Effect of different seed priming techniques on the growth of barley genotypes grown under different levels of drought stress

Treatments	2014-2015						2015-2016					
	Well-watered		Moderate drought		Severe drought		Well-watered		Moderate drought		Severe drought	
	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	62.88d-h	64.66 def	59.24ghi	63.89 d-g	51.14 l	52.49kl	61.31 fg	67.90 cd	58.10 f-i	67.67 cde	53.00 i	58.51 f-i
Hydropriming	64.17 def	71.58 bc	58.88hi	62.75 e-h	53.32 jkl	52.70 kl	68.07 cd	68.76 cd	63.38 def	71.02 bc	55.39 hi	56.26 ghi
Osmopriming	67.72 cd	86.43 a	65.26 de	71.16 bc	57.36 ijk	55.84 i-l	71.78 bc	72.47 bc	61.00 fg	74.86 b	63.65 def	71.26 bc
Biopriming	71.73 bc	73.70 b	60.29 f-i	65.32 de	57.82 ij	60.44 e-i	68.25 cd	87.74 a	60.05 fgh	75.14 b	62.08 ef	60.29 fgh
LSD value (p 0.05)	4.92						5.59					
Leaf area (cm^2)												
Control	507.14 cd	592.56 b	449.06 fgh	435.26 gh	298.86 m	318.93 lm	506.34 ef	529.58 def	419.71 j	435.20 ij	332.09 m	321.97 m
Hydropriming	523.10 cd	597.05 b	419.81 hi	452.00 fgh	330.95 lm	345.94 kl	548.50 cd	535.59 cde	459.66 hi	448.68 hij	327.63 m	348.02 lm
Osmopriming	516.12 cd	643.39 a	471.96 ef	462.05 efg	394.13 ij	368.92 jk	561.10 c	542.85 cd	501.79 fg	522.27 def	337.48 m	371.41 kl
Biopriming	529.91 c	656.27 a	490.56 de	439.35 fgh	336.87 kl	439.53 fgh	595.99 b	647.84 a	476.13 gh	447.53 hij	386.74 kl	452.87 hi
LSD value (p 0.05)	32.86						29.85					

Means sharing the same letter for a parameter during a particular year, don't differ significantly at $p \leq 0.05$; H-93 = Haider-93, F-87 = Frontier-87

The decrease in the growth of both genotypes increased with increasing severity of drought stress (severe drought > moderate drought > control); however, Haider-93 had less plant height and leaf area than Frontier-87 (Table 2). The longest plants were recorded for osmo- and bioprimed seeds of Frontier-87 under moderate drought stress. The leaf area was improved by biopriming of Haider-93 and osmopriming of Frontier-87 during first and second year, respectively. Plant height during first year and leaf area during both years was mostly improved by biopriming of Frontier-87 under severe drought stress, while plant height was improved by osmopriming of Frontier-87 during second year (Table 2).

Yield and Related Traits

Drought stress negatively affected yield and related traits of barley genotypes during both years (Table 3). The reduction in yield and related traits of both genotypes was increased with increasing severity of drought stress; however, the deleterious effects were more prominent in Frontier-87 (Table 3). Seed priming improved yield and related traits of both genotypes under drought stress. The number of productive tillers was improved by biopriming and osmopriming of Haider-93 under moderate drought stress during first and second year, respectively. Moreover, biopriming of Haider-93 improved number of grains per spike, 100 grain weight and grain yield during both years. Harvest index was improved by biopriming and osmopriming of Haider-93 during first and second year, respectively. Biopriming of Frontier-87 and Haider-93 observed the highest increase in productive tillers under severe drought stress during first and second year, respectively. Number of grains per spike were enhanced by osmopriming of Haider-93 during both years. The most improvement in 100 grain weight and yield was recorded by osmopriming and biopriming of Haider-93 during first and second year, respectively. Osmopriming of Fronteirs-87 and biopriming of Haider-93 caused the highest increase in harvest index during first and second year, respectively (Table 3).

