



Available Online at EScience Press

## Plant Protection

 ISSN: 2617-1287 (Online), 2617-1279 (Print)  
<http://esciencepress.net/journals/PP>

### PROLINE INDUCED CHANGES IN REDOX BALANCE AND PHOTOSYNTHETIC ACTIVITY OF WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALINE CONDITIONS

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#### ARTICLE INFO

##### Article history

Received: 16<sup>th</sup> February, 2022

Revised: 30<sup>th</sup> March, 2022

Accepted: 6<sup>th</sup> April, 2022

##### Keywords

Chlorophyll fluorescence

Growth

Proline

Salinity

Wheat

Yield

#### ABSTRACT

Salinity is a major environmental constraint that reduces plant development, growth, and yield. To appraise the potential role of foliar-applied proline as an alternative shotgun approach to ameliorate the adverse effects of salinity on wheat, a pot experiment was conducted under controlled environmental conditions using two wheat cultivars (Galaxy-13 salt-sensitive) and (Pasban-90 salt-tolerant). The experiment was laid out in a completely randomized design with the factorial arrangement and four replications. After ten days of germination, the plants were thinned to maintain six plant seedlings in each pot and the germinating seedlings were irrigated weekly with full strength Hoagland's nutrient solution and salinity stress (NaCl) was applied after three weeks of germination. Foliar spray of proline (100 mM) was applied with the treatment of salinity. Salinity stress caused a significant reduction in morphological attributes like lengths and fresh weights of root and shoot, number of leaves and leaf area as well as yield attributes like spike length, number of spikelets per spike, number of grains per spike, yield per plant and 100 seed weight. Furthermore, chlorophyll fluorescence, chlorophyll contents, quantum yield, and electron transport rate (ETR) were also reduced by salt stress. However, foliar spray of 100 mM proline was the most effective to ameliorate the toxic effects of salinity by improving biomass production, chlorophyll fluorescence, chlorophyll contents, and quantum yield in both the cultivars. The findings confirmed the ability of foliar spray of proline to stimulate salt tolerance in wheat plants.

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#### INTRODUCTION

Salinity is one of the most important abiotic stresses that limit the productivity of crops in arid and semi-arid regions of the world (Hussain et al., 2010). Apparently, more than 60% of the probable production of crops is damaged by salt stress (Xie et al., 2016). More than 900 million hectares of land world-wide, approximately 20%

of the total cultivated land and more than 6% of the total world land are affected by salinity. In Pakistan, about 10 million hectares of land is severely affected by salinity which covers 12.9 percent of the state land. The main causes of salinity are: poor irrigation practices, high rate of evapotranspiration, actual soil structure and the surface deepness of the water table (Mahboob et al.,

2016; Sattar et al., 2010).

High levels of soil salinity badly affect the quality and quantity in crop production by preventing seed propagation and seedling development due to the collective effects of high osmotic potential and specific ion toxicity (Hussain et al., 2013). Salinity interrupts plant physiology at both cellular and whole plant levels through osmotic and ionic stress. Physiological processes which are harshly affected by salt stress comprise changes in plant development, mineral distribution and membrane variability resulting from calcium dislocation by sodium, membrane permeability and reduced productivity of photosynthesis (Shahid et al., 2013). High level of salinity increases  $\text{Na}^+$  and  $\text{Cl}^-$  and declines in  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Mg}^{2+}$  levels in a number of plants (Bilkis et al., 2016).

In addition, salt stress also alters a wide range of metabolic processes in growing plants and induces changes in contents and activities of several enzymes. Salinity causes the inhibition of crop growth by decreasing hormone distribution from roots to leaves. It also induces changes in ultrastructure of chloroplast (Sivasankaramoorthy, 2013). Moreover, salt stress induced the reduction in chlorophyll fluorescence efficiency, PS-II activity and decreased pigment content in leaves (Datta et al., 2009; Shaheen and Shahbaz, 2012).

Proline provides a resistance against salinity by supporting redox homeostasis. Under salt stress, exogenous proline up-regulates stress-protective proteins and reduces protein oxidation and lipid peroxidation. It plays a significant role in cell osmotic potential, stability of membrane and purification of poisonous ions in plants under salt environments (Khan et al., 2014).

Wheat (*Triticum aestivum* L.) belongs to the family Poaceae and is generally known as "King of Cereals". It is grown in all temperate and subtropical countries of the world (Zeb et al., 2006) while Pakistan is the 4<sup>th</sup> largest wheat producer in Asia (Zafar et al., 2015). It supports 12.5% to the value added in agriculture and 2.6% to GDP (Hussain et al., 2013). Wheat is considered a good source of minerals, protein, B-group vitamins and dietary fiber because it provides approximately 20% of the food calories and 55% of carbohydrates. It is also a good source of traces minerals like magnesium and selenium, nutrients significant to good health. The young stems are used in the treatment of vomiting and intoxication while

the fruit is antipyretic and sedative (Kumar et al., 2011). Foliar application of proline is a shotgun method in the reduction of toxic effects of salinity. Exogenous applications of proline are considered as a significant agent to retain osmotic potential of the plant cell and also as an antioxidant agent through its potential in increasing the capability of plant to tolerate salinity. However, the evidence pertaining to the role of foliar application of proline on growth, yield and photosynthetic activity is limited. Thus, the aim of the present work was to determine whether foliar application of proline could act as protectant against salt stress and that which wheat cultivar is more tolerant against salinity.

#### **MATERIALS AND METHODS**

The experiment was set-up in Bio Park of Bahauddin Zakariya University, Multan, during the winter season of 2016. Seeds of both the wheat cultivars were obtained from Ayub Agriculture Research Institute Faisalabad. A pot experiment was conducted to examine the influence of foliar spray of proline on two wheat cultivars (Galaxy-13 salt sensitive) and (Pasban-90 salt resistant) under saline and non-saline conditions. The experiment was laid out in a complete randomized design (CRD) with factorial arrangement and four replications. Twenty seeds of each cultivar were sown in plastic pots having diameter of 30 cm and depth of 24 cm as well as containing 9 kg well mixed dry sand. After ten days of germination, six plants of constant size per pot were retained. Full strength Hoagland's nutrient solution was used to provide sufficient nutrients to the plants. Treatment of NaCl (150 mM) through rooting medium and proline (100 mM, Daijing) as foliar spray were applied after three weeks of germination with interval of seven days when 3-5 leaves arose. The growing pots were irrigated at regular interval till the termination of the experiment. There were following six treatments including control each with three replicates with and without sodium chloride and proline as given below:

- 1) Control (0 mM) + (Non- spray)
- 2) Control (0 mM) + (Water spray)
- 3) Control (0 mM) + Proline (100 mM)
- 4) Saline (150 mM NaCl) + (Non- spray)
- 5) Saline (150 mM NaCl) + (Water spray)
- 6) Saline (150 mM NaCl) + Proline (100 mM)

#### **Harvesting and measurement of morphological attributes**

The pot grown plants were carefully uprooted from

the pots after three weeks of treatment. For each treatment, two plants were uprooted, washed with distilled water and separated into root and shoot. Shoot and root lengths of two randomly selected plants from every pot were measured in cm and their means were calculated. Root and shoot fresh weights (g) were taken instantly by the help of an electrical balance after uprooting the plants. The number of leaves and number of tillers per plant were calculated with common observation and mean values were recorded. Dry weights of root and shoot (g) were recorded by placing same samples of root and shoot in an oven at 75°C for three days. The dry weights were calculated by the help of an electrical balance and leaf area was measured by multiplying maximum length, maximum width and correction factor.

