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Research article

Spermine application alleviates salinity induced growth and photosynthetic inhibition in *Solanum lycopersicum* by modulating osmolyte and secondary metabolite accumulation and differentially regulating antioxidant metabolism

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ABSTRACT

Influence of exogenously applied spermine (Spm) on growth and salinity stress tolerance in tomato was investigated. Salinity reduced growth, chlorophyll synthesis and mineral uptake leading to significant reduction in photosynthesis, however Spm application proved beneficial in alleviating the decline to considerable extent. Applied Spm improved nitrate reductase activity, δ-amino levulinic acid content and gas exchange parameters more apparently at 100 μM than 50 μM concentrations. Spm application enhanced the accumulation of compatible osmolytes including proline, glycine betaine and sugars leading to greater tissue water content and photosynthesis. Salinity stress induced oxidative effects were mitigated by Spm treatment reflected interms of reduced accumulation of reactive oxygen species and the activities of protease and lipoxygenase, hence leading to membrane strengthening and protection of their function. Differential influence of exogenous Spm was evident on the functioning of antioxidant system with SOD, GR and APX activities much higher in Spm treated seedlings than CAT and DHAR. Increased synthesis of GSH, AsA and tocopherol in Spm treated seedlings was obvious thereby helping in maintaining the redox homeostasis and the enzymatic antioxidant functioning. Interestingly Spm application maintained the nitric oxide levels higher than control under normal condition while as lowered its concentrations in salinity stressed seedlings depicting existence of probable interaction. Activities of polyamine metabolizing enzymes was up-regulated and the accumulation of secondary metabolites including phenols and flavonoids also increased due to Spm application. Further studies are required to understand the mechanisms clearly.

1. Introduction

Salinity stress is considered as one of the detrimental abiotic environmental factor imparting significant decline in growth and development of crop plants (Khan et al., 2014; Ahanger and Agarwal, 2017a). Globally significant area of agricultural land have been rendered saline affected unproductive, and agricultural malpractices including excessive use of saline waters for irrigation have further aggravated the situation (Turkan and Demiral, 2009). Excess availability of toxic salts affect cellular and whole plant functioning by influencing uptake and assimilation of mineral ions, enzyme activity, photosynthetic functioning etc (Khan et al., 2014; Ahanger and Agarwal, 2017a). Salinity induces ionic and osmotic stress altering the structural and functional stability of plants. Stress exposed plants exhibit significantly increased accumulation of ROS resulting in alteration in the functioning of organelles like chloroplast, mitochondria etc. In addition excess ROS induce damage to proteins, nucleic acids and impair activity of enzymes (Ahanger et al., 2017a). Stress induced enhancement in ROS damages membrane integrity resulting in leakage of electrolytes and alters redox homeostasis thereby causing photoinhibition (Nazar et al., 2011). ROS triggered ion leakage activates proteases and endonucleases causing programmed cell death (Demidchik et al., 2014). In order to ameliorate the oxidative effects of stresses plants up-regulate the indigenously existing defense mechanisms. These mechanisms include

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antioxidant system, osmolyte and secondary metabolite accumulation, and ion compartmentation (Khan et al., 2014; Ahanger et al., 2017a, 2018a). Prime functioning of antioxidants and osmolytes include ROS elimination and osmoregulation respectively thereby contributing to maintenance of redox homeostasis and protection of growth under stress (Ahmad et al., 2010). Antioxidants include both enzymatic and non-enzymatic components occurring in different cellular organelles (Bose et al., 2014), and increased accumulation of compatible osmolytes and key secondary metabolites strengthens the antioxidant system (Hernandez et al., 2009; Ahanger et al., 2017a).

