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Proline Induced Modulation in Physiological Responses in Wheat Plants

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Abstract

Proline is multifunctional amino acid and has roles in inducing salt stress tolerance. Contrasting reports are available on its mechanism of stress tolerance in crop plants such as osmoprotectant, osmotic adjustment, ROS scavenging, ion uptake, and photosynthesis. A pot experiment was conducted to assess proline-induced changes in physiological processes in four wheat cultivars differing in salinity tolerance. Shoot and root fresh and dry weights, length and width of leaves, quantum yield and chlorophyll contents increased by the foliar application of proline. Proline also affected the absorption, trapping and electron transport per reaction center maximaly in S-24 as compared to other three cultivars. Proline modulate the physiological and biochemical processes in wheat cultivars.

Keywords: foliar spray, osmoprotectant, antioxidant, photosynthesis, water potential

Introduction

Wheat (*Triticum aestivum* L.) is major cereal crop and used as a staple food in the world (FAO, 2018). In view of rapidly increasing world population and climatic changes which largely affects crop productivity, it is urgently require to increase the crop productivity. However, crop productivity including that of wheat is mainly hampered by abiotic stresses such as salt stress (Munns & Tester, 2008). Salinity has two major impacts on plants growth by salt induced osmotic and toxic effect (Hasegawa et al., 2000; Munns & Tester, 2008). Salinity also limits the nutrients uptake such as K^+, Ca^{2+}, Mg^{2+}etc., reduces enzymatic activities and membrane permeability through stomatal closure (Ashraf, 2004; Munns & Tester, 2008; Roy, 2014; Horie and Ismail, 2017).Excessive accumulation of Na^+in photosynthetic tissues can cause oxidative stress, which lower photosynthesis by the loss of chlorophyll, and quantum yield of PSII (Ayesha et al., Chaves et al., 2009; Foyer & Shigeoka, 2011; Foyer & Noctor, 2015). These physiological changes impair the cellular structures and diminish growth and yield. Salt tolerant plants are provided with several resistance mechanisms to subsist with salt stress like osmoregulation, ion homeostasis, antioxidant and hormonal regulation (Flowers & Colmer, 2015; Slama et al., 2015). For example, plants accumulate large quantities of different types of compatible solutes to cope with salinity. Compatible solutes are low molecular weight, highly soluble organic compounds that are usually non-toxic at high cellular concentrations such as proline, glycinebetaine, trehalose, polyols etc. These solutes contribute to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization in plants (Ashraf et al., 2007). Of these osmoprotectants, proline is an important compatible solute which protects membranes from lipid peroxidation, and photosystem II (PSII) from photo-damage and scavenge free radicals such as hydroxyl (OH.) and peroxide ion (Ashraf & Foolad, 2007).It may also help in maintaining appropriate $NADP^+/NADPH$ ratios compatible with metabolism (Hare & Cress, 1997). In addition, rapid breakdown of proline upon relief from stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages (Hare and Cress, 1997; Hare et al., 1998). Furthermore, proline is known to induce expression of salt stress responsive genes, which possess proline responsive elements (e.g. PRE, ACTCAT) in their promoters (Satoh et al., 2002; Chinnusamy et al., 2005).

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Thus, engineering or breeding plants with higher proline is one of the suggested way to improve salt tolerance in crops. For example, it has been observed that plants accumulated greater proline were more tolerant to salt stress (Kavi-Kishore et al., 1995; Ashraf and Harris, 2004). Similarly, the ameliorative and osmoprotective role of proline has been appreciably shown in overproducing proline transgenics of **wheat/rice/tomato** (Molinari et al., 2007). However, success in developing salt tolerant plants is very poor (Mansour et al., 2017). Endogenous level of proline can be enhanced by exogenous foliar application of proline (Ashraf and Foolad, 2007). This alternative approach can provide osmoprotection and improve growth under salt stress such as in canola (Athar et al., 2009). However, some controversial reports are available regarding proline induced salt tolerance. For example, while working with radish Ceppi et al. (2012) did not find any improvement in salt tolerance in rice and barley plants. They suggested that accumulated proline is not sufficient for osmotic adjustment to trigger stress tolerance responses. In view of these reports, present study was aimed to assess upto what extent exogenously applied proline modulate physiological responses that induce salt tolerance in wheat cultivars differing in salt tolerance.

