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Research Article

Foliar applied proline and acetic acid improves growth and yield of wheat under salinity stress by improving photosynthetic pigments, physiological traits, antioxidant activities and nutrient uptake

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Abstract

Salinity stress (SS) is serious abiotic stress and a major limiting factor for crop productivity and global food security. In this context, the application of osmolytes is considered as an environmental friend approach to improve plant growth under SS. Thus, the present study was conducted to determine the impact of foliar applied proline (Pro) and acetic acid (AA) on growth, yield, physiological traits, photosynthetic pigments, ionic homeostasis and antioxidant activities of wheat under SS. The study contained SS levels 0, 6 and 12 dS m^{-1} and foliar spray of Pro and AA; water spray, Pro (75 mM), AA (15 mM) and AA (30 mM). The study was conducted in a completely randomized design with the factorial arrangement. Salinity stress significantly reduced wheat growth and yield, by decreasing relative water contents (-49.07%), photosynthetic pigments, free amino acids (FAA: -44.79%), total soluble proteins (TSP: -15.94%) and increasing the electrolyte leakage (EL: +27.28%), hydrogen peroxide (H_2O_2: +51.86%), and malondialdehyde (MDA: +36.91%) accumulation. The foliar spray of Pro and AA markedly improved the wheat growth and productivity through enhanced photosynthetic pigments, RWC, FAA, TSP, antioxidant activities (catalase: CAT, ascorbate peroxide: APX: peroxidase: POD), K^+ and Ca^{2+} uptake and decreasing EL, MDA and H_2O_2 accumulation and restricted entry of toxic ions (Na^+ and Cl^{-1}). Therefore, foliar application of AA and Pro effectively improves the growth and yield of wheat under SS by strengthening the antioxidant defense system, and maintaining ionic homeostasis and physiological performance.

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Keywords: antioxidants; growth; osmolytes; wheat; yield

Introduction

Soil salinity (SS) is a serious reason for land degradation and reduced crop productivity globally (Saidi *et al*., 2021). It is prevalent abiotic stress posing a serious threat to crop productivity and global food security (Pan *et al*., 2022). The salt affected area is increasing at the rate of 8-10 million hectares per year which needs serious attention to manage this problem (Ivushkin *et al*., 2019; Machado and Serralheiro, 2017). It is a serious problem in arid as well as semi-arid regions owing to the fact in these regions precipitation surpassed the evaporation and soluble salts accumulations in plant root zone (Machado and Serralheiro, 2017; Shahid *et al*., 2018). The intensity of SS could be aggravated owing to rapid climate change along with a reduction in rainfall and an increase in global temperature (Corwin, 2021). Aside from natural causes of SS, the excessive use of salty water further intensifies this problem (Ikuyinminu *et al*., 2022; Ivushkin *et al*., 2019).

Soil salinity is associated with the accumulation of toxic ions (Na^+ and Cl^-) in the plant root one that induces ionic, osmotic and oxidative stress (Munns and Tester, 2008). SS also induces production of reactive oxygen species (ROS) that damage cellular membranes, mitochondria, proteins, chloroplast, lipids and negatively affect the plant functioning, thus leading to serious growth and yield reductions (Al-Zahrani *et al.,* 2022; Hasanuzzaman *et al*., 2021). Moreover, SS also induced a substantial increase in MDA and H_2O_2 accumulation and production of methylglyoxal (MG) that damages DNA and cellular structures (Parvin *et al.,* 2020). High concentration of salts also induced ionic and osmotic stresses and both these stresses alone and in combination inhibit nutrient and water absorption and resulting in nutrient deficiency, ionic imbalance and physiological drought (Maimaiti *et al*., 2014; Pan *et al*., 2022). Moreover, SS also adversely affects the beneficial root microbes that negatively affect soil fertility and plant production (Hashem *et al*., 2016).

The adverse effect of salinity should be minimized to improve crop production. Globally, different breeding and agronomic approaches are being used to improve the crop production. The application of osmo-protectants has emerged as an excellent strategy to improve crop production in salt affected soils (Al-Zahrani *et al*., 2022). Acetic acid (AA) has emerged as an important substance to improve crop production under stress conditions. Recently it got attention globally owing to its role in the improvement of drought tolerance in *Arabidopsis thaliana*, rapeseed, maize, rice and wheat (Kim *et al*., 2017). The exogenous application of AA mitigated the adverse impacts of SS by increasing Ca^{2+} and Mg^{2+} uptake, water use efficiency, osmolyte accumulation, antioxidant activity and decreasing Na^+ uptake (Rahman *et al*., 2019). Proline (Pro) is also an important osmolyte that can be used to mitigate the adverse effects of SS (Yang *et al*., 2009). Proline is a signaling molecule and it regulates plant growth by triggering the signaling processes (Yildiz and Terzi, 2013). Moreover, Pro, also improves synthesis of photosynthetic pigments, normal photochemical functioning, activities of antioxidants and regulates Na^+ compartmentalization which protects the plants from adverse effects of SS thus resulting in a substantial improvement in growth and development (Alam *et al*., 2016; Demiralay *et al*., 2017; Rady *et al*., 2019).

Wheat is an important source of protein and minerals and it is a staple food of many nations (Hassan *et al*., 2019). However, it is considered to salt sensitive and SS can cause huge growth and yield losses in wheat growth and productivity *(*Seleiman *et al*., 2022). There is no study available about the role of AA and Pro to mitigate adverse effects of SS in wheat crop. We hypothesized that AA + Pro can substantially reduce the harmful effects of SS by reducing the Na^+ uptake and increasing photosynthetic pigments, and strengthening the antioxidant defense system. Therefore, this study was executed to determine the effects of AA and Pro on growth, yield, physiological traits, ionic homeostasis and antioxidant activity of wheat grown under SS.