Chlorophyll Contents

Chlorophyll contents were significantly decreased by drought stress during both years. The genotypes exhibited differential sensitivity to drought stress in terms of chlorophyll synthesis with greater reduction observed in Frontier-87 (Fig. 1). The greatest improvement in biosynthesis of chlorophyll *a* and *b* under moderate drought stress was noted for biopriming of Haider-93 during first year, while osmopriming of Frontier-87 and Haider-93 resulted in the highest chlorophyll *a* and *b* contents, respectively, during second year. Biopriming and

Table 3: Effect of different seed priming techniques on yield and related traits of barley genotypes grown under different levels of drought stress

Treatments	2014–2015						2015–2016					
	Well-watered		Moderate drought		Severe drought		Well-watered		Moderate drought		Severe drought	
	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												
Control	10.73 e	10.54 ef	8.92 hij	8.51 ij	6.98 k	6.84 k	10.79 d	10.29 e	9.05 f	8.03 g	6.65 i	6.47 i
Hydropriming	11.82 bc	10.99 de	9.14 hi	9.37 gh	8.21 j	7.39 k	11.54 c	11.54 c	10.32 e	8.78 f	7.48 h	8.69 f
Osmopriming	13.08 a	11.87 bc	9.97 fg	10.76 de	8.81 hij	8.48 ij	12.30 b	13.05 a	11.44 c	10.05 e	7.69 gh	7.88 gh
Biopriming	12.25 b	12.35 b	11.45 cd	9.87 fg	8.35 j	8.88 hij	13.55 a	11.80 bc	9.82 e	10.04 e	9.05 f	8.11 g
LSD (p 0.05)	0.71						0.52					
Grains per spike												
Control	33.92 de	32.28 fg	28.01 jk	26.73 kl	22.01 n	19.65 p	33.63 c	32.37 c	27.84 f	26.45 g	19.85 jk	17.65 l
Hydropriming	36.27 c	33.58 ef	28.61 ij	28.28 ij	24.07 m	20.37 op	36.28 ab	37.51 a	28.68 ef	27.85 f	20.73 ij	18.80 kl
Osmopriming	35.10 cd	38.24 b	30.89 gh	30.81 gh	25.34 lm	22.42 n	37.38 a	37.26 a	29.56 de	30.48 d	22.52 h	19.50 jk
Biopriming	40.22 a	39.57 ab	34.19 de	29.72 hi	24.79 m	21.78 no	36.99 a	35.55 b	32.89 c	28.43 ef	21.34 hi	19.90 jk
LSD (p 0.05)	1.49						1.34					
100-grain weight (g)												
Control	3.22 e	3.30 de	2.90 hi	2.80 ij	2.55 kl	2.50 l	3.29 ef	3.17 fg	2.93 ij	2.73 k	2.45 lm	2.26 n
Hydropriming	3.61 bc	3.18 efg	3.02 fgh	3.17 ef	2.84 ij	2.69 jk	3.32 e	3.59 bc	3.06 ghi	2.87 jk	2.54 lm	2.40 m
Osmopriming	3.84 a	3.83 a	3.24 e	3.22 e	3.01 gh	2.68 jk	3.79 a	3.47 cd	3.17 fg	3.02 hi	2.85 jk	2.56 l
Biopriming	3.63 b	3.58 bc	3.45 cd	3.30 de	2.88 hi	2.85 hij	3.61 b	3.37 de	3.36 de	3.09 gh	2.86 jk	2.45 lm
LSD (p 0.05)	0.17						0.14					
Grain yield (g pot^{-1})												
Control	8.17 ef	8.08 ef	5.68 i	5.18 j	3.20 m	2.94 m	7.96 f	7.95 f	5.67 k	5.15 l	2.56 qr	2.25 r
Hydropriming	9.77 c	8.49 de	6.03 i	5.87 i	3.70 l	3.13 m	9.09 e	10.81 b	6.61 i	6.11 j	2.86 opq	2.67 pq
Osmopriming	10.41 b	10.22 b	7.97 f	7.28 g	4.35 k	3.92 kl	11.17 a	10.81 b	7.29 g	6.97 gh	3.28 mn	3.05± no
Biopriming	11.06 a	10.21 bc	8.62 d	6.82 h	4.11 kl	3.74 l	10.19 c	9.58 d	7.79 f	6.74 hi	3.55 m	2.93 nop
LSD (p 0.05)	0.45						0.35					
Harvest index (%)												
Control	31.96 d	31.66 d	26.63 gh	25.79 h	20.15 l	20.70 kl	33.95	33.01	28.07	26.39	23.54	24.08
Hydropriming	35.56 ab	30.96 d	26.84 gh	27.16 fg	22.28 j	21.94 jk	35.01	35.83	29.35	26.12	24.58	25.03
Osmopriming	36.02 a	34.58 bc	31.07 d	28.88 e	22.80 ij	23.67 i	38.70	36.44	32.25	29.83	26.64	26.48
Biopriming	36.71 a	33.56 c	31.38 d	28.20 ef	22.83 ij	22.93 ij	36.62	36.88	30.36	27.96	26.96	26.41
LSD (p 0.05)	1.32						NS					

