Research Article

Exogenous Application of Proline Improved Salt Tolerance in Rice through Modulation of Antioxidant Activities

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Abstract | The present study evaluated the role of foliar applied proline to enhance salt tolerance of two rice cultivars with contrasting salt tolerance: Super basmati (salt susceptible) and Shaheen basmati (salt tolerant) in both nursery and field conditions. For pot experiment, salinity stress was imposed by applying 50 mM of NaCl solution to the soil daily for three continuing days, and applications of proline (0, 25, 50, 75 and 100 mM) was applied 25 DAS. For field experiment, the same cultivars were grown in a saline soil (EC = 5.05 d Sm^{-1}) for two growth seasons with or without 50 mM proline applied at seedling stage (15 days after transplanting, DAT), vegetative state (50 DAT) or at both stages. Results from pot experiment revealed that foliar applied 50 mM proline significantly improved the seedling growth under saline stress by reducing electrolyte leakage and improving the water related (relative water contents and water potential), and biochemical attributes (SOD, CAT, MDA, EL and T *CHL*). Under field conditions, exogenous application of optimized dose of proline both at seedling establishment and vegetative stage significantly improved the performance of rice cultivars by improving tillering dynamics, plant water-relations, chlorophyll pigments, photosynthetic pigments, morphological and yield related traits of rice cultivars. Proline application improved the efficiency of salt tolerance of rice cultivars, Shaheen basmati showed better response in terms of yield than Super basmati. In crux, foliar applications of 50 mM proline at seedling and vegetative stages are more effective for achieving the best kernel yield under saline conditions.

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Introduction

Globally plants are exposed to various biotic and abiotic stresses at different growth stages. Among several abiotic stresses, salinity is a major threatening factor limiting agricultural productivity (Munns, 2002; Flowers, 2004). In most field crops, salt stress has deleterious effects on seed germination, seedling establishment, root growth, photosynthesis, tillering, flowering, pollination and grain quality (Zeinali et al., 2002; Sairam and Tyagi, 2004). Higher level of salt concentration in the root zone causes disturbance in plant nutrient uptake, solute potential, water relation and ion toxicity (Munns, 2002). High concentration of Na^+ and Cl^- ions causes membrane disruption, inhibition of cell division and expansion. Moreover, it also inhibits many enzymatic and metabolic activities including photosynthesis rate, stomatal conductance, leaf water potential, protein synthesis and lipid metabolism (Zhu, 2001; Parida and Das, 2005; Lichtenthaler et al., 2005). Prolonged saline conditions disrupt osmotic potential which result in loss of cell turgor pressure, cell disruption, inhibition of water and nutrients absorption and as a result cell is completely destroyed (Cicek and Cakirlar, 2008; Riaz et al., 2018). In order to cope with detrimental effects of salt stress and to sustain cellular functions, plants have adopted various biochemical and molecular mechanisms. Among them osmotic adjustment is considered to be most important mechanism which results in increase level of organic solutes called "osmolytes". These major incompatible solutes include proteins, proline, glycine betaine, proteins, polyamines and carbohydrates (sugars and starch) (Ashraf, 2004; Ashraf and Harris, 2004). Under saline condition, these osmolytes protect the normal functioning of cell either by maintaining cellular osmotic potential and scavenging of reactive oxygen species (ROS). Higher level of ROS can seriously pose threat to normal metabolic activities of the plant through per oxidation of lipids, oxidation of proteins, nucleic acids and ultimately leads to cell death (Hernadez et al., 2001; Arafa et al., 2009).

In order to eliminate the severe challenges of abiotic stresses and to improve the capacity of crop plants to tolerate salt stress several strategies have been projected. Among them, exogenous foliar application of compatible solutes such as proline, glycinebetaine, trehalose, etc., was considered to be most effective to mitigate the damages of salt stress (Ashraf and Foolad, 2007). Under stress condition, foliar application of proline showed improvement of the tolerance capacity of celery seedling (Saranga et al., 1992) and cell culture of tobacco (Okuma et al., 2000). Further, proline application improved the growth rate inhibition of germination in rape seed when exposed to high level of Na^+ and Cl^- ions (Makela et al., 2002), similar observations were also observed in the in wheat (Raza et al., 2006) and maize crops (Ali et al., 2007). Various workers have reported that exogenous foliar application of proline not only effectively regulates solute potential but also plays an important role in enhancing plant growth under stress environment (Ashraf and Foolad, 2007; Hoque et al., 2007).