### **Determination of photosynthetic attributes**

#### **O.J.I.P chlorophyll fluorescence induction kinetics**

By a fluor pen (FP 100), focused on leaf which was dark adapted (So, all PS2 and put in dark condition along with reaction centers open) cover-up by a clip-on area of 4 mm diameter to facilitate with consistent illumination. Fluorescence of chlorophyll a transient was recorded providing with a range of six diodes that are light emitting. From 10  $\mu$ s to 2s, fast fluorescence was measured, and fluor pen was set as:

When all the reaction centers were opened, initial fluorescence  $F_0$  as O (2  $\mu$ s), while L (150 s), K (300 micro  $\mu$ s), J (200  $\mu$ s) and I (30000  $\mu$ s) are intermediates and represented as  $F_l$ ,  $F_k$ ,  $F_j$ ,  $F_i$  and maximum fluorescence  $p$  (500000  $\mu$ s). In first original chlorophyll a transient of different varieties were plotted without normalization. For detailed analysis of data, different normalizations and differential kinetics were measured later. In between initial and maximum fluorescence, transients were double normalized and fluorescence resistant between OP represented as  $V_{ok}$  was found ( $V_{ok} = (f_{tfo}/f_{k-fo})$ ) to know about changing inflorescence at 300  $\mu$ s by double normalizing the data between  $f_0$  and  $f_k$  (0.3 ns). Difference in transients ( $v_{ok}$ ) was designed are well as characterized by L band.

At next, transients of chlorophyll fluorescence dual normalized among  $F_0$  920  $\mu$ s) and  $F_j$  (2000  $\mu$ s) by formula ( $V_{oj} = (F_t - F_0)/(F_j - f_0)$ ). This difference in transients ( $v_{ok}$ ) was calculated and expressed as K band. Data of chlorophyll fluorescence was normalized between the range of 50  $\mu$ s to 1 s, to measure changing during O-I phase and represented as  $v_{ot} (- < 1) = (F_t -$

$F_0)/(F_i - F_0)$  and with respect to varieties/ cultivars differential kinetics was measured. P phase was calculated by these two approaches.

a)  $V_{oi} (>1) = (F_t - F_0)/(F_i - F_0)$  was calculated normalizing fluorescence transients 30000 to 300000 micro s.

b)  $V_{ip} = (F_t - F_i)/(F_m - F_i)$  was calculated by normalizing transients between 30000 to 200000 us.

#### **Estimation of SPAD**

Subsequent to salinity treatment, total chlorophyll contents were recorded with a pocket size transferable chlorophyll meter (Minolta, SPAD- 502 Japan) from the top of fully mature third leaf.

#### **Measurement of Quantum Yield**

Quantum yield (Kharkwal and Shu) was measured of the third fully expanded leaf from the top of the plant by the Fluor Pen FP 100 MX-LM (Photon System International, Czech Republic. Model: FP 100) pocket size instrument after one week of salt application. The dark adapted QY was measured by taking the pre-dawn values.

#### **Determination of yield attributes**

##### **Spike length (cm)**

Spike length of each replicate was measured from the base of spike to the tip of spike and mean values were calculated.

##### **Number of spikelets per spike**

Number of spikelet per spike of each replicate was determined and mean values were calculated.

##### **Number of grains per spike**

Number of grains were determined per spike of each replicate and mean values were calculated.

##### **100 grain weight (g)**

The grains from each treatment were intermingled and were weighed by means of electric balance and average was calculated.

##### **Yield per plant (g)**

Yield per plant was weighed by collecting all the grains produced by all tiller over three plants and the mean values were calculated.

#### **Statistical analysis**

The data from this completely randomized design experiment were statistically analyzed using COSTAT computer software, while the graphical presentation of data was carried out using Microsoft Excel software.

### **RESULTS AND DISCUSSION**

The statistical data for shoot and root fresh and dry weight showed significant results between cultivars and

treatments (Sodium chloride and Proline) while, non-significant results among the interaction between cultivars and treatments. The salt stress caused a significant ( $p \leq 0.05$ ) reduction in shoot and root fresh and dry weight of both cultivars (Table 1). Application of proline caused a significant ( $p \leq 0.05$ ) increase in shoot and root fresh and dry weights of both the cultivars (Table 1). Maximum increase in shoot and root fresh weights were noted in cv. Pasban-90 when 100 mM

proline was applied under non-saline conditions (Control) as compared to salt stress (Figure 1). The maximum shoot and root dry weight reduction was observed in root dry weight in cv. Galax-13 under saline conditions when 0 mM proline was applied (Figure 1). Maximum increase in shoot and root dry weights were observed in cv. Pasban-90 when 100 mM proline was applied exogenously under non-saline (Control) conditions as compared to salt stress (Figure 1).

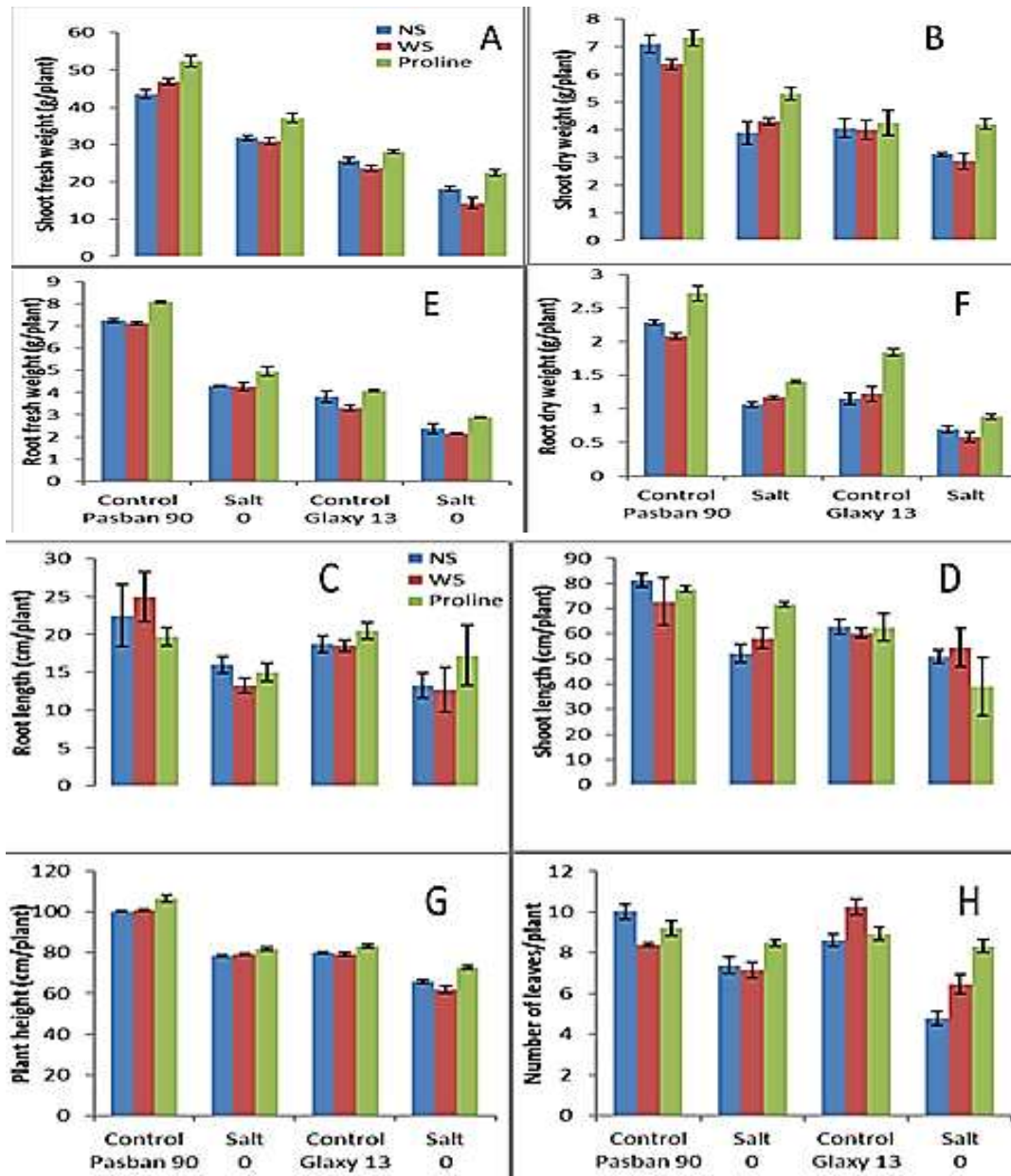


Figure 1: Fresh weight of shoot (A) and Root (E), dry weight of shoot (B) and root (F), root length (C), shoot length (D), Plant height (G) and number of leaves (H) of two wheat cultivars when three-week-old plants were subjected to foliar application of proline and salt stress for further two weeks.