Means sharing the same letter for a parameter during a particular year don't differ significantly at $p \leq 0.05$; H-93 = Haider-93, F-87 = Frontier-87

osmopriming of Hiader-93 exhibited the highest chlorophyll *a* and *b* contents, respectively under severe drought stress during first year, while osmopriming and biopriming of Hiader-93 caused the highest increase in chlorophyll *a* and *b*, respectively, during second year (Fig. 1).

Osmolytes Accumulation

Drought stress elevated the accumulation of osmolytes, during both years (Fig. 2 and 3). Osmolytes accumulation linearly increased in both genotypes with increase in drought stress levels. Hiader-93 accumulated more osmolytes under severe drought stress than Frontier-87 (Fig. 2 and 3). Seed priming enhanced the accumulation of osmolytes in both genotypes under drought stress. Under moderate drought stress, total soluble proteins were increased by osmopriming of Haider-93 and biopriming of Frontier-87 during first year, and biopriming of Haider-93 during second year. The highest accumulation of proline was recorded by osmopriming of Haider-93 during both years. However, total phenolics and glycine betaine contents were increased by biopriming of Haider-93 during first year and by osmopriming of Haider-93 and Frontier-87 during second year. Under severe drought stress, osmopriming of Haider-93 caused the highest accumulation of total phenolics and total soluble proteins during first and second year, respectively, and proline during both years. However, hydro and biopriming of Haider-93 increased total soluble proteins during first year and total phenolics during second year. Glycine betaine content was enhanced by biopriming of Haider-93 and osmopriming of Frontier-87 during first and second year, respectively (Fig. 2 and 3).

Lipid Peroxidation and Cell Membrane Stability

Drought stress increased lipid peroxidation, while decreased the CMS (Fig. 4). The accumulation of MDA was increased with decrease in CMS of both genotypes under increasing severity of drought stress. However, more MDA accumulation and less CMS was recorded for Frontier-87 under drought stress (Fig. 4). Seed priming decreased MDA accumulation, while improved CMS of both genotypes under drought stress. Under moderate drought stress, osmopriming of Frontier-87 caused maximum reduction in MDA accumulation during first year, while biopriming of Haider-93 decreased MDA during second year and improved CMS during both years. However, under severe drought stress, least MDA accumulation occurred by biopriming of Frontier-87 during first year and osmopriming of Haider-93 during second year.

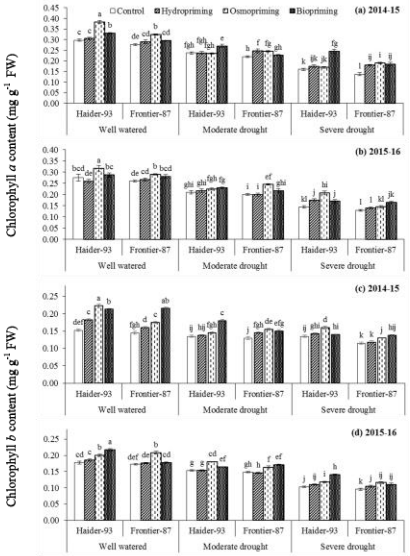

Fig. 1: Influence of different seed priming techniques on chlorophyll *a* (a and b) and chlorophyll *b* (c and d) contents ± SE of barley genotypes under drought stress (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