Rice is very sensitive to high level of salt concentration and its tolerance capacity varies throughout the life cycle of plant. Severe saline stress drastically reduced germination percentage. It is reported that screening of salt tolerant plants at initial stages, e.g. either at germination stage or at seedling stage, would enhance crop stand establishment and uniform growth under salt stress (Munns, 2002; Cuartero et al., 2006). However, little is known about the role of foliar application of proline on early seedling growth and at various stages. Therefore, the present study was conducted in order to examine the response of exogenously applied proline at various growth stages on antioxidant activities and yield components of rice cultivars with contrasting salt tolerance under saline condition.

Materials and Methods

Pot experiment

Study site, experimental treatment and design: A pot experiment was conducted in the glass house of Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. The experiment comprised of two factors: (1) two rice cultivars, Super basmati (salt susceptible) and Shaheen basmati (salt tolerant) (Shabbir et al., 2001), (2) five salt and proline treatments (Nil NaCl + Nil proline, 150 mM NaCl + Nil proline, 150 mM NaCl + 25 mM proline, 150 mM NaCl + 50 mM proline and 150 mM NaCl + 100 mM proline) in 0.1% Tween 20 solution during *kharif*, 2014. The experiment was set-up in completely randomized design (CRD) under factorial arrangement and each treatment was replicated four times. The total pots were 40 (2 cultivars × 5 salt and proline treatments × 4 replicates).

Crop husbandry: Each pot (45 cm × 30 cm diameter) was filled with 10 kg of river sand which was washed with sterilized water. Ten seeds were sown in each pot and thinned to five after germination. Plants were fed with essential nutrients by supplying Hoagland's nutrient solution at initial stage up to 15 days after sowing (DAS), and then salt equivalent to 150 mM NaCl (8.775 g L^{-1}) was applied by adding it to the nutrient solution. The NaCl treatment was applied stepwise, 50 mM (2.925 g L^{-1}) daily for three days. The control plants were supplied with normal Hoagland's nutrient solution. The sand in the pot was moistened daily by adding 250 mL distilled H_2O. Application of proline was done after ten days of salinity stress, i.e. 25 DAS. Proline (25 mL $plant^{-1}$) was applied as foliar application at 09:00 AM according to the designated treatments. The experiment was harvested at 50 DAS.

Data collection: Three plants were selected randomly and tagged, and plant heights were measured using a meter rod. At harvest (50 DAS), the same tagged plants from each pot were used for tillers count. Relative water contents and osmotic potential in leaves were calculated following standard procedures of Weatherly (1950). Chlorophyll content and electrolyte leakage were measured by protocols of Nagata and Yamashita (1992) and Blum and Ebercon (1981). For appraising lipid per oxidation in leaf samples, thiobarbituric acid-based assay was employed by following procedures of Carmak and Horst (1991). Fresh leaf (500 mg) was extracted in 5mL 1.0% (w/v) TCA [Mol. wt. 163.4, MP Biomedicals, de KayserbergIllkirch, France]. The homogenate was centrifuged at 20,000 × *g* for 15 min using a Sigma Model 3K30 (Germany) centrifuge. The supernatant (500 µL) was reacted with 2 mL 0.5% (v/v) 2-thiobarbituric acid (TBA) [Mol. wt. 144.5, Sigma-Aldrich Chemie GmbH, Steinheim, Germany] in 20% TCA. The sample was subjected to 100°C for 1h in a shaking water bath. Thereafter, the reaction stopped by cooling the samples in ice. After cooling, samples were centrifuged at 10,000 × *g* for 10 min and the OD of the filtrate recorded at 532 and 600 nm. The level of TBARS was calculated using the absorption co-efficient, 155 mmol cm^{-1}. MDA = Δ (OD532-OD600)/1.56×105.

Inhibition in photo-reduction of nitro blue tetrazolium (NBT) was used to appraise the activity of SOD. The reaction mixture (1mL) [500 µL phosphate buffer (pH 7.8), 0.5 mL distilled H_2O, 100 µL methionine, 50 µL NBT and 50 µL sample extract] in cuvettes and were kept under light for 20 min. The OD of the irradiated aliquot was read at 560nm. SOD enzyme activity per unit was based on the amount of enzyme that inhibited 50% of NBT photo reduction. The method of Chance and Maehly (1955) was used for appraising catalase (CAT) and peroxidase (POD) activities. The CAT reaction solution (2 mL) [1.9 mL phosphate buffer (50 mM; pH 7.0) and 1mL H_2O_2 (5.9 mM)] and the reaction was initiated by dissolving an aliquot (100 µL) of the enzyme extract. Decreases in OD of the mixture at 240 nm were read spectrophotometerically for 2 min after every 20 seconds. Per unit CAT enzyme activity was based on change in OD per min. For POD one mL reaction contained 750 µL phosphate buffer (50 mM; pH 5.0), 100 µL guaiacol (20 mM), 100 µL H_2O_2 (40 mM) and 100 µL enzyme extract. Increases in OD at 470 nm of the reaction solution were read for 3 min at interval of every 20 sec. per unit POD activity was based on increase in absorbance per min (Mukherjee and Choudhuri, 1983).