Table 1: Analysis of variance for shoot and root fresh and dry weight of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	Df	Shoot fresh wt	Shoot dry wt	Root fresh wt	Root dry wt
Cvs	1	3.51 ***	46.40 ***	100.60 ***	727.25 ***
Salt	1	5.33*	29.47 ***	54.12 ***	3.87ns ***
Proline	2	58.77***	3.52 ***	2.64 ***	55.24 ***
Cvs*Salt	1	3.87ns	8.95 ***	8.87 ***	40.928 ***
Cvs*Proline	2	355.84**	0.04 ns	0.13 ns	36.125**
Salt *Proline	2	19.297*	1.11 *	0.04 ns	185.28***
Cvs*Salt*Proline	2	11.18**	0.50 ns	0.04 ns	0.50 ns
Error	36	1.52	0.25	0.06	3.85

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

The maximum increase in plant height was noted in cv. Pasban under non-saline (Control) conditions when 100 mM proline was applied (Figure 1). On the other hand, maximum decline in plant height was observed in cv. Galaxy under saline conditions when 0 mM proline was applied (Figure 1). Foliar application of proline increased the number of leaves/plant in cv. Galaxy-13 under non-saline (Control) conditions (Figure 1). The number of leaves/plants in cv. Galaxy-13 declined significantly under saline conditions when 0 mM proline was applied (Figure 1). Overall, cv. Pasban-90 showed better results as compared to cv. Galaxy-13 (Figure 1).

Application of salt stress caused a significant ( $p \leq 0.05$ ) reduction in numbers of tillers (Table 3) and the application of proline caused a significant ( $p \leq 0.05$ ) increase in number of tillers (Table 2). Maximum reduction in number of tillers was noted in cv. Pasban-90 under saline conditions when 0 mM proline was applied (Figure 2). Foliar application of proline increased the number of tillers in cv. Galaxy-13 under non-saline (Control) conditions when proline was applied (Figure 2).

In case of number of fertile tillers, the statistical data showed significant results between cultivars and treatments (Sodium chloride and Proline) while non-significant results among the interaction between cultivars and treatments (Table 3). Application of salt stress caused a significant ( $p \leq 0.05$ ) reduction in numbers of fertile tillers (Table 3). Application of proline caused a significant ( $p \leq 0.05$ ) increase in number of fertile tillers (Table 3). Exogenous application of proline enhanced the number of fertile tillers in cv. Pasban-90 under non-saline (Control) conditions (Figure 2).

However, in cv. Galaxy-13 numbers of fertile tillers were reduced under saline conditions when 0 mM proline was applied (Figure 2). In case of leaf area, salt stress decreased the leaf area of plants in cv. Galaxy-13 when 0 mM Proline was applied (Figure 2). Foliar spray of proline enhanced the leaf area of plants in cv. Pasban-90 under non-saline (Control) conditions (Figure 2).

Maximum increase in number of spikelet per spike was noted in cv. Pasban-90 under non-saline (Control) conditions when proline was applied (Figure 2). On the other hand, cv. Galaxy-13 showed a decline in the number of spikelet per spike under saline conditions when 0 mM proline was applied (Figure 2). Overall, foliar application of proline gave better results in both cultivars under saline and non-saline conditions with respect to number of spikelets per spike (Figure 2).

The statistical data for total grain yield, 100 seed weight and spike length showed significant results between cultivars and treatments (Sodium chloride and Proline) while, non-significant results among the interaction between cultivars and treatments (Table 4). In case of total grain yield, application of salt stress caused a significant ( $p \leq 0.05$ ) reduction in total grain yield (Table 4). Application of proline caused a significant ( $p \leq 0.05$ ) increase in total grain yield (Table 4) while maximum reduction was observed in cv. Galaxy-13 under saline conditions when 0 mM proline was applied (Figure 2). Maximum increase was noted in cv. Pasban under control conditions (Figure 2). Overall, when proline was applied under saline and non-saline conditions cv. Pasban-90 gave better results as compared to cv. Galaxy-13 (Figure 2). In case of number of spikes/plant, application of salt stress caused a significant ( $p \leq 0.05$ )

reduction in number of spikes/plant (Table 4). Maximum reduction was observed in cv. Galaxy under saline conditions when 0 mM proline was applied

(Figure 2). Foliar application of proline also enhanced the number of spikes/plant under non-saline conditions in cv. Galaxy-13 (Figure 2).

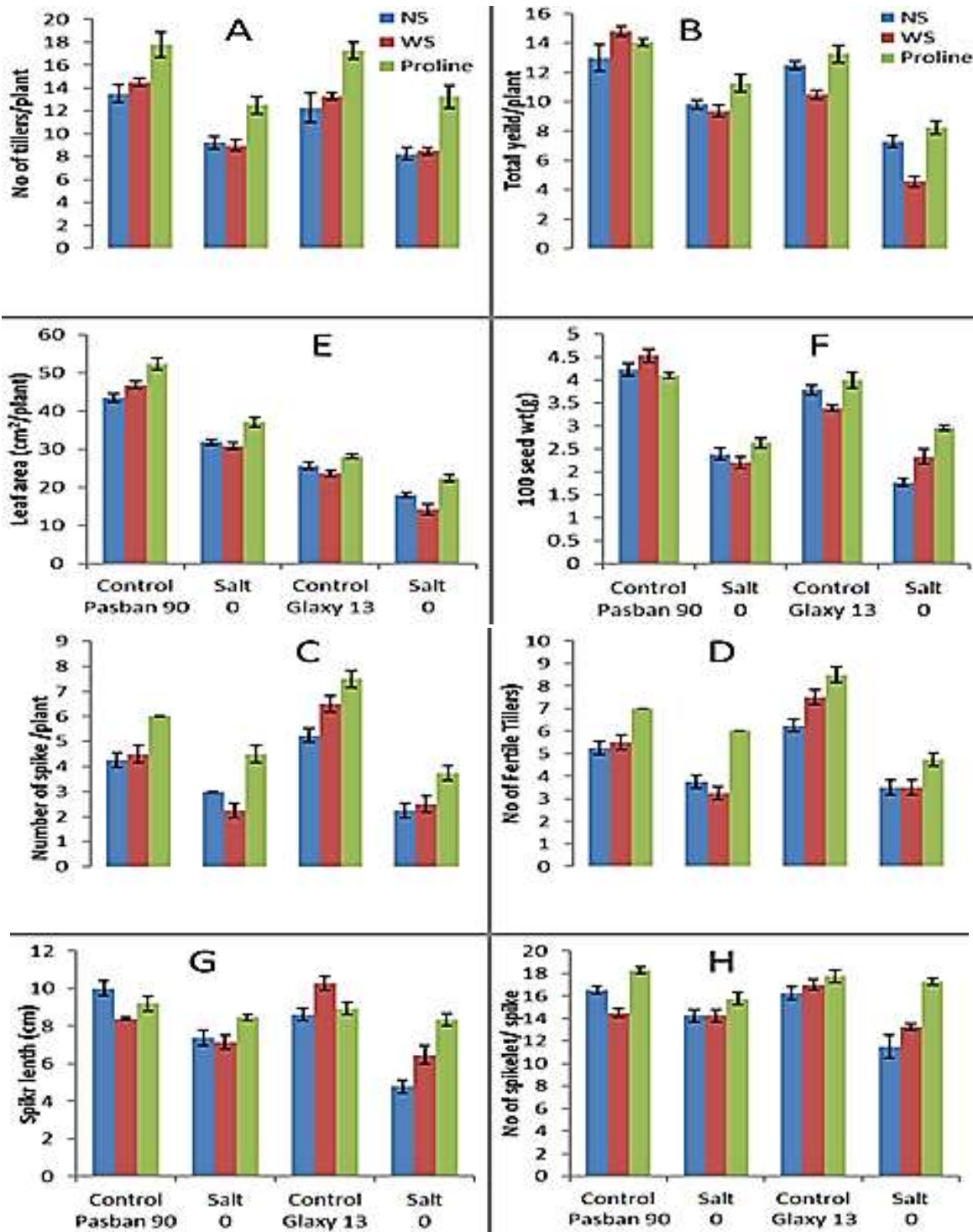


Figure 2: Number of tillers (A), Leaf area (E), total yield(B) 100 seed weight (F), number of spike (C) Number of fertile tillers (D), spike length (G) and number of Spikelet (H) of two wheat cultivars when three-week-old plants were subjected to foliar application of proline and salt stress for further two weeks.