Material and method

Seeds of four wheat (*Triticum aestivum L.*) cultivars (cv. Galaxy-13,cv. Pasban-90,cv. Sahar-06 and S-24) differing in salinity tolerance were obtained from Institute of Pure and Applied Biology,Bahauddin Zakariya University, Multan , Pakistan. The experiment was conducted at the Botanic garden of Bahauddin Zakariya University, Multan, Pakistan. Before experimentation seeds of each wheat cultivar were sterilized with NaHOCl and then washed with distilled water thrice. Seeds of each wheat cultivar were sown in plastic pots (**32 x 12 cm**), each of which filled with 5 kg river sand washed thoroughly with water. Plastic pots have drainage holes at the bottom covered with a piece of muslin cloth. The seeds were allowed to germinate for one week .The ½ L of half strength Hoagland soln. was applied to each pot. Plants were thinned to 8 plants per pot initially and then to 5 plants per pot. Plants of uniform size and placed equidistantly were selected. Four weeks after the start of the experiment, pots were irrigated with full strength Hoagland's nutrient solution containing 0 or 200 mM NaCl. The salinity level was developed by the addition of 50 mM NaCl stepwise. Proline (0 or 100mM) was applied as a foliar spray to plants of each wheat cultivars growing in non- saline and saline conditions. Plants were sprayed in the evening to avoid drying of solution on leaves and allowing maximum proline penetration into the leaf tissue. Hoagland's nutrient solution containing (0 or200 mM NaCl) was replaced every week to replenish nutrients. However, treatment solution was applied in excess to each pot so as to flush through all the salts previously present in the sand and to ensure the desired salt level. After four weeks of proline treatment (2 months old plants), plants were harvested, washed with distilled water, blotted dry and separated into shoots and roots, and data for fresh root and shoot biomass was recorded. These plants were then oven dried at 65C for 72h and dry root and shoot biomass was recorded. However, before harvest quantum yield, SPAD, OJIP, leaf water potential, leaf length and width parameters were also measured. Quantum yield was measured by using Flour Pen FP100 (Photon System Instruments, Bruno, Czech Republic) after 3 weeks of germination. The reading was obtained in sunlight to get the best results. Leaf length was measured from base of the leaf to the tip of the leaf and leaf width was taken from the central part of the leaf to get accurate readings.FlourPen was used to measure different fluorescence parameters i.e. OJIP. After that the recorded data was downloaded to computer and OJIP analysis was taken for Fv/Fm, PI_{ABS}, ABS/RC, TRo/RC, ETo/RC and DIo/RC.Water potential was measured by using water potential apparatus before sunrise to get accurate readings.0.2g of the leaf was taken and placed in acetone. Chlorophyll a, chlorophyll b and carotenoids concentration were measured with a UV-160A UV-Vis recording Spectrometer at 750, 663, 645,652 and 470.

Statistical analysis of data

The data is put into Excel sheets to get the mean, Standard deviation and accurate graphs. The data obtained from all parameters is described as mean value ± SE. By using the statistical software COSTAT, data were subjected to three way analysis of variance (ANOVA).

Results and discussion

Analysis of variance of the data for shoot fresh and dry weight of four cultivars of wheat (Table 1) showed that salt stress significantly reduced fresh and dry weights of shoots of all wheat cultivars. The inhibitory effects of salt stress on plant growth and biomass production are well known (Ma et al., 2013). The reason for decrease in their fresh and dry masses may be due to enhanced osmotic potential by increasing salts, which leads to dehydration, ionic imbalance in transpiring leaves that reduces meristem activity and cell elongation, consequently inhibit the growth of wheat plant (Zhu, 2001; Munns, 2005; Huang et al., 2006).All four wheat cultivars varies in their shoot fresh and dry weights under non-saline or saline conditions.