Field experiment

Study site, experimental treatment and design: The experiment reported in this study was carried out at Proka farm, University of Agriculture, Faisalabad, Pakistan during *kharif*, 2014 and 2015 and was laid out in randomized complete block design (RCBD) in a factorial arrangement with three replications having plot size (8 m × 2.2 m). Each treatment has three plots with average number of plants in each plot was 310. The soil in the experimental site has a EC value of 5.05 d Sm^{-1} (averaged of the two years). The two same rice cultivars were used as in the pot experiment. However, the proline application treatments were control, 50 mM proline at seedling stage [15 days after transplanting (DAT)], 50 mM proline at vegetative state (50 DAT) and 50 mM proline at seedling stage + vegetative stage in 0.1% Tween 20 solution.

Crop husbandry: Seeds were sown on 23rd of June 2014 and 30th June of 2015 using hand drill in a nursery. Thirty-five days old nursery seedlings manually transplanted in puddled fields by maintaining R × R and P × P distance of 22.5 cm. At transplantation, uniform application of fertilizer was applied at the rate of 100, 67 and 60 (N: P: K in kg ha^{-1}) in the form of urea, di- ammonium phosphate and potassium sulphate, respectively. During crop growth, canal water was used to maintain submerged soil conditions. Irrigation water was maintained at a depth of 3–4 cm at transplantation and one week afterwards at a depth of 5–6 cm throughout the growing season till one week before harvesting. For weed control, a mixture two different herbicides Ethoxysulphuran and Phenoxyprop-p-ethyle at 200 g and 370 ml ha^{-1}respectively was applied and hand weeding was also done at specific intervals. Carbofuran was broadcasted at 25 kg ha^{-1} to shield the crop from the insects like stem borers and leaf folders. Crop was harvested manually during November, 2014 and 2015. Each plot was harvested and threshed separately. Plants were harvested after 90 DAT.

Data collection: Penultimate leaves were harvested at after fifteen days of treatment application to determine different parameters of water relations, chlorophyll contents and electrolyte leakage as described in pot experiment. Stomatal conductance (*gs*), photosynthetic rate (*A*), and transpiration rate (*E*), on the 3rd leaf from top of every plant were recorded by utilizing IRGA (infrared gas analyzer) (model, LCA-4; Analytical Development Company, Hoddesdon, England). All these determinations were recorded at 10:00 AM to 01:00 PM. At maturity, plant height was measured with a meter rod from the base of plant to the tip of flag leaf. Number of tillers was counted from a unit area selected randomly from each plot. Panicle length was measured by tape from 20 randomly selected panicles from each plot. After harvesting and threshing of crop, samples were randomly taken from each treatment and 1000 kernels were weighed with an electronic balance. The rest plants in each plot were harvested and threshed and air dried. Yield of each plot was weighed in kg and later on converted into t ha^{-1}.

Statistical analysis

Two sets of data from each experiment were analyzed using Fisher's analysis of variance and treatment means were compared using least significant difference (LSD) test at 5% probability level.

Results and Discussion

Pot experiment

All the proline levels significantly improved agronomic, water related and biochemical attributes under salinity stress (Table 1). However, application of 50 mM proline was more effective in improving the plant height, tillers per plant, relative water contents, and the measured biochemical attributes (Table 1). Maximum plant height (61.3 cm), tillers (6.2 per plant), RWC (57.53%), water potential (- 1.17 MPa), was recorded with 50 mM proline application under salinity stress of 150 mM NaCl. Similarly, the maximum total chlorophyll contents (6.69 mg L^{-1}), SOD (15.12 units mg^{-1} proteins), CAT (10.59 units mg^{-1} proteins) and MDA (27.64 μ mol g^{-1}) while minimum electrolyte leakage (19.99%) was observed with 100 mM proline in those pots where 150 mM NaCl stress imposed. Regarding rice cultivars, Shaheen basmati showed the promising results for agronomic, water related and biochemical attributes as compared to the super basmati under saline conditions. The interactive effect of salinity and proline application and rice cultivars for agronomic, water related and biochemical attributes was found non-significant (Table1).