Table 2: Analysis of variance for shoot and root length and number of leaves of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	Shoot length	Root length	Plant length	Number of leaves
Cvs	1	6463.52 ***	133.33 ***	7225.07 ***	1.02 ns
Salt	1	3855.67 ***	522.72 ***	250.00 ***	3834.19 ***
Proline	2	94.88 ***	34.26 ***	446.64 ***	270.81 ***
Cvs*Salt	1	838.34 ***	1.61 ns	63.77 ***	42.19 **
Cvs*Proline	2	13.76 ns	0.34 ns	69.34 ***	24.15 **
Salt *Proline	2	17.20 *	2.89 ns	6.61 ns	58.19 ***
Cvs*Salt *Proline	2	7.11 ns	0.24 ns	3.94	6.94 ns
Error	36	5.07	1.03	2.98	3.74

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

Table 3: Analysis of variance for number of tillers, number of fertile tillers, flag leaf area and number of spikelet per spike of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	Number of tillers	Number fertile tillers	Flag Leave Area	Number of spikelet per spike
Cvs	1	4.69 ns	3.52 ***	4061.06 ***	0.083ns
Salt	1	256.69 ***	77.52 ***	1430.19 ***	65.33***
Proline	2	91.75 ***	16.58 ***	172.91 ***	35.08***
Cvs*Salt	1	1.69 ns	11.02 ***	137.40 ***	5.33*
Cvs*Proline	2	1.75 ns	1.08 *	20.37 **	6.08**
Salt *Proline	2	1 ns	1.08 *	9.50 ns	4.33**
Cvs*Salt *Proline	2	0.25 ns	0.59 ns	7.76 ns	8.58***
Error	36	1.65	0.24	2.98	0.80

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

In case of 100 seed weight, Application of salt stress caused a significant ( $p \leq 0.05$ ) reduction in 100 seed weight (Table 4). Application of proline caused a significant ( $p \leq 0.05$ ) increase in 100 seed weight (Table 4). Salt stress decreased the 100-seed weight in cv. Galaxy when 0 mM proline was applied (Figure 2). However, 100 seed weight were increased in cv. Pasban under control conditions when water spray was applied (Figure 2). Foliar application of proline reduced the effects of salt stress in both cultivars (Figure 2). Overall, cv. Pasban-90 performed as compared to cv. Galaxy-13 (Figure 2). In case of the number of leaves/plant, application of salt stress caused a significant ( $p \leq 0.05$ ) reduction in number of leaves/plant (Table 4). Maximum number of leaves/plant was noted in cv. Galaxy-13 under non-saline (Control) conditions when

water spray was applied (Figure 2). Salt stress decreased the number of leaves/plant in cv. Galaxy-13 when 0 mM proline or water spray was applied (Figure 2).

The statistical data for chlorophyll content (SPAD) showed significant results between cultivars and treatments (Table 5). Application of salt stress caused a significant ( $p \leq 0.05$ ) reduction in chlorophyll content (SPAD) (Table 5). Application of proline caused a significant ( $p \leq 0.05$ ) increase in chlorophyll content (SPAD) (Table 5). Imposition of salt stress caused a significant decline in chlorophyll content (SPAD) in cv. Pasban (Figure 3). Exogenous application of proline under non-saline (control) conditions in cv. Pasban enhanced the chlorophyll content (SPAD) (Figure 3). Foliar spray of proline improved the chlorophyll content (SPAD) in both cultivars under saline and non-saline conditions (Figure 3).



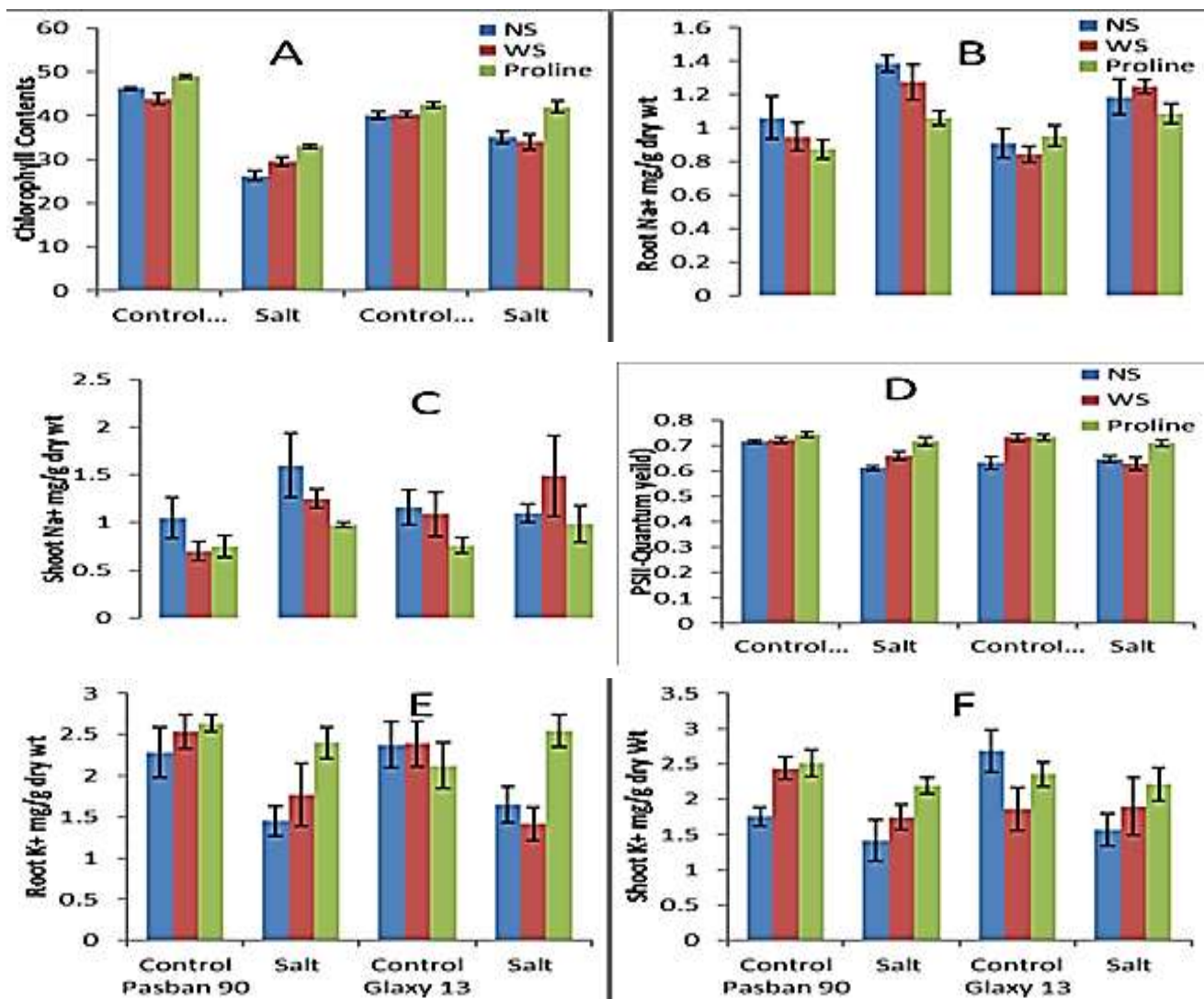


Figure 3: Chlorophyll contents (A), PS-11 quantum yield (D), root Na<sup>+</sup>(B), shoot Na<sup>+</sup>(C), root K<sup>+</sup>(E) and shoot K<sup>+</sup> (F) of two wheat cultivars when three-week-old plants were subjected to foliar application of proline and salt stress for further two weeks.