Polyamines (PAs) include low-molecular-weight compounds having positive charge at the cellular pH and have ubiquitous role for the growth and survival of all organisms (Alcazar et al., 2011). Putrescine, spermidine and spermine (Spm) have been identified as the most abundant PAs in plants that have been opined to function as plant growth regulators and intracellular messengers, and strengthen the stress tolerance mechanisms including antioxidants (Ahmad et al., 2012). Protective role of PAs in photosynthesis have been reported under salinity-alkalinity (Hu et al., 2016), salinity (Zhang et al., 2009) and drought stress (Farooq et al., 2009). Application of PAs has been reported to improve plant performance under stressful conditions by stabilizing the structure and function of components of photosynthetic apparatus like D1 protein (Hu et al., 2016). PAs may exist in free, soluble or insoluble form. In soluble form they remain covalently bound with the small molecules like phenols while as in their insoluble form remain bound to important macromolecules like nucleic acids and proteins (Ahmad et al., 2012). Therefore in addition of stabilizing key molecules like nucleic acids and proteins, PAs have been reported to regulate certain key plant functions including organogenesis, floral initiation and development, fruit development and ripening, and responses to environmental stresses (Bagni and Tassoni, 2001; Alcazar et al., 2010; Ahmad et al., 2012; Li et al., 2015). Alterations in the endogenous concentrations of PAs vary with stress type, duration and plant species thereby imparting significant variations in the response mechanisms initiated (Hu et al., 2012; Puyang et al., 2016). However there is still paucity of research depicting the active involvement of PAs in tolerance of plants to stresses like salinity.

Tomato is an important horticulture crop plant widely cultivated for its food value. Rich in lycopene and anthocyanins tomato has medicinal importance and is often consumed as vegetable and salad. Present study hypothesized that exogenous Spm application can benefit tomato seedlings by strengthening the tolerance mechanisms including antioxidant system and accumulation of osmolytes and secondary metabolites for improved growth and photosynthesis under salinity stress. In order to test this hypothesis, experiments were conducted on salt stressed tomato seedlings and possible modulations by exogenous Spm were observed by investigating the influence on key physiological and biochemical parameters.

2. Material and methods

2.1. Experimental design, plant material and growth conditions

Tomato (*Solanum lycopersicum* L., cultivar Huange 108) seeds were surface sterilized with 0.01% $HgCl_2$. After sterilization seeds were allowed to germinate in petri plates lined with filter paper wetted with distilled water. Ten days after germination seedlings were transferred to bottom perforated pots filled with reconstituted soil containing soil, compost and sand (3:1:1). The soil used was having slightly alkaline pH (7.5) with concentration of available N, P and K as 65.98, 18.78 and 80.67 $mg\,kg^{-1}$ soil respectively. Seedlings were regularly monitored and irrigated. Twenty days after seedling development, two seedlings were maintained in each pot and salinity stress was started by application of 100 mM NaCl on the alternate days. Application of spermine (Sigma Aldrich, spermine tetrahydrochloride, 50 and 100 μM) was done foliarly using a manual sprayer twice a week. Pots were arranged in complete randomized block design with five replicates for each treatment and were kept in green house at the College of Life Science, NorthWest A&F University Yangling Shaanxi China. After fourty days of growth, seedlings were analyzed for photosynthetic parameters, oxidative stress parameters, antioxidant system and osmoregulatory components, and secondary metabolite accumulation.

2.2. δ-Amino levulinic acid (ALA), total chlorophylls and carotenoid content, and photosynthetic parameters

δ-ALA content was measured according to method of Harel and Klein (1972) as described by Dalal and Tripathy (2012). Two sets of leaf samples (200 mg each) were taken from each treatment and one set was incubated for 4 h in 60 mM levulinic acid (LA) under light while another sample set was processed immediately for ALA estimation. Extraction was carried in 5 mL of 1 M sodium acetate buffer (pH 4.6) using pre-chilled mortar and pestle. After centrifugation for 10 min at 15000*g*, 1 mL supernatant was mixed with 4 mL distilled water and 250 μL of acetyl-acetone. After thorough mixing the mixture was heated in a boiling water bath for 10 min and cooled at room temperature followed by addition of Ehrlich's reagent (2 gm dimethyl amino benzaldehyde (DMBA), 30 mL of glacial acetic acid, 16 mL of 70% perchloric acid and final volume was made up to 50 mL with glacial acetic). After vortexing samples were incubated for 10 min and read at 555 nm. ALA synthesized during 4 h incubation period was measured by subtracting the 0 h ALA from 4 h ALA content.

Total chlorophyll and carotenoids were determined in fresh leaf tissues according to method of Arnon (1949) and absorbance of supernatant was recorded at 480, 645 and 663 nm. Photosynthetic efficiency, intercellular CO_2 concentration and transpiration rate were measured in fully expanded leaf using portable photosynthetic apparatus Li-6400 (LI-COR Inc., USA).