Field experiment

Proline application considerably affected the water relations in rice cultivars during both years (Table 2). However, interactive effect of proline application at different growth stages and rice cultivars on water relations was non-significant in both years. Proline application at seedling and vegetative stage showed significant increasing results in terms of water potential during first year while similar trend was also observed during second year. Similarly, proline used at both seedling and vegetative stages significantly decreased osmotic potential than applied at vegetative stage alone, followed by 50 mM proline at seedling in both years. RWC were significantly improved by proline application at both seedling and vegetative stages in both years. Shaheen basmati performed better than super basmati regarding the water potential and RWC. However, osmotic potential was higher in Super basmati than Shaheen basmati (Table 2). Likewise, foliar application of proline at both seedling and vegetative stages significantly improved chlorophyll contents in rice cultivars during both years (Table 2). Total chlorophyll contents were highest in those plots where proline was applied at both seedling and vegetative stages followed by proline at vegetative stage (Table 2). Similar trend was observed for relative water contents. Application of proline significantly reduced the electrolyte leakage in both cultivars. Maximum reduction in the electrolyte leakage was observed in the treatment received proline at both seedling and vegetative stages in both years. Higher reduction in electrolyte leakage was observed in the first year. However, for cultivars minimum electrolyte leakage was observed in Shaheen basmati as compared to Super basmati (Table 2).

Exogenous application of proline under saline condition significantly ($p \leq 0.05$) improved the physiological attributes (Table 3). Proline application at both seedling and vegetative stages appreciably

Table 1: Agronomic, water and biochemical attributes of rice genotypes influenced by different levels of proline in saline condition in the pot experiment.

Factors	Agronomic Traits		Water relations		Biochemical Attributes				
	PH (cm)	T (per plant)	RWC (%)	WP (MPa)	SOD*	CAT**	MDA (µ mol g^{-1})	T CHL (mg L^{-1})	EL (%)
Rice cultivars (C)									
Super Basmati	51.7 B	4.08 B	53.10 B	- 0.96 B	9.87 B	9.94 B	15.41 A	3.87 B	20.47 A
Shaheen Basmati	55.2 A	4.42 A	55.42 A	- 1.02 A	11.41 A	10.12 A	12.34 B	4.34 A	17.59 B
Salinity + Proline Application (S)									
Nil NaCl + Nil proline	57.3 B	5.43 B	55.40 A-C	- 0.95 C	11.45 D	9.41 D	14.56 E	6.34 D	22.93 B
150 mM NaCl + Nil proline	49.9 D	4.66 E	53.26 C-E	- 0.85 E	12.41 C	9.63 C	20.43 D	6.53 C	25.12 A
150 mM NaCl + 25 mM proline	58.0 B	5.66 B	56.53 AB	- 1.06 B	13.43 B	9.87 B	21.27 C	6.61 B	21.96 BC
150 mM NaCl + 50 mM proline	61.3 A	6.20 A	57.53 A	- 1.17 A	14.99 A	10.53 A	23.31 B	6.69 AB	20.89 DE
150 mM NaCl + 100 mM proline	54.5 C	5.00 C	54.37 B-D	- 0.92 D	15.12 A	10.59 A	27.64 A	6.74 A	19.99 DE
LSD (C) ($p \leq 0.05$)	1.55	0.17	1.63	0.03	1.31	0.15	1.98	0.04	0.86
LSD (S) ($p \leq 0.05$)	2.13	0.31	2.85	0.02	1.45	0.07	0.83	0.07	1.51
C×S ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	NS

PH: Plant height; *T:* Tillers; *EL:* Electrolyte leakage; *RWC:* Relative water contents; *WP:* Water potential; *T CHL:* Total chlorophyll contents; *MDA:* Malondialdehyde; for each trait, data between cultivars, and among the treatments followed by the same letters are not significant ($p \leq 0.05$). *n:* 4. *NS:* non-significant; *SOD (Super oxide dismutase) (unit mg^{-1} proteins); **CAT (Catalase Activity) (unit mg^{-1} proteins).

Table 2: Influence of proline application on water-related and biochemical traits of different rice genotypes under saline conditions in field experiment.