Table 4: Analysis of variance for total yield of plant, number of spike, 100 seed weight and spike length of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	Total grain yield per	Number of spike	100 seed Weight	Spike length
Cvs	1	130.11***	3.52***	1.154***	3.52**
Salt	1	387.19***	82.68***	31.611***	54.76***
Proline	2	22.13***	14.33***	0.642***	14.79***
Cvs*Salt	1	11.18**	11.02***	0.761***	4.29**
Cvs*Proline	2	16.75***	1.08*	0.550***	3.31***
Salt *Proline	2	16.74**	1*	0.468***	1.42*
Cvs*Salt *Proline	2	5.68ns	1.08*	0.526***	0.64ns
Error	36	1.35	0.24	0.526	0.34

Salt = Salt stress wt = weight

ns = non-significant; \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability



Table 5: Analysis of variance for shoot and root fresh and dry weight of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	Df	SPAD	Quantum yield
Cvs	1	281.33 ***	0.00 ns
Salt	1	120.33 ***	0.03 ***
Proline	2	494.08 ***	0.02***
Cvs*Salt	1	0.75 ns	0.00 ns
Cvs*Proline	2	18.08**	3.27e-4 ns
Salt *Proline	2	17.58 **	0.00 *
Cvs*Salt *Proline	2	3.19**	0.00 ***
Error	36	7.08e-4	7.08e-4

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

The statistical data for PS-II quantum yield showed significant results among the interaction between cultivars and treatments while non-significant results between cultivars (Table 5). Application of proline caused a significant ( $p \leq 0.05$ ) increase in PS-II quantum yield (Table 5). Maximum increase was noted in cv. Pasban-90 under non-saline (control) conditions when proline was applied as foliar spray (Figure 3). A minimum decline was noted in PS-II quantum yield in cv. Galaxy-13 under non-saline (control) conditions (Figure

3). Overall, foliar application of proline enhanced the PS-II quantum yield in both cultivars under saline and non-saline (control) conditions (Figure 3).

The statistical data for shoot leaf  $\text{Na}^+$  showed significant results between cultivars and treatments (Sodium chloride and Proline) and among the interaction between cultivars and treatments (Table 6, Figure 3). Application of salt stress caused a significant ( $p \leq 0.05$ ) increase in leaf sodium  $\text{Na}^+$  (Table 6).

Table 6: Analysis of variance for leaf sodium, root sodium, leaf potassium and root potassium contents of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	Leaf $\text{Na}^+$	Root $\text{Na}^+$	Leaf $\text{K}^+$	Root $\text{K}^+$
Cvs	1	0.8546672***	1.549***	0.112ns	0.093ns
Salt	1	1.6446505***	0.022ns	3.234*	2.172**
Proline	2	0.2419068***	0.185***	1.08***	0.901**
Cvs*Salt	1	0.0423047**	0.051**	0.106**	0.005ns
Cvs*Proline	2	0.1066797***	0.125***	0.176ns	0.6468*
Salt *Proline	2	0.294688***	0.755***	1.134ns	0.279ns
Cvs*Salt *Proline	2	0.1314984***	0.078***	0.1938**	0.572*
Error	36	0.0095491	0.0059	0.180	0.171

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

Application of proline caused a significant ( $p \leq 0.05$ ) reduction in leaf sodium  $\text{Na}^+$  (Table 6). Maximum increase was observed in cv. Pasban-90 under saline conditions when water spray was applied (Figure 3). Foliar spray of water and proline under non-saline (control) conditions in cv. Pasban-90 decreased the concentration of leaf sodium  $\text{Na}^+$  (Figure 3). Foliar spray

of proline in both the cultivars declined the concentration of leaf sodium  $\text{Na}^+$  under saline conditions (Figure 3). Application of proline caused a significant ( $p \leq 0.05$ ) reduction in root sodium  $\text{Na}^+$  (Table 6). Maximum increase was observed in cv. Pasban-90 under saline conditions when 0 mM proline was applied (Figure 3). Maximum reduction was noted in cv. Galaxy-13 under

non-saline (control) conditions when water spray was applied (Figure 3). Application of proline caused a significant ( $p \leq 0.05$ ) increase in leaf potassium  $K^+$  (Table 6). Maximum increase was noted in cv. Galaxy-13 under non-saline (control) conditions when 0 mM proline was applied (Figure 3). Salt stress decreased the leaf potassium  $K^+$  in cv. Pasban-90 when 0 mM proline was applied (Figure 3). Foliar application of proline in both cultivars under saline and non-saline conditions enhanced the leaf potassium  $K^+$  (Figure 3). Application of salt stress caused a significant ( $p \leq 0.05$ ) reduction in root potassium  $K^+$  (Table 9). Application of proline caused a significant ( $p \leq 0.05$ ) increase in root potassium  $K^+$  (Table 6). Exogenous application of proline caused a maximum increase in root potassium  $K^+$  in cv. Pasban-90 under non-saline (control) conditions (Figure 3). Imposition of salt stress reduced the root potassium  $K^+$  in cv. Galaxy-13 when water spray was applied (Figure 3). Exogenous application of proline under saline and non-saline (control) conditions enhanced the root potassium  $K^+$  in both cultivars (Figure 3).

Analysis of variance of the data for minimal fluorescence from the dark-adapted leaf ( $F_o$ ) of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 7. The statistical data for minimal fluorescence from the dark-adapted leaf showed significant results between

cultivars and treatments while, non-significant results among the interaction between cultivar and treatment (Table 7). Analysis of variance of the data for fluorescence at phase J ( $F_j$ ) of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in (Table 7). The statistical data for fluorescence at phase J ( $F_j$ ) showed significant results between cultivars and treatment (Sodium chloride) while showed significant results between cultivars and treatments (Table 7). Analysis of variance of the data for fluorescence at phase I ( $F_i$ ) of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 7. The statistical data for fluorescence at phase I ( $F_i$ ) showed significant results between cultivars and treatment (Sodium chloride) and also showed significant results between cultivars and treatments (Table 7). Analysis of variance of the data for maximum fluorescence from the dark-adapted leaf ( $F_m$ ) of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 7. The statistical data for maximum fluorescence from the dark-adapted leaf ( $F_m$ ) showed significant results between cultivars and treatments (Sodium chloride and proline) and also showed non-significant results among the interaction between cultivars and treatments (Table 7).

Table 7: Analysis of variance of  $F_o$ ,  $F_m$  and  $F_v$  in leaves of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	$F_o$	$F_j$	$F_i$	$F_m$
Cvs	1	27297142***	3.767***	5.33***	93312 ns
Salt	1	7710803.4**	8542954.7 ns	1.691**	4167384.5 ns
Proline	2	4099508*	42558424 ns	21159867ns	2723778 ns
Cvs*Salt	1	32580.25 ns	2.22**	2349578ns	3130002 ns
Cvs*Proline	2	2797274.1ns	7839302.5 ns	2349578ns	1.17244***
Salt *Proline	2	4150271.2*	2.42***	5.348**	6745464.5 ns
Cvs*Salt *Proline	2	711506.08 ns	47974974 ns	42069391ns	17659624.5*
Error	36	1069239.9	23175144	20605887	3698947.8

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

$F_o$  = minimal fluorescence from the dark-adapted leaf,  $F_j$  = fluorescence at phase J,  $F_i$  = fluorescence at phase I,  $F_m$  = maximum fluorescence from the dark-adapted leaf.