2.3. Determination of leaf water content, soluble sugars, free proline and glycine betaine content

Relative water content (RWC) of leaves was determined by following Smart and Bingham (1974). For measurement of sugar content dry powdered samples were extracted in ethanol and centrifuged at 5000 g for 10 min. Thereafter supernatant was reacted with anthrone reagent and absorbance was taken at 620 nm (Fong et al., 1953; Jain and Guruprasad, 1989). For estimation of free proline method of Bates et al. (1973) was employed while as content of glycine betaine was estimated following Grieve and Grattan's (1983) method.

2.4. Hydrogen peroxide, superoxide, lipid peroxidation and electrolyte leakage

H_2O_2 was estimated by macerating 100 mg fresh leaf tissue in 5 mL trichloro acetic acid (0.1% TCA) and homogenate was centrifuged at 10,000 g for 10 min. Thereafter 500 μL supernatant was mixed with 500 μL of potassium phosphate buffer (pH 7.0) and 1 mL potassium iodide. Absorbance was taken at 390 nm (Velikova et al., 2000).

For determination O_2^- content, 100 mg fresh tissue was macerated in potassium phosphate buffer (65 mM, pH 7.8). After centrifugation at 5000*g*, supernatant was mixed with 10 mM hydroxylamine hydrochloride and mixture was allowed to stand for 20 min. Thereafter sulfanilamide and naphthylamine were added to each sample and incubated at 25 °C for 20 min and absorbance was recorded at 530 nm (Yang et al., 2011) and calculations were done using standard curve of $NaNO_2$.

For measurement of lipid peroxidation, content of malonaldehyde (MDA) formation was determined in accordance with the method of Heath and Packer (1968). Briefly, 100 mg fresh tissue was extracted in 1% TCA using pestle and mortar. After centrifugation at 10,000 g for 5 min, 1.0 mL supernatant was mixed with 4 mL of 0.5% thiobarbituric acid and mixture was boiled at 95 °C for 30 min. After cooling the tubes on ice bath samples were again centrifuged for 5 min at 5000 g and optical density of supernatant was measured at 532 and 600 nm.

Electrolyte leakage (EL) was measured following method of Dionisio-Sese and Tobita (1998). Fresh leaf discs (twenty) were immersed in test tubes filled with deionized water and the electrical conductivity (ECa) was recorded. The same samples were heated at 50 °C for 25 min in a water bath and electrical conductivity (ECb) was measured. Thereafter the same samples were again heated at 100 °C for 10 min and again the electrical conductivity (ECc) was recorded. Percent EL was calculated using following formulae:

$$\text{EL}\,(\%) = \frac{\text{EC}_b - \text{EC}_a}{\text{EC}_c}\ \text{X}\ 100$$

2.5. Lipoxygenase and protease activity

For assay of LOX (EC 1.13.11.12) activity method of Doderer et al. (1992) was followed using linoleic acid as substrate and change in absorbance was recorded at 234 nm. For calculation of activity extinction coefficient of 25 $\text{mM}^{-1}\,\text{cm}^{-1}$ was used. Assay of protease (EC 3.4.21.40) was done adopting method of Green and Neurath (1954). Fresh plant tissue was homogenised in chilled sodium potassium buffer (50 mM, pH 7.4) supplemented with 1% PVP using prechilled pestle and mortar. Homogenate was centrifuged at 5000 g for 5 min at 4 °C. Supernatant (1 mL) was incubated with casein at 40 °C and amount of tyrosine released was determined by reacting the mixture with Folin Ciocalteu's reagent in alkaline medium. Optical density was recorded at 660 nm and activity was expressed as μg tyrosine released mg^{-1} protein.

2.6. Estimation of nitric oxide

For determination of nitric oxide (NO) method described by Zhou et al. (2005) was followed. 500 mg fresh leaf tissue was macerated in ice-cold acetic acid buffer (50 mM, pH 3.6), containing 4% zinc diacetate using pestle and mortar. After centrifugation for 15 min at 11,500 g pellet was resuspended in 1 mL extraction buffer and after centrifugation supernatants were pooled together. Supernatant was neutralised by addition of charcoal followed by vortexing and filtration. To 1 mL filtrate, 1 mL Greiss reagent was added and mixture was incubated for 30 min at room temperature followed by measurement of absorbance at 540 nm. Concentration of NO was calculated from a standard curve of sodium nitrite ($NaNO_2$).