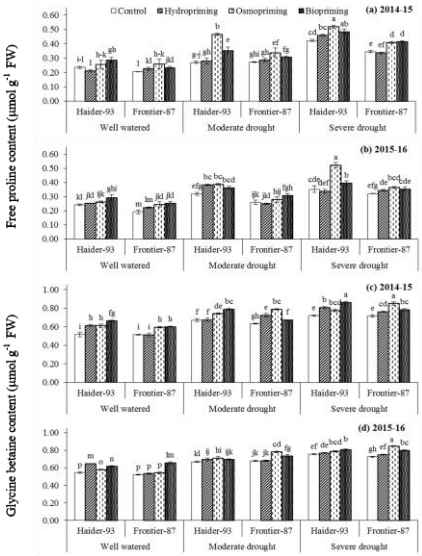

Fig. 3: Influence of different seed priming techniques on free proline (a and b) and glycine betaine contents (c and d) of barley genotypes ± SE under drought stress (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

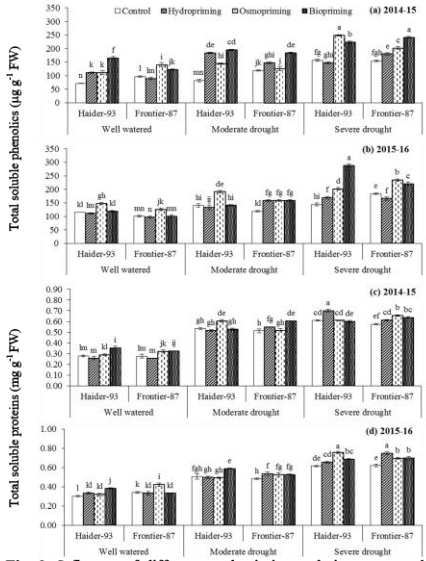

Fig. 2: Influence of different seed priming techniques on total soluble phenloics (a and b) and total soluble proteins contents (c and d) of barley genotypes± SE under drought stress(n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

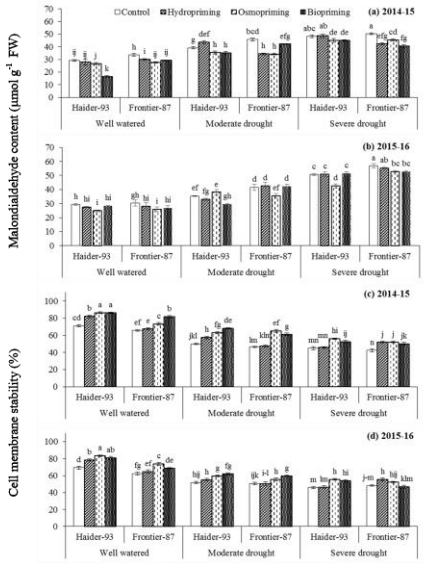

Fig. 4: Influence of different seed priming techniques on malondialdehyde content (a and b) and cell membrane stability (c and d) of barley genotypes ± SE under drought stress (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

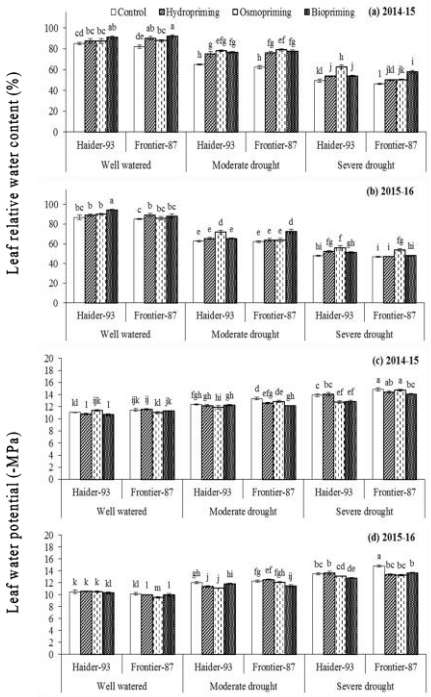

Fig. 5: Influence of different seed priming techniques on leaf relative water contents (a and b) and leaf water potential (c and d) of barley genotypes ± SE under drought stress (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$).