Factors	Water related traits						Biochemical traits			
	Water Potential (MPa)		Osmotic Potential (MPa)		Relative water contents (cm)		Total chlorophyll contents (mg L^{-1})		Electrolyte leakage(%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Rice cultivars (C)										
Super Basmati	- 1.95 B	- 2.02 B	- 2.51 A	- 2.39 A	55.04 B	54.96 B	3.81 B	3.85 B	20.34 A	19.89 A
Shaheen Basmati	- 2.07 A	- 2.12 A	- 2.04 B	- 2.10 B	57.07 A	57.81 A	4.40 A	4.43 A	16.54 B	15.12 B
Salinity + Proline Application (S)										
Control	- 1.51 C	- 1.51 C	- 2.03 A	- 2.07 A	45.23 D	47.56 D	5.90 D	5.95 D	23.12 A	22.92 A
50 mM proline at seedling stage (SS)	- 1.61 B	- 1.66 B	- 1.86 C	- 1.89 C	53.63 B	54.84 B	6.34 B	6.40 B	17.10 C	16.82 C
50 mM proline at vegetative stage(VS)	- 1.59 B	- 1.55 C	- 1.91 B	- 1.94 B	50.32 C	52.50 C	6.19 C	6.24 C	20.13 B	20.02 B
SS + VS	- 1.70 A	- 1.81 A	- 1.75 D	- 1.79 D	57.43 A	60.53 A	6.54 A	6.59 A	13.02 D	12.94 D
LSD (C) ($p \leq 0.05$)	0.10	0.08	0.11	0.08	1.96	1.45	0.23	0.21	2.56	2.10
LSD (S) ($p \leq 0.05$)	0.05	0.06	0.04	0.06	2.02	1.90	0.07	0.08	0.85	1.05
C×S ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

For each trait; data between cultivars and among the treatments followed by the same letters are not significant ($p \leq 0.05$). *n:* 3; *NS:* non-significant.

improved all the measured physiological attributes in both cultivars. Transpiration rate, stomatal conductance and photosynthetic rate were the highest in the treatment where proline was applied at both seedling and vegetative stages followed by proline at vegetative stage in both years. Shaheen basmati performed better for all these physiological attributes than Super basmati (Table 3). Application of proline under saline condition improved the antioxidant activity like CAT, SOD and MDA contents (Figures 1, 2 and 3). Proline application at both seedling and vegetative stages appreciably improved the antioxidant activity in both cultivars. Maximum response in CAT, SOD and MDA contents were observed in that treatment where proline was applied at both seedling

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Table 3: Influence of proline application on physiological traits of different rice genotypes under saline conditions in field experiment.

Factors	Physiological traits					
	PR (A) (μ mol $m^{-2}s^{-1}$)		TR (E) (m mol $m^{-2}s^{-1}$)		SC (g_s) (mol $m^{-2}s^{-1}$)	
	2014	2015	2014	2015	2014	2015
Rice cultivars (C)						
Super Basmati	13.21 B	13.78 B	7.45 B	7.65 B	0.24 B	0.26 B
Shaheen Basmati	14.95 A	15.01 A	8.34 A	8.82 A	0.33 A	0.35 A
Salinity + Proline Application (S)						
Control	9.26 D	9.72 D	5.22 D	5.39 D	0.11 D	0.14 D
50 mM proline at seedling stage (SS)	10.83 C	11.32 C	6.57 C	6.87 C	0.29 C	0.31 C
50 mM proline at vegetative stage (VS)	12.29 B	12.63 B	7.19 B	7.63 B	0.36 B	0.38 B
SS + VS	13.71 A	14.06 A	8.04 A	8.21 A	0.44 A	0.49 A
LSD (C) ($p \leq 0.05$)	0.51	0.71	0.21	0.51	0.06	0.09
LSD (S) ($p \leq 0.05$)	0.43	0.61	0.37	0.43	0.03	0.05
C×S ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS

PR: Photosynthetic rate; *TR:* Transpiration rate; *SC:* Stomatal conductance; for each trait, data between cultivars and among the treatments followed by the same letters are not significant ($p \leq 0.05$). *n:* 3; *NS:* non-significant.

and vegetative stages followed by proline at vegetative stage in both years. Super basmati performed better for all these attributes than Shaheen basmati (Figures 1, 2 and 3).