Analysis of variance of the data for relative variable fluorescence at phase J of fluorescence transient curve ( $V_j$ ) of two wheat cultivars with or without foliar spray

of proline under saline and non-saline conditions is presented in Table 8. The statistical data for relative variable fluorescence at phase J of fluorescence transient

curve ( $V_j$ ) showed non-significant results between cultivars and treatments and among the interaction between cultivars and treatments (Table 8). Analysis of variance of the data for relative variable fluorescence at phase I of fluorescence transient curve ( $V_i$ ) of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 8. The statistical data for relative variable fluorescence at phase I of fluorescence transient curve ( $V_i$ ) showed significant results between cultivars while, non-

significant results among the interaction between cultivars and treatments presented in Table 8. Analysis of variance of the data for maximum yield of photochemistry of PSII ( $F_v/F_m$ ) of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 8. The statistical data for maximum yield of photochemistry of PSII ( $F_v/F_m$ ) showed non-significant results between cultivars and among the interaction between cultivars and treatments (Table 8).

Table 8: Analysis of variance for  $F_v$ ,  $V_j$ ,  $V_i$  and  $f_m/F_o$  of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	$F_v$	$V_g$	$V_i$	$F_m/F_o$
Cvs	1	3.001***	3.179***	0.04***	0.115ns
Salt	1	1.125***	1.968***	7.20ns	0.3177ns
Proline	2	11.18**	18973323ns	0.003ns	0.454*
Cvs*Salt	1	16.75***	7194489ns	8.3136ns	0.3177ns
Cvs*Proline	2	16.74**	3209588.1ns	5.753ns	0.161ns
Salt *Proline	2	4.29**	3.178**	0.003ns	0.195ns
Cvs*Salt *Proline	2	0.0023*	34.065747ns	0.0035ns	0.61ns
Error	36	4.5569e-4	14522808	0.0016883	0.1220

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

$V_j$ , = relative variable fluorescence at phase J of fluorescence transient curve,  $V_i$  = relative variable fluorescence at phase I of fluorescence transient curve,  $F_v/F_o$ =maximum primary yield of photochemistry,  $F_v/F_m$ = maximum yield of photochemistry of PSII.

Analysis of variance of the data for  $F_v/F_o$  of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 9. The statistical data for  $F_v/F_o$  showed significant results between cultivars and among the interaction between cultivars and treatments (Table 9). Analysis of variance of the data for  $F_v/F_m$  of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 9. The statistical data for  $F_v/F_m$  showed non-significant results between cultivars and among the interaction between cultivars and treatments (Table 9). Analysis of variance of the data for  $M_o$  of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 9. The statistical data for  $M_o$  showed significant results between cultivars and treatments (sodium chloride and proline) while, non-significant results among the interaction between cultivars and treatments (Table 9). Analysis of variance

of the data for area of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 9. The statistical data for area showed significant results between cultivars and among the interaction between cultivars and treatments while, non-significant results between treatments (Table 9).

Analysis of variance of the data for fix area of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 10. The statistical data for fix area showed significant results between cultivars and treatments (sodium chloride and proline) while, among the interaction between cultivars and treatments (Table 10). Analysis of variance of the data for  $S_m$  of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 10. The statistical data for  $S_m$  area showed significant results between cultivars while, non-significant results among the interaction between

cultivars and treatments (Table 10). Analysis of variance of the data for Ss of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 10. The statistical data for Ss showed significant results between cultivars while, non-significant results between cultivars and treatments

(sodium chloride and proline) and among the interaction between cultivars and treatments (Table 10). The statistical data for N showed significant results between cultivars and treatments (sodium chloride and proline) while non-significant results among the interaction between cultivars and treatments (Table 10).

Table 9: Analysis of variance for Fv/Fo, Fv/Fm, Mo and area of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	Fv/Fo	Fv/Fm	Mo	Area
Cvs	1	5.335***	0.159 ns	0.359***	4.182***
Salt	1	1.690**	0.122 ns	0.008025 ns	6.279 ns
Proline	2	21159867 ns	0.4336*	0.0279106 ns	2.610 ns
Cvs*Salt	1	2349578 ns	0.3554 ns	0.039*	9.296*
Cvs*Proline	2	531855 ns	0.0997 ns	0.0409*	4.395 ns
Salt *Proline	2	1.1511**	0.2009 ns	0.007ns	1.7405 ns
Cvs*Salt *Proline	2	2.22**	0.0702*	0.0080ns	37.38***
Error	36	2060587	0.0988838	0.0096257	1.7764

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

Area= size of reduced plastoquinone pool or the area above the chl. fluorescence between FO and FM, SM=multiple turnover number, SS=single turnover number, N= number of QA- redox turnover until FM.

Table10: Analysis of variance for fix area Sm, Ss and N of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	Fix Area	Sm	Ss	N
Cvs	1	1.2142***	268725.34***	0.0165551	6099687.03**
Salt	1	5.498***	228956.11***	0.004378*	1103412.1***
Proline	2	4.8106***	889.44284 ns	0.0031595*	14657.124 ns
Cvs*Salt	1	8.7725***	81426.382*	3.4844ns	24.7651.96*
Cvs*Proline	2	7.5819***	41922.905 ns	5.9511ns	175905.36*
Salt *Proline	2	6.1586***	1040.3ns	0.0030489*	5905.1599 ns
Cvs*Salt *Proline	2	2.1788***	2.8888628	6.6878ns	1491.8385 ns
Error	36	2.1768	14009.162	8.1302	56461.359

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

$\Phi$ PO = maximum quantum yield of primary photochemistry,  $\psi_o$ =capacity of PS II to transfer trapped excitation,  $\phi E_o$ =quantum yield of electron transport,  $\phi D_o$ = quantum yield of dissipation energy

Analysis of variance of the data for  $\phi_{P_o}$  of two wheat cultivars with or without foliar spray of proline under saline and non- saline conditions is presented in Table 11. The statistical data for  $\phi_{P_o}$  showed significant results among the interaction between cultivars and treatments (sodium chloride and proline) while, non-significant results between cultivars and treatments

(Table 11). Analysis of variance of the data for  $\psi_o$  of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 11. The statistical data for  $\psi_o$  showed significant results between cultivars and treatments (sodium chloride and proline) and among the interaction between cultivars and treatments

(Table 11). Analysis of variance of the data for  $\varphi_{Eo}$  of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 11. The statistical data for  $\varphi_{Eo}$   $\psi_o$

showed significant results between cultivars and treatments (sodium chloride and proline) while, non-significant results among the interaction between cultivars and treatments (Table 11).