Materials and Methods

Growth conditions and salinity development

The pot present study was laid out in a completely randomized design (CRD) with a factorial arrangement using four replications. Pots were filled with 10 kg of soil with 1:3 sand and soil composition. Fifteen seeds of wheat were sown in each pot during first week of November 2019. NaCl and Na_2CO_3 in 9:1 was mixed in soil and sand mixture to maintain salinity levels of 6 dSm^{-1} and 12 dSm^{-1} for salinity stress.

For obtaining different levels of SS, Na_2CO_3 and NaCl were used. The salinity levels were maintained using the formula given by Khaliq *et al*. (2015).

$$\text{Salt req. (g/kg)} = \frac{Tss \times mol.weight \times saturation(\%)}{100 \times 1000}$$

To calculate soil saturation percentage, distal water was added to the saturated soil and soil paste was made and soil was mixed well with a spatula. This mixture was left for 2 hours to reach equilibrium. This mixture was filtered with filter paper and after that this soil mixture was oven dried (105 °C) and soil saturation was calculated by the following formula;

$$saturation\ (\%) = \frac{loss\ in\ soil\ weight\ on\ drying}{weight\ of\ soil\ after\ drying} \times 100$$

Plant sampling and analysis

Three plants were randomly selected from each pot to determine the shoot length and shoot fresh weight. These leaves from these selected three plants were counted and the average was taken. Moreover, these plants were oven dried (70 °C) and weighed from determination of shoots dry weight.

Determination of photosynthetic pigments

For photosynthetic pigments; 0.5 g of fresh plant sample leaves were weighed and ground in 80% solution of 5 ml acetone. Afterward, the grinded sample was centrifuged for 3 minutes. A clear supernatant was collected after centrifugation and its absorbance was measured using a spectrophotometer at three wavelengths i.e., 645nm for Chl a, 663nm for chl. b and 470nm for carotenoids. Chl a and b content was measured according to the (Arnon, 1949) method.

Determination of relative water content and electrolyte leakage

Fresh leaves (1g) were taken and weighed on an electrical balance. After that, they were dipped in the distal water (H_2O) for 24 h. Then leaves were removed from the water and excess water was wiped out and weighed again. Afterward, the leaves were sun dried and packed in small paper bags. The same leaves were oven dried (70 °C for 24 h) to determine dry weight. Leaf RWC was evaluated by the formula as stated by: RWC = (FW-DW) / (TW-DW) × 100. Electrolyte leakage (EL) was recorded by using the method of Yan *et al*. (1996). A fresh leaf sample (0.5 g) was chopped into small pieces and then dipped in distilled water for a half an hour and EC_1 was measured by using EC meter. EC_2 was recorded by heating the samples in water bath at 90 °C for 50 minutes and EL was measured with following formula: EC%= EC1/EC2×100.

Determination of antioxidant activities and oxidative stress markers

For determination of CAT activity leaf sample (0.1 g) was taken for grinding in 2.5 ml of 50 mM K-buffer. Afterwards samples were centrifuged and supernatant was taken. Furthermore, 0.1 ml plant crude extract was added in 0.1 ml of H_2O_2 (5.9 mM) and 2.5 ml of 5% TCA buffer. The absorbance was measured at 240 nm on a spectrometer to determine CAT activity (Chance, 1955). The technique of (Chance, 1955) was used for the determination of POD activity. 0.1 ml H_2O_2 was added in 2.7 ml phosphate buffer then shakes it well. After shaking, 0.1 ml of guicol and 0.1 ml of enzyme extract were added and shaken again. Absorbance change was recorded at 470 nm after every 20 sec for 3 mints. APX activity in wheat plant was measured by

using the following protocol Asada (1987). 1.0 g of leaf sample was ground in 10 ml of 50 mM K-buffer and centrifuged at 4 °C for 30 minutes and supernatant was taken. 100 µl of 0.5 M ascorbic acid, 100 µl of 6.5 mM of H_2O_2 and 500 µl of potassium phosphate buffer of (50 mM) were treated in a test tube and absorbance was noted at 290 nm. H_2O_2 concentration in the wheat sample was determine by following the method of (Velikova *et al*., 2000). Plant leaves sample of 0.25 g was grinded in 5 mL of 5% TCA. After that, centrifugation was done at 10,000 rpm for 5 min to take the supernatant. In 1mL of crude sample, 1 mL of KI (1M) and 100 µL phosphate buffer was added and kept at room temperature. At 390 nm, the absorbance was taken to assess the amount of H_2O_2. To determine MDA; we took plant samples (0.5 g) and ground them with 5 ml of TCA and centrifuged for 15 minutes at 12000 rpm. Later on, mixture having 1 ml plant extract and 1 ml TCA was heated at 100 °C and then cooled in ice bath and absorbance was noted at 532 nm to determine MDA concentration (Rao and Sresty, 2000).