Table 1. Mean squares from analysis of variance of data for shoot and root fresh and dry weight of four cultivars of wheat (*Triticum aestivum* L.) treated with or without foliar spray of Proline to salt stressed and non-stressed plants.

Source of variance	df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Leaf length	Leaf width
Salt	1	259.2***	170.4***	3.603***	2.153***	954.8***	4.51***
Cultivars	3	36.00***	49.42***	1.450***	0.239***	208.6***	1.80***
Proline	1	12.78***	5.8**	0.065*	5.60ns	105.0***	0.36**
Salt x Cvs	3	4.17***	9.51***	0.106***	0.062***	22.9*	0.11*
Salt x Proline	1	0.47ns	0.21ns	0.007ns	0.002ns	0.45ns	0.04ns
Proline x Cvs	3	0.31ns	0.18ns	0.024ns	0.001ns	8.38ns	0.025ns
Salt x Cvs x Proline	3	0.26ns	0.38ns	0.005ns	0.005ns	7.09ns	0.01ns
Error	48	0.57	0.53	0.013	0.006	6.46	0.02
Total							

Salt = Salt stress wt = weight; ns = non-significant; *,**,*** significant at 0.05, 0.01 and 0.001 probability

Exogenous foliar spray with proline increased the shoot fresh and dry weight of the wheat cultivars except cultivar S-24.Root fresh weight of all four wheat cultivars increased with foliar spray of proline but root dry weight remained unchanged. Salt decreases the leaf length and leaf width of all the four cultivars. There is significant increase in leaf length and leaf width after foliar spray of proline. Proline application increased the length and width of leaf in both control and saline conditions. This increase in growth in wheat plants by foliar application of proline is similar to some earlier studies such asof wheat (Talat et al., 2013), barley (Agami, 2013) and sunflower (Khan et al., 2014).A number of scientists reasoned to its physiological functions such as osmoprotectant (Yancey, 1994), membrane stabilizing (Bandurska, 2001), and ROS scavenger (Matysik et al., 2002).

Analysis of variance of the data for chlorophyll a, chlorophyll b, chlorophyll a/b, total chlorophyll carotenoids, chlorophyll/carotenoid and chlorophyll contents from SPAD(Table 2) show that salt stress reduced photosynthetic pigments significantly in all wheat cultivars. However, proline treatment did not increase these photosynthetic pigments except that of chlorophyll a under saline or non-saline conditions. Similarly, carotenoids remained unchanged in all wheat cultivars due to salt stress and proline treatment. It has different effect in all four wheat cultivars under saline and non-saline conditions. In contrast, chlorophyll content measured as SPADincreased with proline treatment under both salt stress and non-stress conditions, particularly in cultivars Galaxy-13 and Sahar-06. Growth of plants is associated with a decrease in photosynthetic pigments and this reduction in chlorophyll contents due to salt stress is revealed in wheat, maize and canola (Ali et al., 2007; Raza et al., 2006).Foliar applied proline significantly improved the chlorophyll contents of salt stressed wheat plants, either through stimulating its biosynthesis and/or inhibiting its degradation and consequently increase the rate of CO2 diffusion and allow higher photosynthetic rate (Ali et al., 2007; Sharkey et al., 2007). Similar results were also reported by Khan et al. (2010) in *Brassica campestris*, Abdelhamid et al. (2010) in bean, and Abd El-Samad et al. (2011) in maize.

Table 2. Mean squares from analysis of variance of data for chl. a, chl. b, total chl. and chl. a/bof four cultivars of wheat (*Triticum aestivum* L.) treated with or without foliar spray of Proline to salt stressed and non-stressed plants.