Table 11: Analysis of variance of data for  $\varphi_{Po}$ ,  $\psi_o$ ,  $\varphi_{Eo}$ ,  $\varphi_{Do}$  and  $\varphi_{PAV}$  of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	$\varphi_{Po}$	$\psi_o$	$\varphi_{Eo}$	$\varphi_{Do}$	$\varphi_{PAV}$
Cvs	1	4.4451ns	0.0198106***	0.0279726***	4.4451ns	525.45 ns
Salt	1	1.5417ns	0.0064133*	3.7056ns	1.542 ns	3150.2027***
Proline	2	7.2552**	0.0060603*	0.0015022 ns	1.861 ns	45.707 ns
Cvs*Salt	1	3.4534ns	0.0098837**	0.00539*	3.454 ns	99.754*
Cvs*Proline	2	2.1072ns	0.0040926*	0.005601**	2.4909 ns	252.79 ns
Salt *Proline	2	2.2799ns	0.0028224*	9.8502 ns	4.1318 ns	4.68**
Cvs*Salt *Proline	2	2.0213**	5.8663*	9.0859 ns	3.1284 ns	23.50 ns
Error	36	1.4838	0.0012746	9.8663	1.4838	178.93431

Salt = Salt stress wt = weight

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

$\varphi_{Po}$ = maximum quantum yield of primary photochemistry,  $\psi_o$ =capacity of PS II to transfer trapped excitation,  $\varphi_{Eo}$ =quantum yield of electron transport,  $\varphi_{Do}$ = quantum yield of dissipation energy

The statistical data for  $\varphi_{Eo}$   $\psi_o$  showed non-significant results between cultivars and treatments (sodium chloride and proline) and among the interaction between cultivars and treatments (Table 11). Analysis of variance of the data for  $\varphi_{PAV}$  of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 11. The statistical

data for  $\varphi_{PAV}$   $\psi_o$  showed significant results between treatments (sodium chloride and proline) while, non-significant results among the interaction between cultivars and treatments (Table 11).

Analysis of variance of the data for ABS/RC of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 12.

Table12: Analysis of variance of data for ABS/RC, TRo/RC, ETo/RC and Dlo/RC in leaves of two wheat cultivars grown under salt stress conditions with foliar application of proline.

Source of variance	DF	ABS/RC	TRo/RC	ETo/RC	Dlo/RC
Cvs	1	57.99***	0.6730***	0.2612***	0.1297***
Salt	1	614512	0.0250ns	0.0684*	0.0048**
Proline	2	0.388ns	0.1748**	0.0537*	0.0040**
Cvs*Salt	1	45.0498***	0.0030ns	0.033ns	0.0023*
Cvs*Proline	2	0.0522ns	0.0838*	0.0119ns	0.0033*
Salt *Proline	2	0.1772ns	0.0189ns	0.04341*	0.0023*
Cvs*Salt *Proline	2	0.3361*	0.0174ns	0.0110ns	0.0023*
Error	36	0.1481	0.722	0.01244	4.5569e-4

ns = non-significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 probability

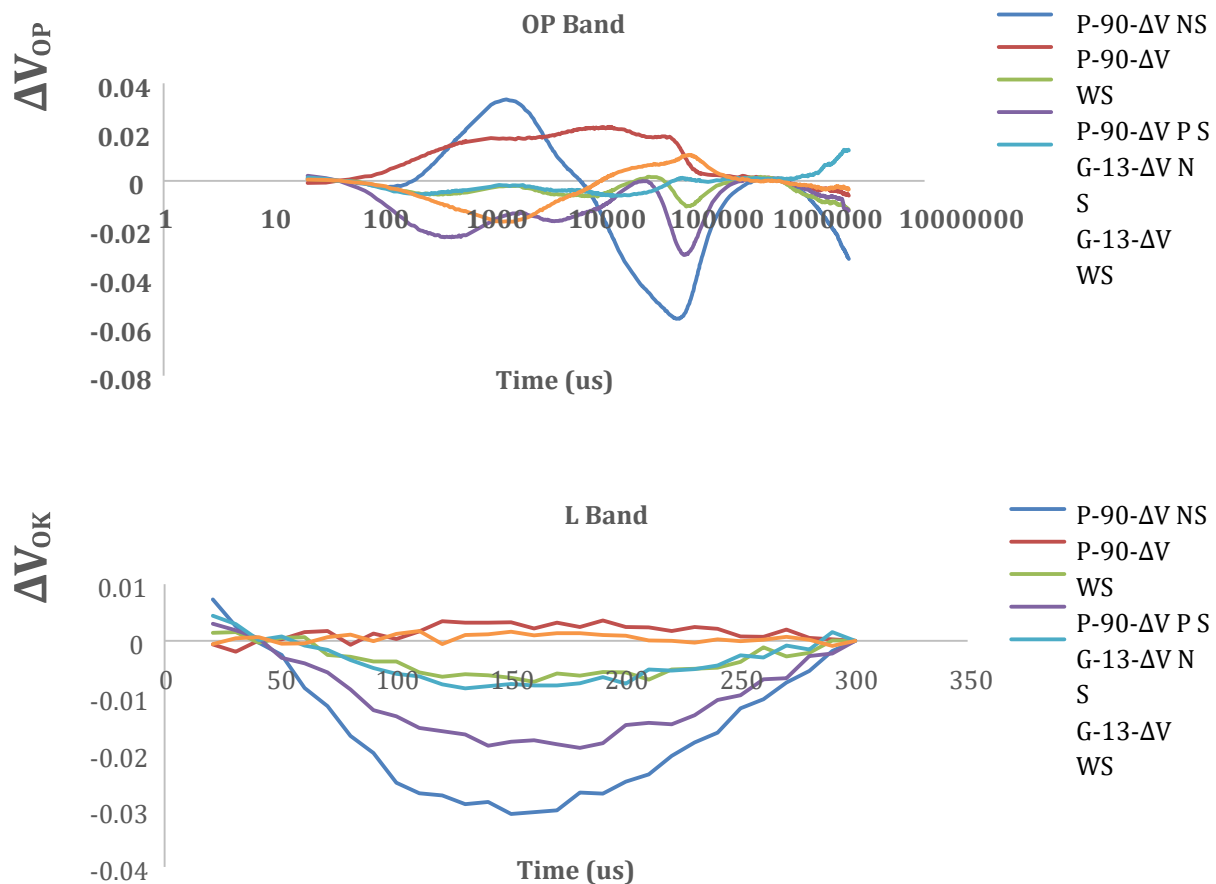
GB = Glycine betaine, Salt = Salt stress wt. = weight

ABS/RC=absorbance flux per reaction center, TRo/RC= trapped energy flux per reaction center, ETo/RC= electron transport flux per reaction center, Dlo/RC=dissipation energy flux per reaction center.

The statistical data for ABS/RC showed significant results between cultivars and among the interaction between cultivars and treatments while, non-significant results between treatments (sodium chloride and proline) (Table 12). Analysis of variance of the data for TR<sub>o</sub>/RC of two wheat cultivars with or without foliar spray of proline under saline and non-saline conditions is presented in Table 12. The statistical data for TR<sub>o</sub>/RC showed significant results between cultivars while, non-significant results among the interaction between cultivars and treatments (Table 12).

Polyphasic chlorophyll a fluorescence transient was measured to evaluate the proline effect on wheat cultivars under saline and non-saline condition on photochemical efficiency of PSII. The time course of fluorescence yield in dark adapted leaves plotted on logarithmic time scale clearly showed the separation of OJIP phase of two wheat cultivars. A comparison was made among the raw OJIP transient measured under

saline and non-saline condition with or without exogenous foliar spray of proline of both cultivars of wheat. In cv. Pasban-90 Ft of the dark-adapted control plants exhibited a typical OJIP transient with Fo of ~8466, Fm of ~40717 and variable fluorescence Fv of ~32251. Similarly, in cv Glaxy-13 Ft of the dark-adapted control plants exhibited a typical OJIP transient with FO of ~8153.2, Fm of ~40017.6 and variable fluorescence Fv of ~31864.4. Salt stress decreased the fluorescence at O and J phases of both cultivars of wheat. Proline application significantly increased the fluorescence at O phase of both cultivars of wheat under saline condition. Salt stress decreased the fluorescence at J phase, while Proline application ameliorates the salt effect on fluorescence at J phase. Proline application had no effect on J, I and P phases of both cultivar of wheat under non-saline condition. Exogenous foliar application of proline reduces the effect of salinity on fluorescence at O, J, I and P phases of both cultivars of wheat (Figure 4 and 5).