Determination of total soluble protein and free amino acids

For measurement of FAA, we took wheat plant samples (0.5 g) and ground them using potassium buffer (5 ml) then centrifuged them for 5 minutes and supernatant was taken. After that 1 ml of extract was taken in test tubes comprising of 2% ninhydrine solution (1 ml) and 10% pyridine solution (1 ml) and heated (90 °C) for 30 minutes. Then add distal water was added to maintain volume up-to 15ml. The absorbance was checked on spectrophotometer at 570nm (Hamilton and Van Slyke, 1943). Total soluble proteins were measured by the protocol of Bradford (1976). 0.5 g fresh leaves were grinded in potassium phosphate buffer of (50 mM). Afterward, it was centrifuged at 15,000 rpm for 15 minutes at 4°C. 100 µl of supernatant was taken in a test tube and 2 ml of Bradford reagent was added and allowed for 15 minutes then absorbance was noted at 590 nm.

Ionic analysis

The samples of wheat plants were taken, and dried (70 °C) afterward they were milled to make powder. The samples were digested by using a mixture of acids (HCl and HNO_3; 1:2) on a hot plate. Later on, distilled water was added and diluted samples and Cl^- concentration was measured by a chloride analyzer whereas concentration of Ca^{2+}, Na^+ and K^+ was measured with a flame photo-meter.

Determination of yield traits

The productive tillers were counted from every replication of each treatment pot. The length of the selected spikes was measured and spikelet's/spike were counted. The manually harvested wheat plant samples from every replication of treatment threshed manually to separate grains to determine the grain yield. Moreover, 1000-grain weight was recorded by taking a random sample of 1000 grains of wheat.

Statistical analysis

A computer-based software; STATISTIX 8.1 was used to analyze the observed data for two-way analysis of variance and LSD test was used to check the significance of treatment means at a probability level of 5% (Steel *et al*., 1980).

Results

Growth traits

The results indicated that SS and foliar application of Pro and AA had a significant impact on the growth traits of wheat (Table 1). SS induced a marked reduction in growth traits including plant height, SFW, SDW and LPP and this reduction was significantly increased with increasing SS (Table 1). The foliar of application

of both Pro and AA markedly improved the growth traits of wheat plants. The foliar application of Pro remained the top performer in this context followed foliar application of AA (30 mM) (Table 1).

Photosynthesis pigments

The data showed that the SS and foliar application of Pro and AA significantly affected the chlorophyll and carotenoid contents of wheat plants (Table 2). The decrease of 16% and 44% in chlorophyll-a contents were recorded at 6 and 12 dS m^{-1} SS. Conversely, an increase of 43.31% and 25% and 11.11% in chlorophyll a content was recorded with foliar applied Pro and AA (30 mM) under strong SS (Table 2). Similarly, chlorophyll-b and carotenoid contents also showed a reduction of 42.80% and 40.50% at both SS levels (6 and 12 dS m^{-1}). The foliar spray of Pro (75 mM) markedly improved the chlorophyll-b and carotenoid contents by 43.30% and 65.07% at strong SS (Table 2).

Table 1. Effect of foliar application of proline and acetic acid on growth traits of wheat under salinity stress

Salinity stress	Application of AA and Pro	PH (cm)	SFW (g)	SDW (g)	LPP
Control	Water spray	70.40c±0.88	5.10bc±0.11	0.94cd±0.066	8.00c±0.33
	Pro (75 mM)	76.35a±0.49	7.55a±0.25	2.77a±0.11	11.00a±.42
	AA (15 mM)	72.82b±0.13	5.05bc±0.17	1.12cd±0.025	10.33ab±0.32
	AA (30 mM)	74.07b±0.29	5.62bc±0.33	1.72bc±0.028	9.33b±0.52
6 dSm^{-1}	Water spray	58.25g±085	4.30cd±0.29	0.42de±0.10	7.67cd±0.49
	Pro (75 mM)	67.77d±0.40	6.35ab±0.26	2.37ab±0.14	8.33c±0.40
	AA (15 mM)	62.20f±0.16	4.85bc±0.09	1.00cd±0.11	8.00c±0.38
	AA (30 mM)	64.50e±0.28	5.45bc±0.033	1.52c±0.029	8.00c±0.28
6 dSm^{-1}	Water spray	39.40j±0.50	3.10d±0.066	0.27e±0.014	6.66e±0.40
	Pro (75 mM)	45.12h±0.23	5.32bc±0.25	1.50c±0.33	7.66d±0.33
	AA (15 mM)	41.50ij±0.57	3.97cd±0.32	0.57de±0.28	7.33de±0.52
	AA (30 mM)	43.00hi±0.47	4.40cd±0.23	1.25cd±0.22	7.33de±0.22

PH: plant height, SFW: shoot fresh weight, SDW: shoot dry weight, LPP: leaves per plant. The data is the mean of four replications with S.E. (±) and diverse letters indicating significance at 0.05 P level. AA: acetic acid, Pro: proline.

Table 2. Effect of foliar application of proline and acetic acid on photosynthetic traits of wheat under salinity stress

Salinity stress	Application of AA and Pro	Chlorophyll-a (mg/g FW)	Chlorophyll-b (mg/g FW)	Carotenoids (mg/g FW)
Control	Water spray	2.30cd±0.072	2.10ef±0.047	0.63d±0.033
	Pro (75 mM)	3.29a±0.062	4.53a±0.078	1.43a±0.074
	AA (15 mM)	2.54bc±0.047	2.89cd±0.081	0.90bcd±0.039
	AA (30 mM)	2.71b±0.054	3.82b±0.045	1.09ab±0.10
6 dSm^{-1}	Water spray	1.94ef±0.048	1.52gh±0.049	0.59d±0.070
	Pro (75 mM)	2.60bc±0.072	3.20bc±0.025	1.17ab±0.047
	AA (15 mM)	2.13de±0.078	2.22de±0.070	0.70cd±0.081
	AA (30 mM)	2.39cd±0.076	2.62cde±0.051	1.03bc±0.092
6 dSm^{-1}	Water spray	1.17g±0.081	1.14h±0.053	0.16e±0.049
	Pro (75 mM)	1.84ef±0.028	2.62cde±0.049	1.04bc±0.049
	AA (15 mM)	1.34g±0.032	1.75fgh±0.051	0.64d±0.061
	AA (30 mM)	1.66f±0.039	2.12efg±0.50	0.83bcd±0.089

The data is the mean of four replications with S.E. (±) and diverse letters indicating significance at 0.05 P level. AA: acetic acid, Pro: proline.