Source of variance	df	Chl.a	Chl.b	Chl a/b	Total chl.	Carotenoids	Crtnd/chl	SPAD
Salt	1	0.15*	1.26**	1.08*	1.63*	1.04ns	9.16***	1615.3***
Cultivars	3	0.55***	2.98***	4.95***	6.72***	91.0***	3.93***	2073.4***
Proline	1	0.23**	0.004ns	0.02ns	0.15ns	13.7ns	0.16ns	302.1***
Salt x Cvs	3	0.09*	0.75**	0.84*	0.18ns	6.67*	0.39ns	1881.6***
Salt x Proline	1	0.27**	0.50ns	0.44ns	1.17ns	4.67ns	2.12ns	16.37ns
Proline x Cvs	3	0.11*	0.25ns	0.03ns	0.57ns	11.4ns	0.17**	12.71ns
Salt x Cvs x Proline	3	0.04ns	0.26ns	0.34ns	0.08ns	1.39ns	0.05ns	44.89***
Error	48	0.02	0.15	0.21	0.31	3.73	0.27	5.47
Total								

Salt = Salt stress wt = weight; ns = non-significant; *,**,*** significant at 0.05, 0.01 and 0.001 probability

Analysis of variance of the data for quantum yield (Table 03) shows that salt stress reduced the quantum yield of all the four wheat cultivars. Proline has increased the quantum yield of all four cultivars significantly. Exogenous proline application neutralize the harmful effects of salinity on carbohydrate metabolism, resulting in improved entire plant growth (Nessim et al., 2008; Abd El-Samad 2011). Similar results were also reported by Agami, (2013) in barely seedlings. When salt is applied to the cultivars the water potential decreases in all the four cultivars. After the application of proline water potential has increased in all the cultivars in control and salt conditions.

Table 3. Mean squares from analysis of variance of data for carotenoid, chlorophyll/carotenoid, carotenoid/chlorophyll and SPADof four cultivars of wheat (*Triticum aestivum* L.)treated with or without foliar spray of Proline to salt stressed and non-stressed plants.

Source of variance	Df	Water Potential	Quantum yield	Fv/Fm	PI_{ABS}
Salt	1	15.58***	0.039**	0.005*	3.81ns
Cultivars	3	1.18***	0.008ns	0.02***	41.9***
Proline	1	0.61***	0.078***	9.75	0.005ns
Salt x Cvs	3	0.06ns	0.001ns	0.003*	3.51ns
Salt x Proline	1	0.005ns	0.001ns	0.001	5.08ns
Proline x Cvs	3	0.04ns	0.005ns	1.32	1.93ns
Salt x Cvs x Proline	3	0.003ns	0.003ns	0.001	1.41ns
Error	48	0.02	0.005	0.001	1.32
Total					

Salt = Salt stress wt = weight ns = non-significant; *,**, *** significant at 0.05, 0.01 and 0.001 probability

Absorbtion capacity per reaction center (ABS/RC), trapping per reaction center (TRo/RC), electron transport per reaction center (ETo/RC) anddissipation energy per reaction center DIo/RC (Table 04) of S-24 decreased in saline and increased in non-saline conditions with the foliar application of proline while other three cultivars remained unchanged.

Table 4. Mean squares from analysis of variance of data for ABS/RC, DIo/RC, ETo/RC and Fv/Fm of four cultivars of wheat (*Triticum aestivum* L.)treated with or without foliar spray of Proline to salt stressed and non stressed plants

Source of variance	Df	ABS/RC	TRo/RC	ETo/RC	DIo/RC
Salt	1	0.49*	0.08ns	0.03*	0.13ns
Cultivars	3	2.46***	0.57***	0.07***	0.63***
Proline	1	0.04ns	0.02ns	0.02ns	0.003ns
Salt x Cvs	3	0.25ns	0.05ns	0.01ns	0.09ns
Salt x Proline	1	0.58*	0.19**	0.08***	0.10ns
Proline x Cvs	3	0.01ns	0.007ns	0.003ns	6.57ns
Salt x Cvs x Proline	3	0.29ns	0.07*	0.04***	0.07ns
Error	48	0.11	0.02	0.006	0.03
Total					

Salt = Salt stress wt = weight; ns = non-significant; *,**,*** significant at 0.05, 0.01 and 0.001 probability

Table.(a). Chlorophyll contents measured as SPAD values of four wheat cultivars when three weeks old plants grown under saline and non-saline conditions were sprayed with proline.