2.7. Determination of nitrate reductase

Activity of nitrate reductase (NR) was measured by incubating 300 mg freshly cut leaf tissue in potassium phosphate buffer (100 mM, pH 7.5) supplemented with 200 mM KNO_3 and 0.5% n-propanol (v/v) for 3 h in dark at 30 °C. Aliquot (1 mL) was taken and 1 mL of each sulphanilamide (1%) and 1-nephthylethylene diamine dihydrochloride (0.2%) was added. After 20 min absorbance was recorded at 540 nm (Srivastava, 1974).

2.8. Assay of antioxidant enzymes

Antioxidant enzymes were extracted by macerating 1.0 gm fresh leaf tissue in pre-chilled pestle and mortar using 100 mM phosphate buffer (pH 7.8) supplemented with 1% polyvinyl pyrolidine, 1 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation at 12,000 g for 15 min at 4 °C supernatant was collected and used for enzyme assay.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed following method of Bayer and Fridovich (1987) and ability of enzyme to inhibit the photochemical reduction of nitroblue tetrazolium chloride was monitored at 560 nm after incubating the assay mixture for 15 min under florescent light. For determination of catalase (EC 1.11.1.6) activity, method of Aebi (1984) was followed and decrease in absorbance was recorded at 240 nm for 2 min in assay mixture containing 50 mM phosphate buffer (pH 7.0), H_2O_2 and 100 μL enzyme extract. Activity of ascorbate peroxidase (APX, EC 1.11.1.11) was assayed by monitoring the disappearance of H_2O_2 at 290 nm for 3 min according to Nakano and Asada (1981). Glutathione reductase (GR; EC 1.6.4.2) activity was assayed by monitoring the glutathione dependent oxidation of NADPH as change in absorbance at 340 nm for 2 min (Foyer and Halliwell, 1976). Activity of DHAR (EC: 1.8.5.1) was assayed in accordance of Nakano and Asada (1981) and change in absorbance was recorded at 265 nm for 2 min in an assay mixture containing 50 mM potassium phosphate buffer (pH 7.0), 2.5 mM GSH and 100 μM DHA. Activities of antioxidant enzymes were expressed as EU mg^{-1} protein.

2.9. Estimation of ascorbate, reduced glutathione, and tocopherol

Method described by Mukherjee and Choudhuri (1983) was employed for determination of ascorbate (AsA). After extraction in 5% TCA supernatant was mixed with dinitrophenylhydrazine (2%) and thiourea (10%) followed by incubation in water bath for 15 min. Thereafter samples were cooled and 5 mL cooled 80% H_2SO_4 was added. Absorbance was recorded at 530 nm and standard curve of AsA was used for calculation.

Reduced glutathione (GSH) was estimated in accordance with Ellman (1959). Briefly 100 mg fresh tissue was homogenised in phosphate buffer (pH 8.0). After centrifugation supernatant (500 μL) was mixed with equal volume of 5, 5-dithiobis-2-nitrobenzoic acid and optical density was read at 412 nm. Concentration of GSH was calculated from the standard curve of GSH.

Content of tocopherol was extracted in ethanol and petroleum ether (1.6:2) and optical density was measured at 520 nm after incubating the supernatant with 2% of 2, 2-dipyridyl in dark (Backer et al., 1980). Calculation was done using standard curve of tocopherol.