Relative water contents and electrolyte leakage

The results depicted that SS and application of osmolytes showed marked effects on the RWC and EL of wheat plants (Figure 1). RWC showed a significant decrease with increasing SS and a reduction of 9% and 28.90% in RWC contents was recorded at 6 and 12 dS^{-1} SS (Figure 1). In the case of foliar application of Pro and AA maximum RWC (81.16%) was recorded with Pro application (75 mM), followed by 30 mM acetic acid application (72.83%) and 15 mM acetic acid application (69%) and lowest RWC were recorded in control (Figure 1). An increasing trend in EL (%) was observed with increasing SS and it has been reported that EL was increased by 35.10% and 45% under at 6 and 12 dS m^{-1} SS (Figure 1). However, EL was decreased by 37%, 26% and 17.1% due to foliar applied Pro (75 mM), AA (30 mM) and AA (15 mM) respectively as compared to control (Figure 1).

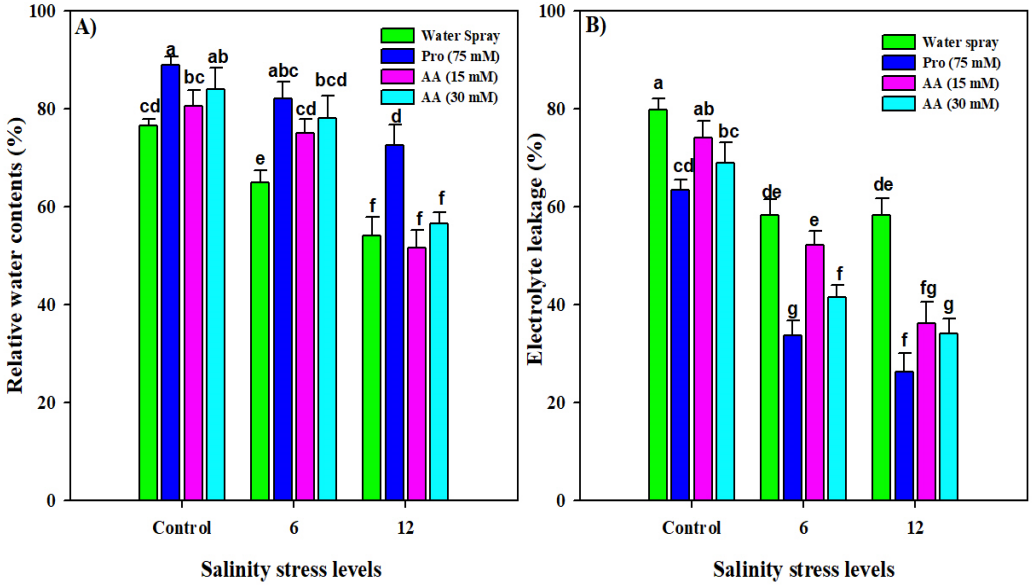

Figure 1. Effect of foliar application of proline and acetic acid on RWC (a) and EL (b) wheat under salinity stress

The data is the mean of four replications with S.E. (±) and diverse letters indicating significance at 0.05 P level.

Antioxidant activities and oxidative stress markers

CAT activity of wheat plants was increased under different levels of salt stress. An increase of 37.6% and 56.4% at 6 dS m-1 and 12 dS m-1 was observed respectively compared to control (Table 3). The different Pro and AA concentration markedly improved the CAT activity of wheat plants under control and SS conditions (Table 3). APX activity of wheat plants was also increased under SS and an increase of 38.6% and 78% in APX activities were observed at both SS (Table 3). An increase of 19%, 12.9% and 6.3% was recorded APX activity with foliar applied Pro (75 mM) and AA (30 and 15 mM) (Table 3). The results depicted that foliar SS showed a marked increase POD activities under control and SS conditions. POD activity was increased by 2 times and 3 times at 6 and 12 dS m^{-1} (Table 3). The foliar spray of Pro (75 mM) showed maximum POD activity, followed by 30- and 15-mM application of AA (Table 3). H_2O_2 concentration of salt stressed plants wheat plants was gradually increased with increasing concentrations of salts in growing medium (Table 3). H_2O_2 concentration was increased by 36.40% and 67.80% at both SS levels as compared to normal conditions (Table 3). The different levels of Pro and AA helped in decreasing H_2O_2 levels and a reduction of 16.10%, 11.10% and 5%, in H2O2 concentration was recorded with foliar spray of Pro (75 mM) and AA (30 and 15 mM) (Table 3). MDA concentration was significantly increased under SS and it was significantly increased with increasing SS (Table

3). The foliar spray of Pro and AA significantly decreased the MDA concentration and, in this regard, foliar application of Pro (75 mM) remained the top performer followed by AA application (30 mM) (Table 3).