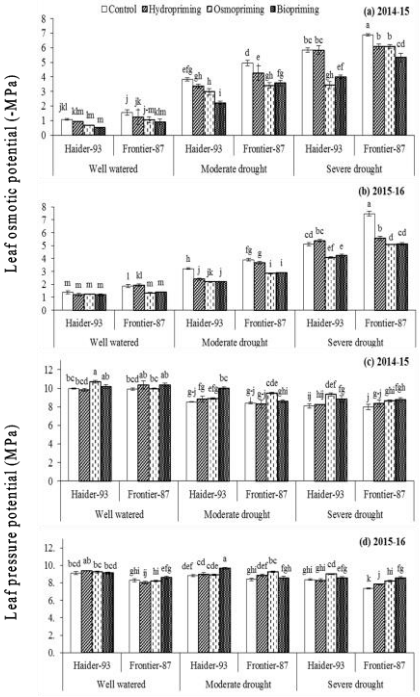

Fig. 6: Influence of different seed priming techniques on leaf osmotic potential (a and b) and leaf pressure potential (c and d) of barley genotypes ± SE under drought stress (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$).

Fig. 5: Influence of different seed priming techniques on leaf relative water contents (a and b) and leaf water potential (c and d) of barley genotypes ± SE under drought stress (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

Likewise, osmopriming of Haider-93 improved CMS during both years. However, the effect of hydropriming of Frontier-87 on CMS was similar during second year (Fig. 4).

Water Relations

The water relation traits of both genotypes were hampered by drought stress with more negative effects with increasing severity during both years (Fig. 5 and 6). Although water relations of both genotypes were affected, adversities of drought stress were more prominent in Fronties-87 (Fig. 5 and 6). Nonetheless, seed priming improved water relations of both genotypes under drought stress. Under moderate drought, the highest improvement in relative water contents was recorded for osmopriming and biopriming of Frontier-87 during first and second year, respectively. Water potential was improved by osmopriming of Haider-93 during both years. However, osmotic and pressure potentials were improved by biopriming of Haider-93 during both years, while osmopriming of Hiader-93 produced similar results for osmotic potential during second year. Under severe drought, osmopriming of Hiader-93 exhibited

Fig. 6: Influence of different seed priming techniques on leaf osmotic potential (a and b) and leaf pressure potential (c and d) of barley genotypes ± SE under drought stress (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

maximum improvement in water potential during first year, and relative water contents, osmotic potential and pressure potential during both years. However, water potential was improved by biopriming followed by osmopriming of Haider-93 during second year (Fig. 5 and 6).

Grain Nutrient Contents

The interaction among genotypes, drought stress and seed priming techniques was non-significant for seed nutrient contents; however, the individual effects of drought stress, seed priming techniques, genotypes and interaction between genotypes and drought stress were significant (Table 4). Drought stress significantly decreased grain Zn, B and Mn contents during both years (Table 4). The negative effects of drought stress on grain nutrient contents in both genotypes were increased with increasing severity. However, Haider-93 accumulated more Zn, B and Mn than Frontier-87 under control as well as moderate and severe drought stress (Fig. 7). However, biopriming followed by hydropriming partially improved the seed nutrients contents, as compared to non-primed control (Table 4).

Table 4: Effect of different seed priming techniques on seed mineral nutrients of barley genotypes under different levels of drought stress