2.10. Determination of activities of ornithine decarboxylase (ODC), arginine decarboxylase (ADC) and polyamine oxidase (PA)

500 mg fresh plant tissue was homogenised in potassium phosphate buffer (100 mM, pH 8.0) containing PMSF (0.1 mM), pyridoxal phosphate (1 mM PLP), dithiothreitol (5 mM, DTT), 1 mM EDTA, 10 mM ascorbic acid and 0.1% PVP. After centrifugation at 12,000 g for 40 min at 4 °C supernatant was dialyzed and used for assaying the enzyme activity. Activities of ADC or ODC were determined according to Zhao et al. (2003) in an assay mixture containing tris-buffer (100 mM, pH 7.5), EDTA, pyridoxal phosphate (50 mM), DTT and 300 μL enzyme extract. After incubation at 37 °C for 2 min, 200 μL of L-arginine (for ADC) or 200 μL of L-ornithine (for ODC) were added and mixtures were again incubated for 1 h at 37 °C. Thereafter, 5% perchloric acid was added and centrifuged again at 3000 g for 10 min. To 500 μL supernatant were added 2 mM NaOH and benzoyl chloride, and mixture was thoroughly mixed and incubated at 37 °C for 30 min. Thereafter NaCl (2 mL) and ether (3 mL) were added and mixture was centrifuged again at 1500 g for 5 min. After extraction with ether, evaporated ether phase was redissolved in methanol (60%) and read at 254 nm.

For determination of PAO activity fresh leaf tissue was homogenised in 100 mM phosphate buffer (pH 6.5) and homogenate was centrifuged for 20 min at 10,000 g at 4 °C. Reaction mixture contained 100 mM phosphate buffer (pH 6.5), 200 μL 4-aminoantipyrine/N, N-dimethylaniline solution, 100 μL horseradish peroxidase and 200 μL enzyme extract. Change in optical density was monitored at 254 nm after initiating reaction by adding 20 mM of each spermidine and spermine (Su et al., 2005).

2.11. Estimation of total phenols, flavonoids and assay of phenylalanine ammonia lyase

Total phenol content was determined following Malick and Singh (1980). Dry powdered (500 mg) sample was extracted in 80% ethanol. After centrifugation at 10,000 g for 10 min supernatant was evaporated to dryness and volume was made to 10 mL by distilled water. Known volume of aliquot was made to 2 mL followed by addition of 1N Folin–Ciocalteu reagent and 1 mL Na_2CO_3. After incubation at room temperature absorbance was read at 650 nm and calculation was done from the standard curve of catechol.

Content of flavonoids was estimated by following method of Zhishen et al., 1999a. 100 mg dry plant sample was extracted in methanol (3 mL) followed by centrifugation at 10000*g* for 10 min. Thereafter, 1 mL supernatant was made to 4 mL using distilled water followed by addition of 5% $NaNO_2$ and 10% $AlCl_3$. After 5 min, 2 mL of NaOH and 2.4 mL distilled water were added. Absorbance was recorded at 510 nm and calculation was done using standard curve of catechin.

Activity of phenylalanine ammonia lyase (PAL) was assayed in accordance with Zucker (1965) and formation of trans-cinnamic acid was measured at 290 nm.

2.12. Estimation of Na, K and N

Na and K were estimated flame photometrically described by Ahanger et al. (2015). Micro-Kzeldahl's method suggested by Jackson (1973) and modified by Iswaran and Marwaha (1980) was employed for estimation of N content.

2.13. Statistical analysis

Mean (± SE) of three replicates have been presented for each parameters. Significance of data was tested by performing Duncan's Multiple Range Test using One Way ANOVA and least significant difference (LSD) was calculated at $p < 0.05$.

3. Results

3.1. Spm application improves pigment synthesis and photosynthetic functioning

Exogenous application of Spm at 100 μM significantly increased the synthesis of δ-ALA, total chlorophylls and carotenoids under normal conditions and resulted in maximal amelioration of the decline induced by NaCl stress. Relative to control, 100 μM Spm increased δ-ALA by 16.72%, total chlorophylls by 27.42% and carotenoids by 27.85% over the control. Relative to NaCl stressed plants, an amelioration of 35.74% for δ-ALA, 46.10% for total chlorophylls and 40.06% for carotenoids was observed due to application of 100 μM Spm (Fig. 1A–C). In addition, application of 100 μM Spm increased the net photosynthesis (41.66%), intercellular CO_2 (29.11%) and transpiration rate (32.43%) over control more evidently than 50 μM Spm concentration. Seedlings exposed to NaCl treatment exhibited apparent decline in the net photosynthesis (30.69%), intercellular CO_2 (32.76%) and transpiration rate (40.00%) over the control, however Spm application at both concentrations imparted amelioration of the salinity mediated decline with much evident effect in 100 μM Spm treated plants. Relative to NaCl stressed plants, net photosynthesis, intercellular CO_2 and transpiration rate increased by 47.76, 39.31 and 49.16% respectively in NaCl +100 μM Spm treated plants (Fig. 2A–C).