Table 3. Effect of foliar application of proline and acetic acid on photosynthetic traits of wheat under salinity stress

Salinity stress	Application of AA and Pro	CAT (U/mg protein)	APX (U/mg protein)	POD U/mg protein)	H_2O_2 (µ mol g^{-1} FW)	MDA (µ mol g^{-1} FW)
Control	Water spray	22.01l±1.13	23.50hi±0.36	0.76f±0.027	9.77f±0.22	6.89±0.26
	Pro (75 mM)	24.41i±0.78	17.50k±0.89	3.17de±0.068	7.92h±0.17	5.22±0.14
	AA (15 mM)	22.89k±0.82	21.50ij±1.11	1.48ef±0.15	9.01fg±0.30	6.22±0.56
	AA (30 mM)	23.76j±0.99	19.50jk±0.48	2.19ef±0.13	8.43gh±0.49	6.00±0.60
6 dSm^{-1}	Water spray	30.73h±0.54	31.50de±0.92	2.17ed±0.22	12.91cd±0.15	9.78±0.31
	Pro (75 mM)	33.35e±1.19	25.50gh±0.99	6.02bc±0.34	11.18e±0.31	8.70±0.19
	AA (15 mM)	31.61g±0.78	29.50ef±1.12	3.24de±0.12	12.39d±0.49	9.25±0.29
	AA (30 mM)	32.48f±1.44	27.25fg±1.16	5.06cd±0.58	11.44e±0.23	9.00±0.34
6 dSm^{-1}	Water spray	35.09d±1.13	39.50a±1.29	3.48de±0.19	16.03a±0.37	13.61±0.41
	Pro (75 mM)	37.71a±1.39	33.50cd±0.78	8.73a±0.90	13.35c±0.32	11.84±0.56
	AA (15 mM)	35.97c±1.11	37.50ab±1.72	5.64bc±0.82	15.21ab±0.49	12.33±0.62
	AA (30 mM)	36.84b±1.89	35.50bc±1.42	7.60ab±0.70	14.38b±0.61	12.82±0.41

CAT: catalase, APX: acerbate peroxidase, POD: peroxidase, H_2O_2: hydrogen peroxide, MDA: malondialdehyde. The data is the mean of four replications with S.E. (±) and diverse letters indicating significance at 0.05 P level. AA: acetic acid, Pro: proline.

Total soluble proteins and free amino acids

TSP of salt stressed wheat plants gradually decreased with increasing concentrations of salts (Figure 2). The reduction of 20.80% and 38.70% in TSP was recorded at 6 and 12 dS m^{-1} SS as compared to the control (Figure 2). The foliar applied Pro and AA significantly increased the TSP of wheat plants under SS (Figure 2). Maximum TSP (4.82 mg g^{-1} FW) was recorded in Pro (75 mM) application followed by 30 mM acetic acid (4.39 mg g^{-1} FW) and 15 mM acetic acid (3.51 mg g^{-1} FW) while minimum TSP contents was recorded in control (2.58 mg g^{-1} FW) (Figure 2). TFA of salt stressed plants also gradually decreased with increasing concentrations of salts. The reduction of 24% and 40.6% in TFA was recorded under both SS levels (Figure 2). An increase of 21.1%, 14% and 6.6% in TFA was recorded with Pro (75 mM), AA (30 mM) and AA (15 mM) (Figure 2).

Yield traits

The results indicated that productive tillers decreased by 17.10% and 45.30% under both SS levels as compared to control (Table 4). The foliar application of Pro and AA markedly increased the PT of wheat plants grown under normal and SS conditions (Table 4). The results indicated that SLPS decreased by 7.60% and 28.60% under both SS levels as compared to control. The maximum SLPS (20.75) was recorded in Pro (75 mM) application followed by 30 mM acetic acid application and minimum PT were recorded in control conditions. SS also caused a marked reduction GPS however; foliar applied Pro and AA appreciably improved the GPS of wheat crops (Table 3). The foliar spray of Pro increased the GPS by 40.03% whereas foliar spray of 30- and 15-mM acetic acid increased GPS by 24.56% and 9.92% (Table 3). TGW of wheat plants was decreased with increasing SS. The results showed that maximum TGW (26.70 g) was recorded with foliar spray of Pro (75 mM) after that 30 mM acetic acid and lowest TGW (21.04 g) was recorded in control. The BY and GY of wheat were decreased by increasing SS (Table 4). The maximum BY (35.67g) was recorded with 75 mM Pro, followed by 30 mM acetic acid (30.66 g) and lowest BY was noted in control (Table 4). GY also significantly decreased with increasing SS. However, foliar applied Pro and AA mitigated the adverse effects of SS. The

maximum grain yield was obtained with foliar spray of Pro (75 mM), followed by 30 mM acetic acid and the lowest GY was recorded in control (Table 4).