Treatments	Seed Zn content (µg g^{-1} DW)		Seed Mn content (µg g^{-1} DW)		Seed B content (µg g^{-1} DW)	
	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Genotypes (V)						
Haider-93	37.52a	37.83 a	111.85 a	111.19 a	1.68 a	1.64 a
Frontier-87	34.93b	36.29 b	105.66 b	105.04b	1.57 b	1.58 b
LSD (p 0.05)	0.77	0.80	2.22	1.97	0.03	0.03
Drought stress (D)						
Wellwatered	39.81 a	41.70 a	120.48 a	119.86 a	1.83 a	1.86 a
Moderate drought	36.96 b	37.52 b	112.51 b	114.73 b	1.70 b	1.65 b
Severe drought	31.90 c	31.97 c	93.29 c	89.76 c	1.34 c	1.31 c
LSD (p 0.05)	0.95	0.98	2.72	2.41	0.04	0.03
Seed priming (T)						
Control	35.24 c	35.94b	105.58 c	104.65c	1.58 c	1.56 c
Hydropriming	36.84 b	36.86b	109.67 b	109.88 b	1.66 b	1.62 b
Osmopriming	34.55 c	36.61b	104.28 c	102.68 c	1.51 d	1.54 c
Biopriming	38.28 a	38.84a	115.50 a	115.25 a	1.74 a	1.72 a
LSD (p 0.05)	1.09	1.13	3.14	2.78	0.04	0.04

Means in a column sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

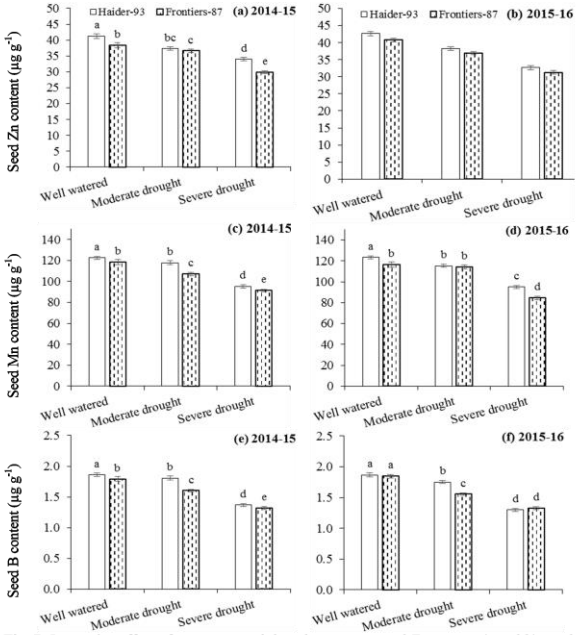

Fig. 7: Intercative effect of genotypes and drought stress on seed Zn content (a and b), seed Mn content (c and d) and seed B content (e and f) of barley genotypes ± SE (n=4).Means sharing the same letter for a parameter during a particular year don't differ significantly (p ≤0.05).

Fig. 7: Intercative effect of genotypes and drought stress on seed Zn content (a and b), seed Mn content (c and d) and seed B content (e and f) of barley genotypes ± SE (n=4). Means sharing the same letter for a parameter during a particular year don't differ significantly ($p \leq 0.05$)

Discussion

Drought stress suppressed the barley performance as indicated by decreased growth, yield and related traits. However, seed priming techniques improved growth, water relations and chlorophyll contents, which resulted in improved yield of both barley genotypes under well-watered and drought stress conditions. The improved growth and yield by osmopriming with $CaCl_2$ under drought stress is attributed to enhanced accumulation of phenolics, total soluble proteins, proline and glycine betaine (Fig. 2 and 3), which resulted in improved chlorophyll contents (Fig. 1), water relations (Fig. 5 and 6), cell membrane stability and decreased MDA accumulation (Fig. 4). Enhanced accumulation of osmolytes by osmopriming might be due to upregulation of gene expressions for osmolytes, heat shock proteins and antioxidants due to induced osmotic stress during seed priming (Kibinza *et al*., 2011; Chen *et al*., 2012; Chen and Arora, 2013). These gene expressions decrease lipid peroxidation and improve water relations, consequently improving growth and stress tolerance (Tabassum *et al*., 2017). Moreover, Ca^{2+} used in osmopriming regulates calmodulin like proteins and acts as a secondary messenger, which enhances gene expression for osmolytes accumulation (White and Broadley, 2003; Sarwat *et al*., 2013).