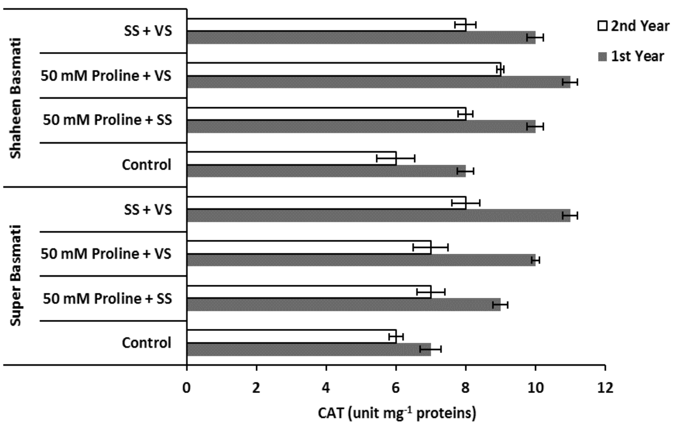

Figure 1: *Influence of proline application on CAT activity of different rice genotypes under saline conditions in field experiment.*

Foliar application of proline under saline conditions considerably affected plant height during the two years (Table 4). In the first year, maximum plant height was found in the plots where proline was applied at both seedling and vegetative stages (Table 4), followed by application of proline at vegetative stage during both years. Similarly, the proline application significantly ($p \leq 0.05$) affected the total number of tillers, panicle length, 1000-kernels weight and kernel yield during both years (Table 4). Likewise, Shaheen performed significantly better than Super basmati in terms of yield and yield related attributes.

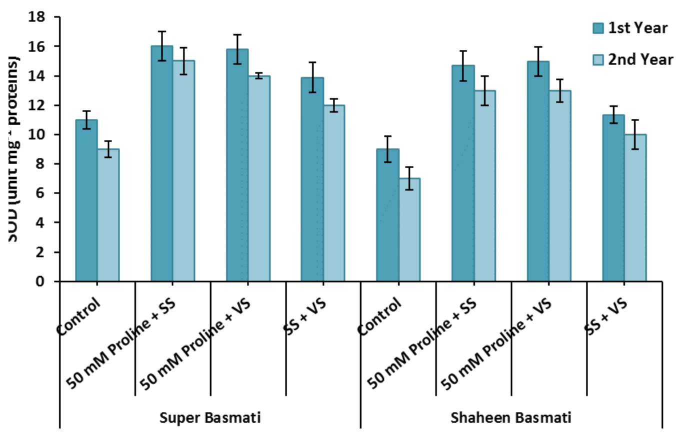

Figure 2: *Influence of proline application on SOD activity of different rice genotypes under saline conditions in field experiment.*

This study has given encouraging results of application of foliar proline to improve the crop stand establishment, water relations, photosynthetic pigments and tillering number in rice cultivars. Application of proline in rice grown under saline conditions significantly increased agronomic traits ($p \leq 0.05$). Present study, demonstrated that salinity stress caused a significant reduction in growth of rice cultivars. Globally saline stress is a major issue (Munns et al., 2010), which causes alteration in a multitude of molecular, biochemical and physiological phenomena in plants resulting in a marked reduction in agricultural

Table 4: Influence of proline application on morphological and yield-related traits of different rice genotypes under saline conditions in field experiment.

Factors	Yield related traits									
	Plant height (cm)		Total tillers (m^{-2})		Panicle length (cm)		1000-kernels weight (g)		Kernel yield (t ha^{-1})	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Rice cultivars (C)										
Super Basmati	141.6 A	140.8 A	424.68 B	429.12 B	21.3 B	21.8 B	22.75 B	22.81 B	4.09 B	4.17 B
Shaheen Basmati	137.5 B	137.9 B	449.45 A	452.45 A	25.4 A	26.1 A	24.03 A	24.06 A	4.49 A	4.61 A
Salinity + Proline Application (S)										
Control	129.6 D	130.4 C	413.12 C	418.35 D	21.3 D	21.7 D	20.41 D	20.52 D	3.36 C	3.41 D
50 mM proline at seedling stage (SS)	132.4 C	132.6 C	418.31 B	427.84 C	23.6 C	23.9 C	21.16 C	21.23 C	3.44 C	3.49 C
50 mM proline at vegetative stage (VS)	136.7 B	137.3 B	435.31 A	445.32 B	24.5 B	24.9 B	21.69 B	21.89 B	3.56 B	3.61 B
SS + VS	140.3 A	141.3 A	436.83 A	452.48 A	26.4 A	26.8 A	23.32 A	23.37 A	4.24 A	4.38 A
LSD (C) ($p \leq 0.05$)	2.12	1.72	3.10	2.81	0.34	0.74	0.56	0.49	0.19	0.29
LSD (S) ($p \leq 0.05$)	2.31	2.84	2.62	3.73	0.71	0.69	0.31	0.25	0.11	0.05
C×S ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

For each trait, data between cultivars and among the treatments followed by the same letters are not significant ($p \leq 0.05$). *n:* 3, *NS:* non-significant.

productivity (Ashraf et al., 2010). Reduction in plant height, reduced numbers of tillers were observed in the pot study under saline conditions which might be due to NaCl stress (Munns and Tester, 2008; Turan et al., 2010; Flowers et al., 2010).