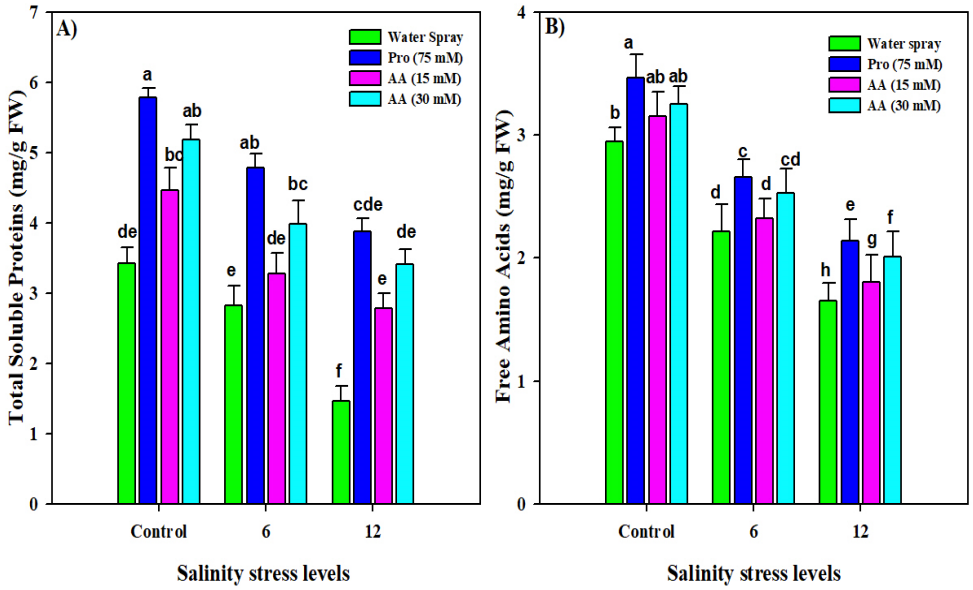

Figure 2. Effect of foliar application of proline and acetic acid on TSP (a) and FAA (b) wheat under salinity stress

The data is the mean of four replications with S.E. (±) and diverse letters indicating significance at 0.05 P level.

Table 4. Effect of foliar application of proline and acetic acid on photosynthetic traits of wheat under salinity stress

Salinity stress	Application of AA and Pro	PT (m^{-2})	SLPS	GPS	TGW (g)	GY/pot (g)	BY/pot (g)
Control	Water spray	22.25a±0.14	15.25de±0.55	36.75de±2.56	24.62cd±0.62	23.41c±0.43	35.50cd±0.33
	Pro (75 mM)	22.25a±0.15	20.75a±0.59	48.75a±3.32	29.65a±0.48	34.34a±0.48	55.00a±0.47
	AA (15 mM)	22.25a±0.19	17.00cd±0.47	39.00cd±3.28	25.32bc±0.32	25.54c±0.29	40.75c±0.55
	AA (30 mM)	22.00a±0.22	19.00abc±0.50	44.75b±2.99	26.15b±0.39	27.81b±0.22	49.00b±0.78
6 dSm^{-1}	Water spray	18.50b±0.29	12.50fgh±0.33	28.50g±3.49	20.30f±0.47	17.62e±0.31	20.00fg±0.47
	Pro (75 mM)	18.50b±0.26	20.25ab±0.86	41.00c±3.95	26.12b±0.77	22.33c±0.29	30.75d±1.42
	AA (15 mM)	18.00b±016	14.75def±0.28	31.50f±1.24	23.85d±0.36	20.27d±0.27	25.25f±0.89
	AA (30 mM)	18.50b±0.10	18.00bc±0.46	35.750e±1.28	25.77bc±0.20	18.26e±0.22	27.50e±0.89
6 dSm^{-1}	Water spray	11.75d±0.19	10.50h±0.57	20.25j±1.78	18.20g±0.42	8.94g±0.20	10.25i±0.72
	Pro (75 mM)	12.00cd±0.4	15.50de±0.33	30.00fg±1.35	24.32cd±0.52	15.45e±0.40	18.25fg±0.99
	AA (15 mM)	12.50c±0.28	11.50gh±0.39	23.50i±0.46	20.17f±0.69	11.33f±0.56	13.75hi±0.70
	AA (30 mM)	12.25c±0.32	13.50efg±0.22	26.00h±1.23	21.77e±0.65	12.32f±0.52	15.50gh±0.58

PT: productive tillers: SLPS: spikelets/spike, GPS: grains/spike, TGW: thousand grain weight, GY: grain yield, BY: biological yield. The data is the mean of four replications with S.E. (±) and diverse letters indicating significance at 0.05 P level. AA: acetic acid, Pro: proline.

Discussion

Salinity stress is serious abiotic stress that is negatively affecting crop production; therefore, appropriate measures must be adopted to mitigate the adverse effects of SS on plants (Kim *et al.*, 2021; Kim *et al.*, 2017). The results indicated SS induced a marked reduction in growth traits of wheat (Table 1). SS negatively affects plant physiological functioning, nutrient and water uptake, assimilates translocation, photosynthesis and respiration thereby, hinder plant growth (Hasanuzzaman *et al.*, 2021; Iqbal *et al.*, 2018). SS also significantly

increased the accumulation of toxic ions (Na^+ and Cl-) around roots of plants which reduce the water uptake and cause a serious reduction in plant growth (Kosová *et al*., 2011). The increase in toxic ion (Na^+) perturbs the K^+ accumulation which makes ionic homeostasis is challenging and induces substantial decrease in plant growth (Ivushkin *et al*., 2019; Negrão *et al*., 2017). The foliar spray of Pro and AA significantly improved the growth of wheat plants (Table 1). The foliar spray of Pro and AA reduced the Na^+ accumulation (Figure 3) and increased accumulation of K^+, Ca^{2+} which caused a marked improvement in plant growth (Farooq *et al.,* 2015; Rady *et al*., 2019). Pro also improves plant physiological functioning, antioxidant activities, and osmolytes accumulation which in turn improved the plant growth under SS (Rady *et al*., 2019). The foliar spray of AA also mitigated the adverse effects of SS as evidenced by improved shoot growth and LPP (Table 1). AA application improves photosynthetic pigments, antioxidant activities and synthesis of stress protection osmolytes and leading to a marked increase in plant growth (Rahman *et al*., 2019).