	Cultivars			
	Galaxy-13	**Pasban-90**	**Sahar-06**	**S-24**
Control	45.45±1.39b XY	44.20±1.31a Y	48.20±1.60ab X	42.92±0.73b Y
Proline	49.50±0.50a X	46.27±1.18a X	46.65±1.45ab X	46.50±0.47a X
Saline	39.52±3.14c YZ	37.65±1.57b Z	45.25±1.05b X	41.95±1.74b XY
Saline+ Proline	50.32±1.26a X	46.25±0.98a Z	49.90±1.56a XY	46.62±0.68a YZ

Figure with same letters in column (a-b) and rows (x-y) did not differ significantly at probability 0.05 level.

$LSD_{0.05\%}$ Salinity x Cultivars x Proline = 3.32

Table.(b) .LSD of TRo/RC of four wheat cultivars when three weeks old plants grown under saline and non-saline conditions were sprayed with proline.

	Cultivars			
	Galaxy-13	**Pasban-90**	**Sahar-06**	**S-24**
Control	1.40±0.03a Y	1.49±0.02a Y	1.54±0.06a Y	1.87±0.11a X
Proline	1.55±0.03a Y	1.54±0.02a Y	1.55±0.04a Y	1.93±0.20a X
Saline	1.45±0.03a Y	1.50±0.04a Y	1.65±0.10a XY	1.85±0.13a X
Saline+ Proline	1.51±0.04a Y	1.44±0.00a Y	1.55±0.02a XY	1.72±0.13a X

Figure with same letters in column (a-b) and rows (x-y) did not differ significantly at probability 0.05 level.

$LSD_{0.05\%}$ Salinity x Cultivars x Proline = 0.20

Table.(c). Means of energy flux for trapping per reaction center (ETo/RC) of four wheat cultivars when three weeks old plants grown under saline and non-saline conditions were sprayed with proline.

	Cultivars			
	Galaxy-13	**Pasban-90**	**Sahar-06**	**S-24**
Control	1.04±0.04a X	1.03±0.02a X	0.99±0.04a X	1.09±0.06b X
Proline	1.07±0.01a Y	1.06±0.01a Y	1.01±0.02a Y	1.21±0.12a X
Saline	0.99±0.03a Y	0.98±0.04a Y	1.06±0.04a XY	1.13±0.07ab X
Saline+ Proline	1.02±0.02a X	1.01±0.02a X	1.02±0.02a X	1.06±0.06b X

Figure with same letters in column (a-b) and rows (x-y) did not differ significantly at probability 0.05 level.

$LSD_{0.05\%}$ Salinity x Cultivars x Proline = 0.11

Conclusion

The foliar spray of proline ameliorate the harmful effects of 200 mM NaCl stress in wheat plants. Proline application improved the shoot and root fresh and dry weights, length and width of leaves, quantum yield,leaf chlorophyll content and photosynthetic efficiency.

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Fig.1. Fresh and dry weight of root and shoot and leaf length and width of four wheat cultivars when three weeks old plants grown under saline and non-saline conditions were sprayed with proline.