3.2. Effect of Spm application on oxidative stress parameters under NaCl stress

Exogenous application of Spm significantly reduced the accumulation of toxic radicals including O_2^- and H_2O_2under NaCl stress however under normal conditions there was no significant decline in ROS accumulation. Maximal reduction 36.40% for lipid peroxidation and 49.36% for electrolyte leakage was observed in seedlings supplied with 100 μM Spm over the control. Application of 50 μM Spm was not significantly affective in reducing the oxidative stress parameters under normal growth conditions however imparted beneficial effect when supplied to NaCl stressed plants. Relative to NaCl stressed plants, maximal decline of 39.74 and 32.90% in O_2^- and H_2O_2 causing 45.95 and 51.70% amelioration in the lipid peroxidation and electrolyte leakage was observed in NaCl +100 μM Spm treated plants

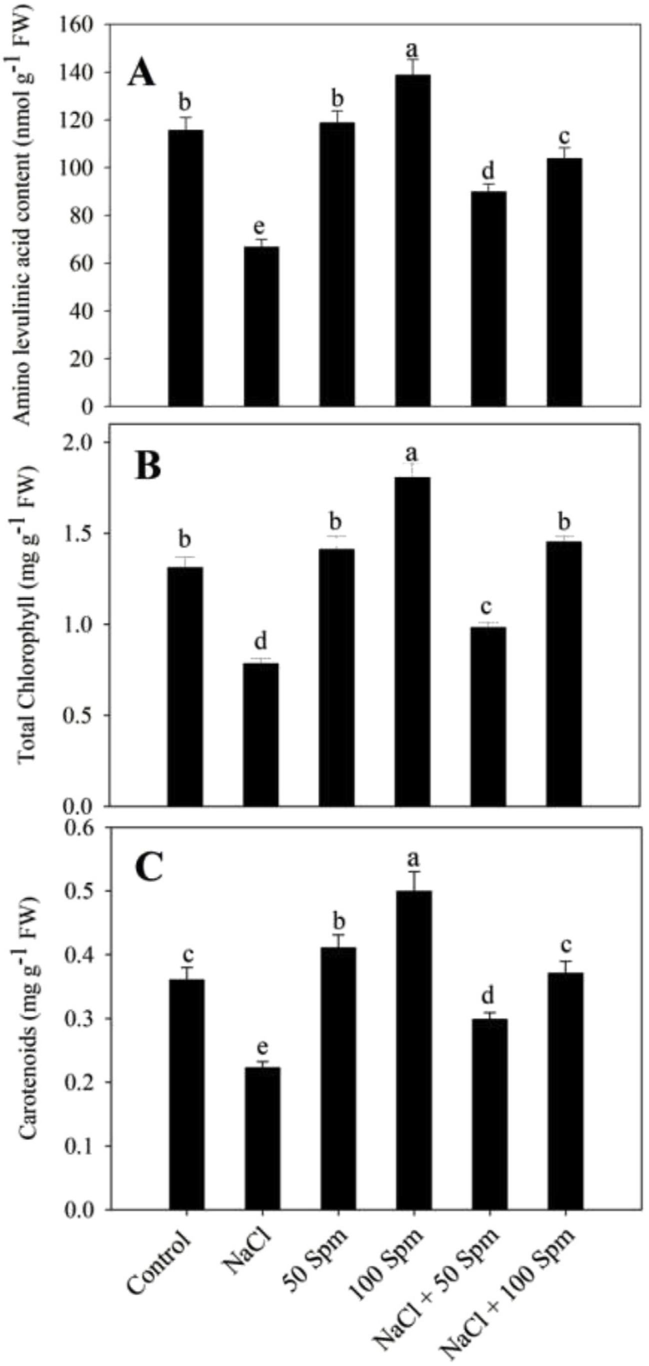

Fig. 1. Effect of Spm (50 and 100 μM) application on (A) δ-amino levulinic acid, (B) total chlorophyll and (C) carotenoid content in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