The most common effect of SS on plants is a reduction in chlorophyll contents (Nahar *et al*., 2016; Taïbi *et al*., 2016) and in the present study, SS also significantly reduced the chlorophyll contents of wheat plants. Salinity stress induced oxidative stress, inhibited chlorophyll synthesis and activate chlorophyll degrading enzyme (chlorophyllase) which cause a reduction in chlorophyll contents under SS (Banakar *et al*., 2022). Carotenoids work as an antioxidant for scavenging of ROS and they also participate in light energy harvesting for photosynthesis. They also stabilize chloroplast membranes and decreases vulnerability to lipid peroxidation (Mi *et al*., 2022; Taïbi *et al*., 2016). The results showed that SS caused a significant reduction in carotenoid contents which in line with the findings of Taïbi *et al.* (2016) they also noted significant reduction in carotenoid contents under salinity stress. The foliar application of Pro and AA significantly improved chlorophyll and carotenoid contents which were linked with improved synthesis of chlorophyll owing to an increase in activities of enzymes involved in chlorophyll synthesis (de Freitas *et al*., 2018; Zali and Ehsanzadeh, 2018).

The initial problem faced by plants under SS is osmotic stress owing to a decrease in water potential outside the plant body. Salinity induced osmotic stress which inhibits the uptake of water thus, reduced the water contents. The reduction in RWC of wheat might be due to salinity induced osmotic stress which reduced the water uptake and entailed the lower RWC (Al-Zahrani *et al*., 2022). Wheat plants under SS showed a significant increase EL which causes substantial damage to plant cells (Parvin *et al*., 2020). The results showed that SS also significantly increased the MDA and H_2O_2 accumulation and both these induced negative impacts on plant physiological and biochemical functioning, membrane integrity and enzymatic activities (Miller *et al*., 2010; Zhao *et al*., 2020). However, the foliar applied Pro and AA maintained higher RWC under SS which can be attributed to the ability of both these osmolytes to mitigate the salinity induced osmotic stress (Ma *et al*., 2016).

SS hinders the uptake of essential nutrients owing competition between Na^+ and ionic forms of nutrients (Hasanuzzaman *et al*., 2021; Parvin *et al*., 2020). SS depolarizes the plasma membranes of roots and induces the function of guard cell outward-rectifying K channels. These changes lead to increase in Na^+ accumulation and reduction in K^+ accumulation (Bose *et al*., 2014). In present study, wheat plants showed a significant increase in Na^+ and Cl- accumulation which consequently reduced the K^+ and Ca^{2+} accumulation (Figure 3).