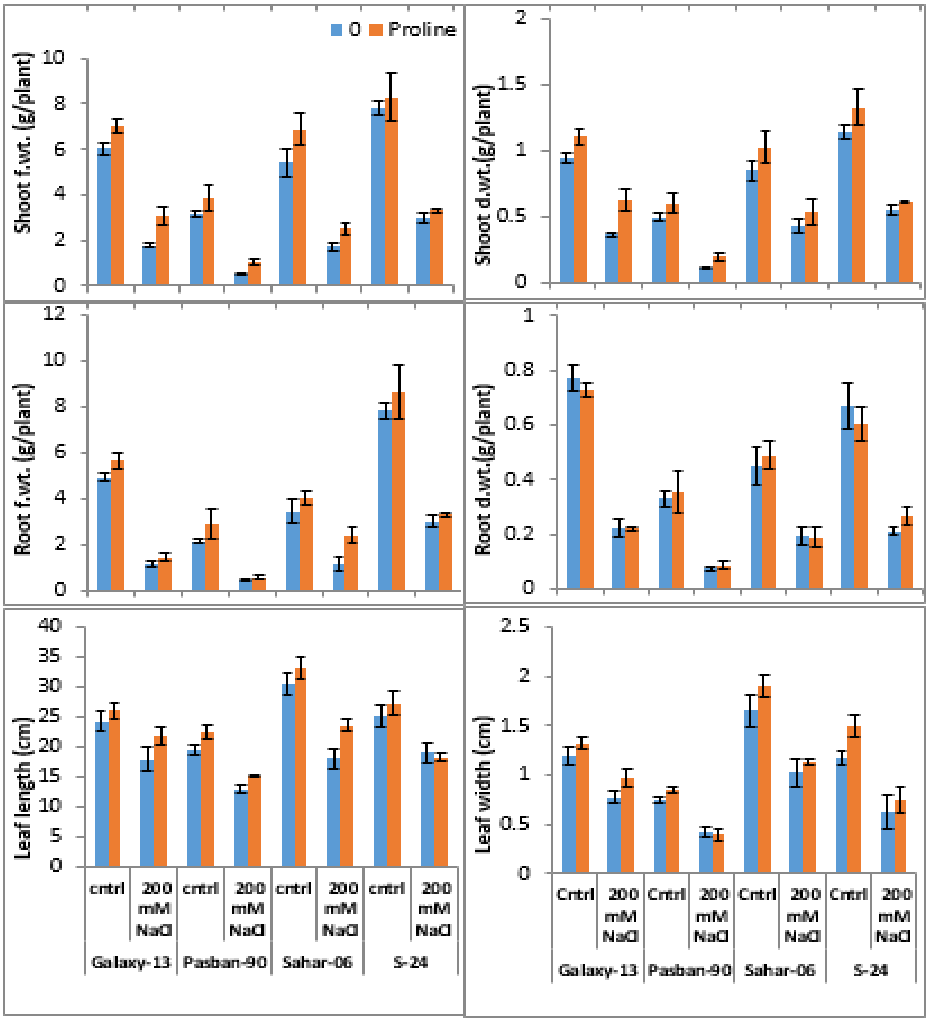

Fig.2. Chlorophyll a, chlorophyll b, chlorophyll a/b ratio, total chlorophyll, carotenoid, carotenoid/chlorophyll and SPAD values of four wheat cultivars when three weeks old plants grown under saline and non-saline conditions were sprayed with proline