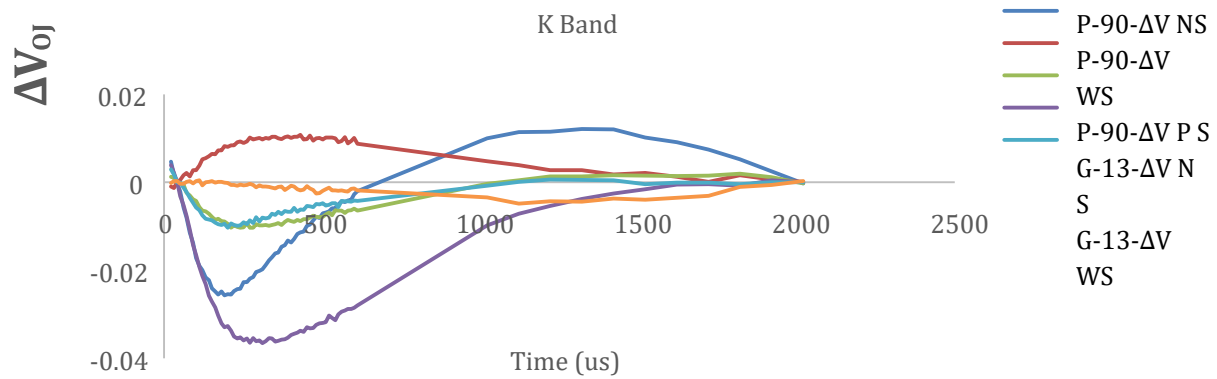


Figure 4: OP, L and K bands of plants of two wheat cultivars when three week old plants were subjected to foliar application of Proline and salt stress for further two weeks. In legends P represents cultivar Pasban and G represents cultivar Glaxy.  $\Delta V_{OK}$  was obtained as follow:  $[VOP\ salt - VOP\ cont] = \Delta V_{OP\ Salt}$ ,  $[VOP\proline - VOP\ cont] = \Delta V_{OP\proline}$ ,  $[VOP\proline + Salt - VOP\ cont] = \Delta V_{OP\proline + Salt}$ ,  $[VOP\proline + Salt - VOP\ Salt] = \Delta V_{OP\proline\ salt}$ . By following similar pattern K, L, OI and IP bands obtain.

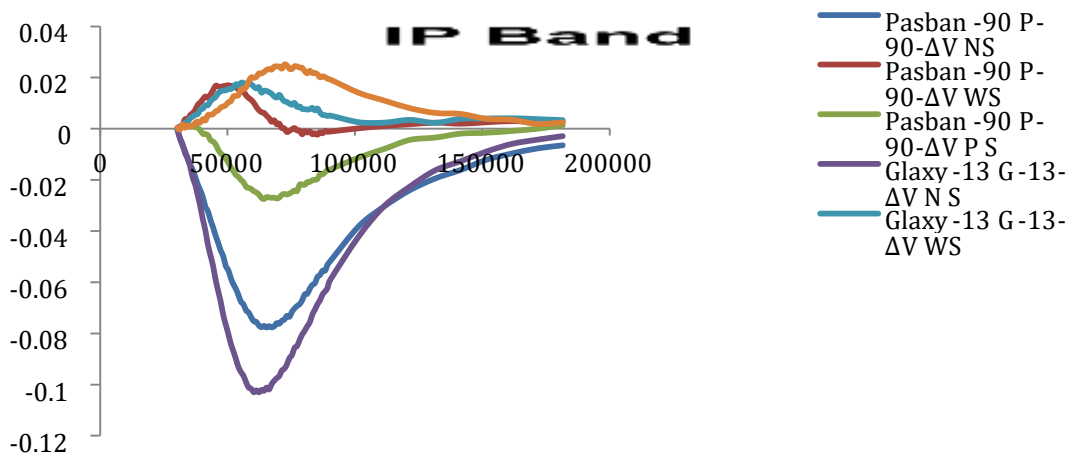
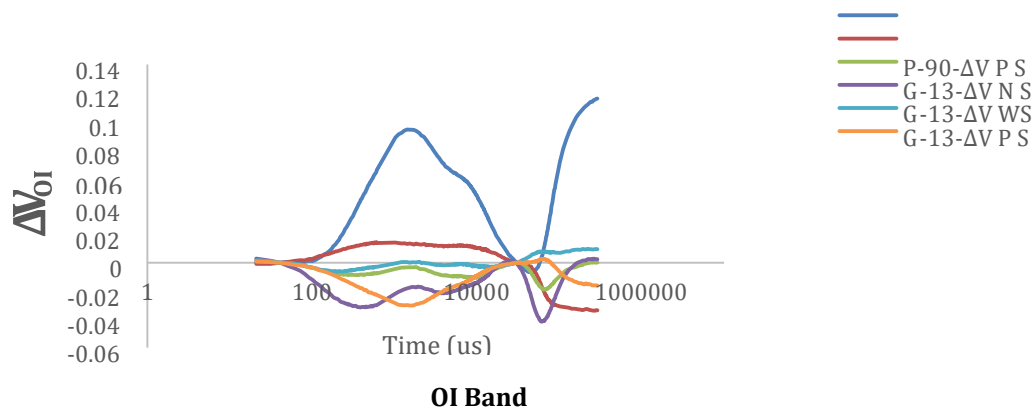


Figure 5. OI Band and IP Band of the plants of two wheat cultivars when three week old plants were subjected to foliar application of glycine betaine and salt stress for further two weeks.

To reveal the detailed fluorescence yield changes during fluorescence kinetics, the OJIP transients were normalized between  $F_0$  and  $F_M$  ( $V_{OP}$ ) in both cultivars of wheat. Changes in chlorophyll fluorescence at each step (K, J, I and P), data was normalized and obtained OP, L, K, OI, IP bands graphs that shown in Figure 4 and 5.  $\Delta V_{OK}$  (L band) was obtained as follow:  $[V_{OK \text{ salt}} - V_{OK \text{ cont}}] = \Delta V_{OK \text{ Salt}}$ ,  $[V_{OK \text{ proline}} - V_{OK \text{ cont}}] = \Delta V_{OK \text{ proline}}$ ,  $[V_{OK \text{ proline}} + \text{Salt} - V_{OK \text{ cont}}] = \Delta V_{OK \text{ proline}} + \text{Salt}$ ,  $[V_{OK \text{ proline}} + \text{Salt} - V_{OK \text{ Salt}}] = \Delta V_{OK \text{ proline salt}}$ . Negative peaks obtained under saline condition revealed that salinity stress greatly affected the fluorescence at 175 $\mu$ s of both cultivars of wheat, while by giving foliar spray of proline, less negative peaks was obtained. However, by giving proline under saline condition, positive peaks were obtained.  $\Delta V_{OJ}$  (K band) of different treatments presented in figure 4 was calculated in a similar way as  $\Delta V_{OK}$  (L band). Plants of both cultivars tolerated the salinity stress till 1000 $\mu$ s after that positive peak obtained under saline condition and similar but slightly lower peak obtained when foliar spray of proline applied under saline condition.  $\Delta V_{OI}$  (OI band) of different treatment presented in (Figure 5) was calculated as  $\Delta V_{OJ}$  (K band). Negative peaks obtained under both saline condition and by foliar application of proline under saline condition.  $\Delta V_{IP}$  (IP band) was also calculated as above.

#### CONCLUSION

It is concluded from the present study that foliar spray of 100 mM proline was the most effective to ameliorate the toxic effects of salinity by improving biomass production, chlorophyll fluorescence, chlorophyll contents, and quantum yield in both the cultivars. The findings confirmed the ability of foliar spray of proline to stimulate salt tolerance in wheat plants.

#### AUTHORS' CONTRIBUTION

SJ, SK and MAA designed, planned and prepared layout of the study, SJ, AH and MSAA conducted greenhouse experiments and recorded the data, SJ, AI and MU compiled and organized the data, SJ, SK and MAA analyzed the data, MU made the graphs, all the authors helped in manuscript write up and formatting and SJ, SK, MSAA and MU proofread the manuscript.

#### CONFLICT OF INTEREST

The authors declare no conflict of interests.

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