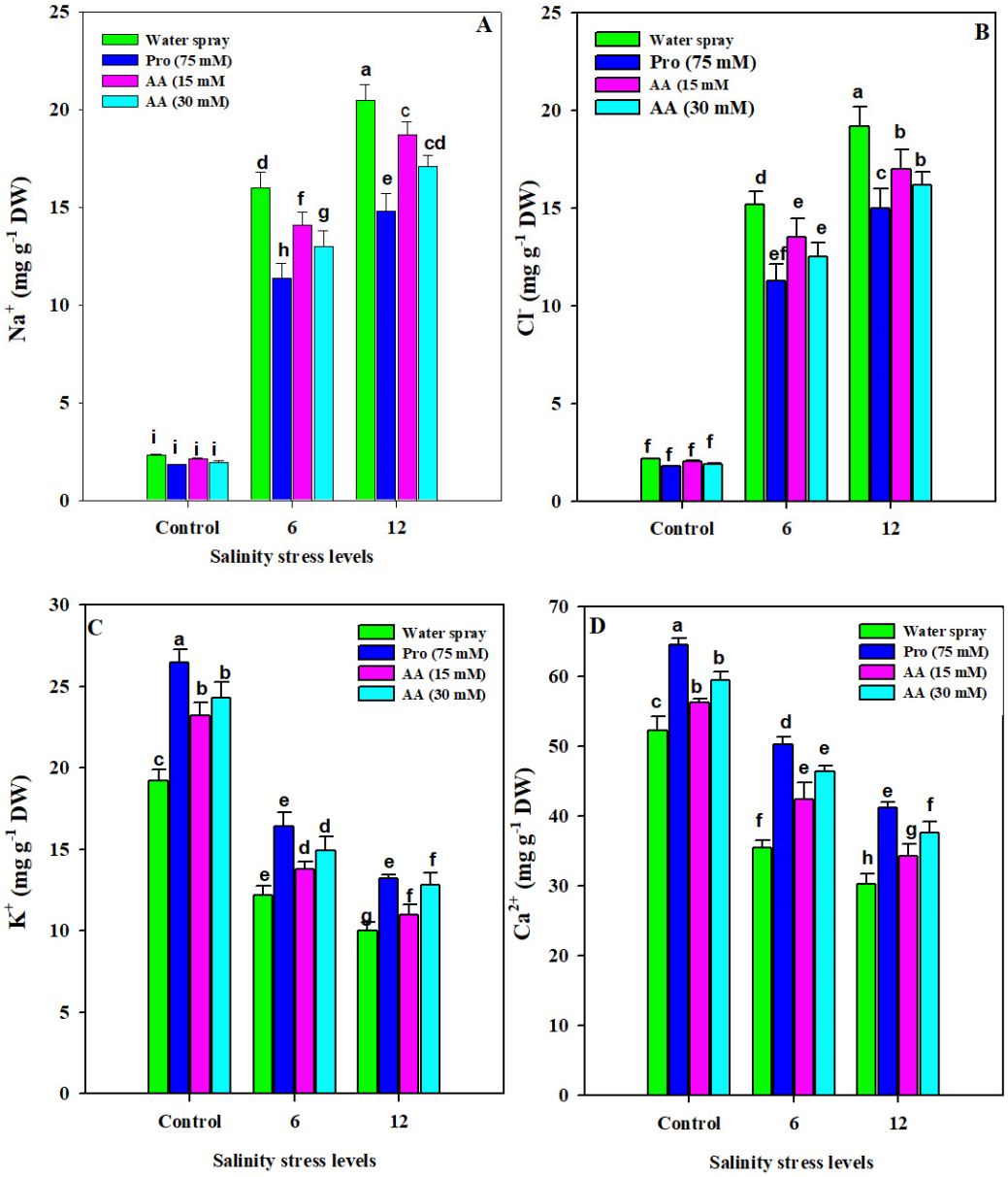

Figure 3. Effect of foliar application of proline and acetic acid on Na+ (a), Cl- (b), K+ (c) and Ca2+ wheat under salinity stress. The data is the mean of four replications with S.E. (±) and diverse letters indicating significance at 0.05 P level.

The higher concentration of Na^+ disturbs Ca^{2+} availability and a particular amount of Ca^{2+} is needed to maintain the integrity of membranes (Hu *et al*., 2007). However, foliar applied Pro and AA significantly improved the accumulation of K^+ and Ca^{2+} while they reduced the accumulation of toxic ions (Na^+ and Cl^-). The application of AA up-regulates the transcription of K^+/Na^+ transporters (HKT1;1) which reduced the accumulation of Na^+ under SS (Zhang *et al*., 2021). The application of AA also improves the uptake of K^+ and Ca^{2+} which is a remarkable feature exerted by AA and Pro to improve plant growth under SS (Rahman *et al*., 2019). The activities of all the antioxidant enzymes were increased in SS conditions which were further increased by application of Pro and AA (Table 3). The increase in antioxidant activities scavenges the ROS which protects the plants from salinity induced oxidative damage (Mahmood *et al*., 2021; Parveen *et al*., 2019).

The results indicated that MDA and H_2O_2 concentration was significantly increased under SS. However, the application of Pro and AA reduced MDA and H_2O_2 accumulation which was linked with the activation APX, CAT and POD enzymes. The activation of CAT, APX and POD plays an important role to eliminate H_2O_2, thereby prevents oxidative damage (Abogadallah, 2010; de Freitas *et al*., 2018). Therefore, present increase in antioxidant activities substantially reduced the toxic effect of SS on wheat. Previously, different authors also noted a significant increase in antioxidant activities with Pro application under SS (de Freitas *et al*., 2018; Nakhaie *et al*., 2020; Patade *et al*., 2014). The foliar spray of AA also increased all antioxidant activities which suggests that AA activates antioxidant enzymes to protect the plants against salinity induced toxic effects. Earlier various researchers also noted a substantial increase in antioxidant (APX, CAT, POD and SOD) activities with AA application under drought and SS (Rahman *et al*., 2021; Zhang *et al*., 2021). SS significantly reduced the accumulation of TSP and TFA. However, foliar application of Pro and AA maintained higher TSP and TFA concentration which improve the salinity tolerance owing to the fact TSP and TFA improve antioxidant activities.

Salinity stress significantly decreased the yield and yield traits of wheat plants. SS reduced the energy conversion into yield owing to the fact a larger part of the energy is used in stress alleviation (Munns and Gilliham, 2015; Zörb *et al*., 2019). Soil salinity also reduced the photosynthesis and translocation of photosynthates into sinks which reduced the grain filling and subsequently reduced the number of seeds (Farooq *et al*., 2015; Sangwongchai *et al*., 2021). Nonetheless, foliar spray of Pro and AA significantly improved the yield traits and yield of wheat. This increase was linked with improved photosynthetic pigments, RWC, nutrient uptake, TFA and TSP accumulation, improved antioxidant activities and reduced and EL, MDA, H_2O_2, Na and Cl- accumulation (Rady *et al*., 2016; Rahman *et al*., 2019).

Conclusions

Salinity stress caused a marked reduction in the growth and yield of the wheat crop. The application of Pro and AA appreciably offset the negative effects of salinity stress by improving the synthesis of photosynthetic pigments, osmo-regulating compounds, nutrient homeostasis and physiological traits. Moreover, the positive effects of Pro and AA on wheat were also linked with restricted entry of toxic ions (Na^+ and Cl^-) and improved uptake of Ca^{2+}, K^+ and Mg^{2+}. Therefore, in light of current findings application of AA and Pro could be a promosing approach to mitigate the adverse effects of SS. However, more studies are direly needed to optimize the dose and timing of AA and Pro application for the wheat crop. Besides this more studies are also needed to underpin the molecular mechanism mediated by AA and Pro to induce salinity tolerance.

Authors' Contributions

Conceptualization: IK and MUC, writing original draft: IK, MUC and MUH. Data collection: MI, writing, reviewing and editing: AM, RM, MA, MTA, MSH, SAK, SN and MM.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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