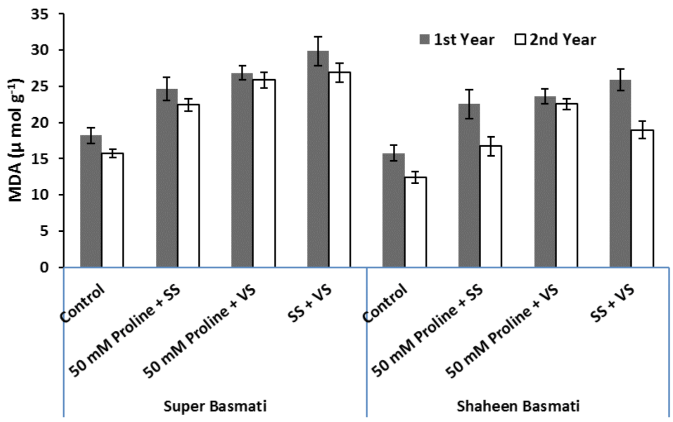

Figure 3: *Influence of proline application on MDA activity of different rice genotypes under saline conditions in field experiment.*

In present study role of exogenously applied proline modulated the water related, physiological and yield responses of rice cultivars to salinity stress. Commonly it is observed that plants grown in stress conditions show reduced shoot and root growth. There is some evidence that salinity causes accumulation of Na^+ and Cl^- in leaf tissues than normal situation as a result water loss is increased and cytoplasm dehydrated. Hydrated cytoplasm can no more perform normal function of cell metabolism and eventually reduced the seedlings growth (Le Rudulier, 2005).

In this study, plants exposure to salinity stress significantly reduced the growth and photosynthetic rates. Studies showed that salinity affects gaseous exchange and inhibits CO_2 diffusion in the leaf tissues which limits stomatal opening and subsequently reduces rate of photosynthesis (Flexas et al., 2004, 2007). Further, Munns et al. (1995) recommended that accumulation of Na^+ ions in the vacuolar cells causes reduction of photosynthesis and translocation of assimilates in young growing cells which ultimately suppress whole plant growth. Several researchers proved that salinity reduced chlorophyll and carotenoids contents of the leaf and cause chlorosis in many field crops such as alfalfa corn and *Triticum* species (Ali et al., 2007). However, application proline at 50 mM to rice plant considerably improved the status of chlorophyll a, b and carotenoids under salty conditions in root zone. Due to exogenous proline application photosynthetic pigments was improved which primarily increased the rate of CO_2 diffusion as well as rate of photosynthesis (Ali et al., 2007; Sharkey et al., 2007). In addition, salt stress reduces synthesis of chlorophyll pigments and suppresses the rate of photosynthesis and other vital processes involved therein. It is now well established that salt stress induced suppress of photosynthesis which depends on the gas exchange characteristics, photochemical quenching, photosynthetic pigments, type of cultivar or species, stomatal and non-stomatal factors, accumulation of various fundamental metabolites (organic and inorganic) and antioxidants (Ashraf, 2009; Doganlar et al., 2010; Liu and Shi, 2010).

Under saline conditions proline application improved the water relations which might be due to increase in water uptake. However, to analyze the positive effect of proline under saline environment, it is imperative to understand the role of proline in plant water status because the initial reduction in plant growth after salt accumulation results from the osmotic effect of salts (Munns and Tester, 2008). The ability of a plant to hold water under saline conditions can improve salt tolerance by eliminating an excessive ion concentration by a dilution effect (Romero-Aranda et al., 2006). In our experimentation, foliar proline could retain maximum level of water content under saline conditions. Same observation was found in *P. maritimum* plants (Khedr et al., 2003). Thus, foliar applied proline improved the salt tolerance ability of rice which is partially due to higher level of relative water content.

Gaseous exchange through stomatal conductance (*gs*), rate of photosynthesis (*A*) and transpiration (*E*) showed significant positive increases by application of proline under saline conditions. In the present study physiological characteristics decreased in rice cultivars due to salt stress. Salt deposition creates reduction in these attributes which occurs due to stomatal closure and limited supply of CO_2 and reduces the efficiency of photosynthetic enzymes (Dubey, 2005). These findings verify that exogenous application of organic solutes, e.g. proline or glycine betaine, may reduce stomatal opening and reduce transpiration rate (Raghavendra and Reddy, 1987). The results of this experiment revealed that foliar treatment of proline under saline conditions is essential for proper maintenance of physiological processes. Proline application helped in improving water use efficiency which ultimately plays a vital role in achieving optimum yield of rice under saline stress.