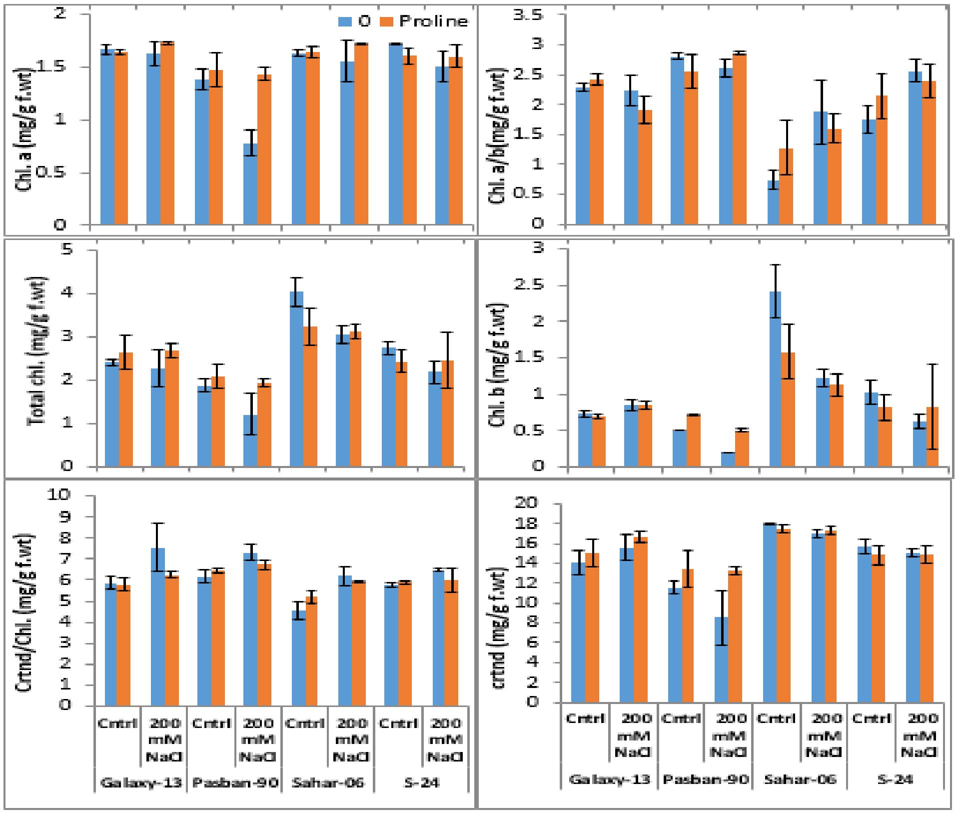

Fig. 3. Water potential, quantum yield of PSII of dark adapted leaves, and light adapted leaves (Fv/Fm) and PI_{ABS} values of four wheat cultivars when three weeks old plants grown under saline and non-saline conditions were sprayed with proline.

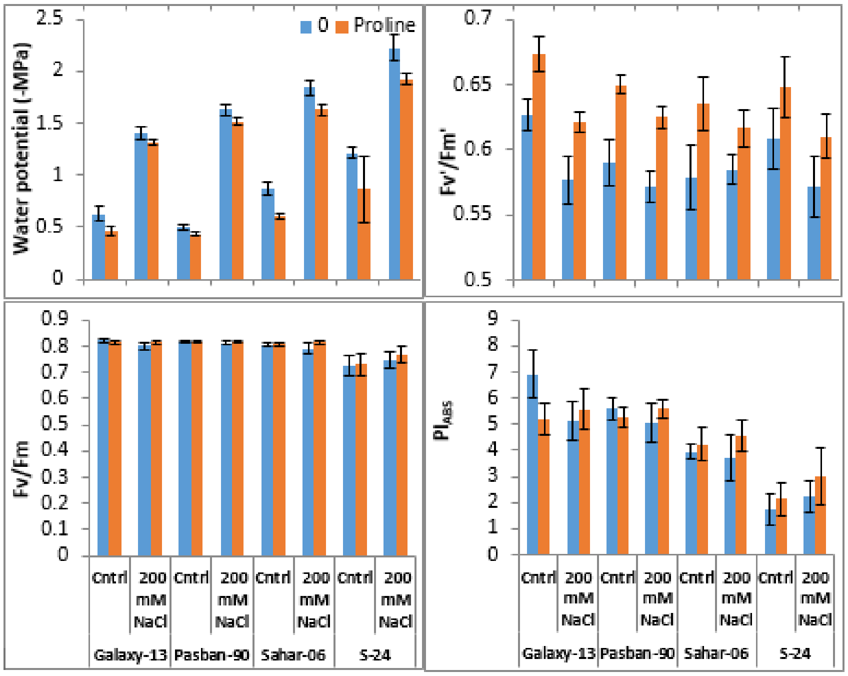

Fig.4. ABS/RC, TRo/RC, ETo/RC and DIo/RC values of four wheat cultivars when three weeks old plants grown under saline and non-saline conditions were sprayed with proline.