Biopriming also improved the growth and yield of both genotypes under each level of drought stress. The improved performance of barley by biopriming under drought stress is attributed to enhanced accumulation of osmolytes (Fig. 2 and 3), which improved chlorophyll contents (Fig. 1), tissue water status (Fig. 5 and 6), cell membrane stability while decreasing the lipid peroxidation (Fig. 4). Improved drought tolerance by biopriming might be due to endophytic bacteria which place the metabolism of plants in primed state, and enable greater and rapid accumulation of transcription factors for osmolytes and stress related gene expressions (Theocharis *et al*., 2012; Miotto-Vilanova *et al*., 2016). These regulations increase the accumulation of osmolytes and result in decreased lipid peroxidation and improved cell membrane stability (Dimkpa *et al*., 2009). Biopriming improved the grain Zn, Mn and B contents (Table 4) across genotypes and drought stress levels. The improved grain nutrients by biopriming might be due to endophytic bacteria which improved water

relations (Fig. 5 and 6), and uptake of nutrients by solubilization and improved root growth by modulation in growth hormones (Mantelin and Touraine, 2004; Kloepper *et al*., 2007). Moreover, plant growth promoting bacteria enhance the mineral nutrients uptake by stimulating the proton ATPase pump (Mantelin and Touraine, 2004).

The plants produced by primed seeds exhibited better protected membranes due to lesser lipid peroxidation in both barley genotypes under all levels of drought stress (Fig. 4). The improved cell membranes stability and decreased MDA due to seed priming is attributed to higher accumulation of phenolics, total soluble proteins, proline and glycine betaine in both genotypes under drought stress (Fig. 2 and 3). The phenolics contain aromatic ring in their structures which protects and stabilizes cellular membranes and enhances ROS scavenging in cells under stressed conditions (Shetty *et al*., 2001; Taiz *et al*., 2015). Likewise, soluble proteins protect the cellular membranes by improving their hydration and avoiding the oxidative damage to lipids, proteins and nucleic acid by ROS under stressed conditions (Wahid and Close, 2007; Arafa *et al*., 2009). Moreover, the proline and glycine betaine improve cell membrane stability by improving tissue water status through osmotic adjustment and quenching the ROS (Anjum *et al*., 2017; Song *et al*., 2017; Tabassum *et al*., 2017).

Drought stress reduced growth, yield and yield related traits of both genotype, as compared to well-watered conditions; however, the effects were more severe on Frontier-87 than Haider-93 at each level of drought stress (Table 2 and 3). This variation among genotypes was associated with production and accumulation of osmolytes in greater quantities which resulted in better chlorophyll contents (Fig. 1), well-maintained water relations (Fig. 5 and 6), cell membrane stability and lower MDA accumulation (Fig. 4) in Haider-93 indicating it more drought tolerant than Frontier-87. Accumulation of compatible solutes or osmolytes is exaggerated under stressed conditions and may be used as an index for stress tolerance (Wang *et al*., 2016). It has been observed that drought tolerant genotypes accumulate osmolytes in greater quantities than sensitive ones (Anjum *et al*., 2017; Farooq *et al*., 2017a).

Yield and related attributes of both genotypes were negatively affected by drought stress. However, Haider-93 showed higher yield and harvest index than Frontier-87, which was associated with higher number of productive tillers, grains per spike and grain weight under drought stress (Table 3). Moreover, seed priming improved yield and related traits in both genotypes under drought stress (Table 3). The improved chlorophyll contents (Fig. 1), water relations (Fig. 5 and 6) and better protection of cellular membranes (Fig. 4) might have improved the pollen viability and assimilate translocation, which resulted in improved number of grains and grain weight (Arshad *et al*., 2017) under drought stress. This improvement by osmopriming and/or biopriming in this study led to improved number of grains and grain weight that produced greater grain yield and harvest index.

Conclusion

Drought stress resulted in decreased growth and yield of both barley genotypes due to decrease in chlorophyll contents and impaired plant water relations. However, seed priming improved the growth, yield and related traits of both barley genotypes through enhanced accumulation of phenolics, total soluble proteins, proline and glycine betaine, which resulted in improved chlorophyll contents, tissue water status and cell membrane stability with concomitant decrease in lipid peroxidation. The order of improvement in yield and related traits under drought stress was biopriming>osmopriming>hydropriming.

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