Generation of free radicals and increased lipid per oxidation under saline stress may have resulted in the loss of membrane integrity and increase in membrane permeability. It is reported that elevated levels of H_2O_2 and O_2 caused by stress assist the formation of active radicals like OH^-. These radicals are usually considered to be the most likely reactive oxygen species (ROS) to initiate the per oxidation destruction of lipids that lead to membrane damage. Lipid per oxidation has been linked with the damage provoked by a variety of abiotic stresses and also indicates oxidation damages of cell caused by salt stress (Elkahoui et al., 2005). Decomposition of polyunsaturated fatty acids present in biological membranes produce malondialdehyde which has been used as an indicator of lipid per oxidation and its accumulation is increased under salinity stress (Zhu et al., 2008). Our findings suggested that foliar proline enhances the amount of MDA in salt-affected plants, which is consistent with the results of Jain et al. (2001) in groundnut.

Under unstressed conditions, the overall balance between ROS production and detoxification must be tightly controlled, however, stress conditions may perturb this balance (Apel and Hirt, 2004). The enzymatic or non-enzymatic defense system of plants can be activated under saline environment, although sometimes the anti-oxidant system is not effective to inhibit oxidative damage (Apel and Hirt, 2004). In recent study, an overall increase in SOD and CAT activities was observed, even if sometimes this increase was not statistically considerable. However, the increase in action is not sufficient to protect rice plants from the damage. The reason behind this could be the significant enhancement of oxidation products induced by salinity. It is recommended that proline application plays an effective cell-protective role by minimizing oxidative stress (Chen and Dickman, 2005). The application of exogenous proline increased SOD activity, regardless of the salinity treatment; the similar result was obtained in grape vine (Ozden et al., 2009).

Application of proline increased the height of rice plant, total tillers, panicle length and kernel yield over control, which may be attributed to the better water balance, enzymatic activity and auxin metabolism in plant by proline. The number of fertile tillers, thousand grains weight and length of panicle were the highest in plants treated with proline at both seeding and vegetative stages. Deivanai et al. (2011) found that salinity significantly decreased plant growth but application of proline increased the growth and yield. Dogan, (2011) also reported that salinity reduced the growth components in soybean but applied proline has reduced the drastic effects of saline stress. Proline application also significantly increased kernel yields of rice cultivars (Table 4). Application of proline at both seedling and vegetative stages produced the highest yield of rice grains of Shaheen basmati while lower yield was observed in control. Islam et al. (2011) also reported that kernel yields of a hybrid rice badly reduced by the saline stress. Ali et al. (2004) observed the parallel result on grain yield of rice. Miah et al. (1992) suggested that rice crop showed reduction in yield attributes due to salinity stress but foliar applied proline reduced the undesirable effects of saline stress. Hayat et al. (2013) observed that the exogenous application of proline at vegetative stage appreciably reduced the injurious effects of salt stress on wheat. Thus, foliar proline improved growth of salt affected rice plants by improving photosynthetic capacity which support the opinion made by Nátr and Lawlor (2005) that under different situations the final biological or economical yield could be increased by enhancing the rate of photosynthesis. Similarly, some earlier studies show a positive association between photosynthetic rate and growth rate in cotton (Faver et al., 1997), maize (Shuting et al., 1997) and wheat (Raza et al., 2006; Arfan et al., 2007).

Conclusions and Recommendations

The current pot and field experiments demonstrated that salt stress imposed negative effects on the growth, morphology and physiology of rice cultivars but exogenous foliar application of proline significantly eliminate the harmful effects of salinity. Foliar application of 50 mM proline at both seedling and vegetative stage was more effective in improving crop stand establishment, water relations, maintaining the membrane permeability, photosynthetic pigments, tillering ability and kernel yield of rice cultivars. Moreover, affirmative effect of foliar applied proline was due to its encouraging effects on photosynthetic capacity by reducing stomatal resistance, improving biosynthesis of photosynthetic pigments, or protecting photosynthetic pigments from salt stress degradation.

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Author's Contribution

Qamar uz Zaman, Ambreen Aslam planned the research and helped in manuscript writing, Fouzia Tabssum conducted the research and collected the data, Umair Riaz and Humera Aziz helped in the statistical analysis of the data, Waqas Ashraf, Nusrat Ehsan made tables and figures. Shamim ul Sibtain Shah technically improved the draft. Rab Nawaz and Yinglong Chen helped in the proof reading of the manuscript.

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