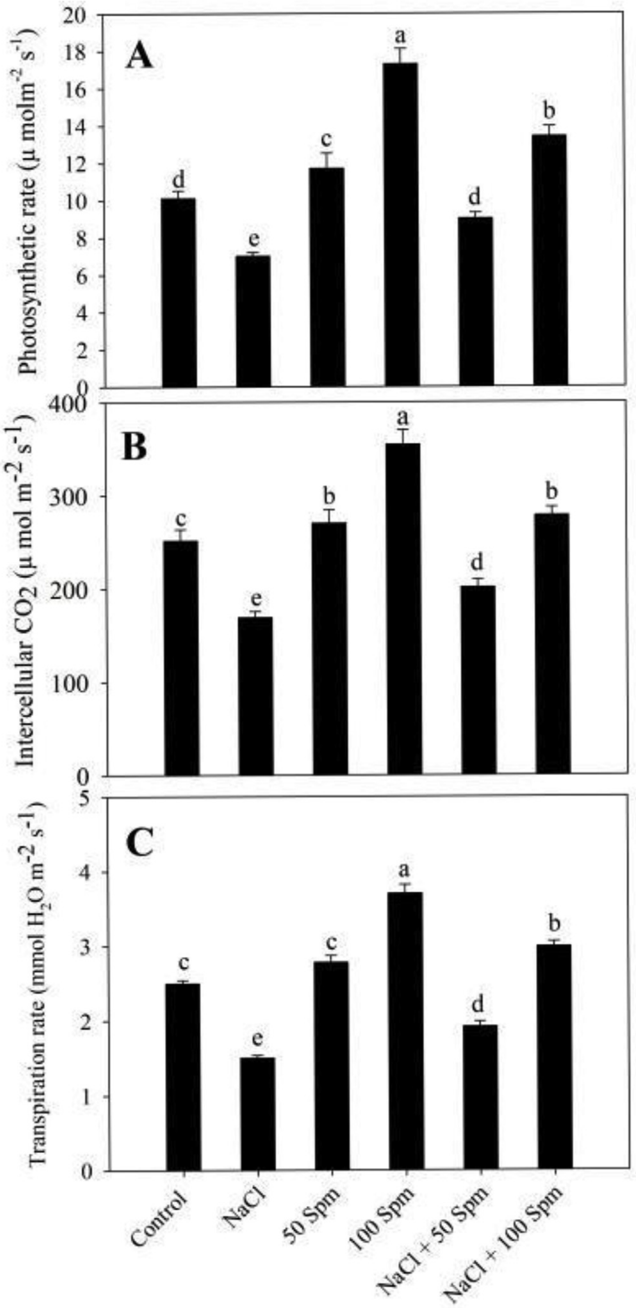

Fig. 2. Effect of Spm (50 and 100 µM) application on (A) photosynthesis, (B) intercellular CO_2 concentration and (C) transpiration rate in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

(Fig. 3A–D).

3.3. Spm application influences the activity of protease and lipoxygenase, and NO production

Salinity stress triggered activity of protease (39.20%) and lipoxygenase (37.97%) while as application of Spm (100 µM) significantly reduced (14.83 and 25.70%) the activities over the control. Application of Spm, at both concentrations reduced the activities of protease and lipoxygenase when applied to NaCl stressed plants. Relative to NaCl stressed counterparts, maximal decline in activities of protease and lipoxygenase was 24.82 and 35.06% respectively in NaCl +100 µM Spm treated seedlings (Fig. 4A and B). NO production exhibited 3.2 fold increase due to salinity stress. However application of Spm to salt stressed plants declined the NO formation by 51.07 and 25.58% at 50 and 100 µM over the NaCl stressed counterparts. Relative to control, Spm application increased NO production by 37.87 and 45.42% at 50 and 100 µM respectively (Fig. 4C).

3.4. Effect of Spm application on the activities of enzymatic and non-enzymatic antioxidant

Exogenous application of Spm differentially influenced the activities of antioxidant enzymes assayed. Under normal conditions, among the antioxidant enzymes assayed increase in the activities due to Spm application was much obvious in SOD, APX and GR compared to CAT and DHAR. Salinity stress resulted in increased activities of SOD (31.74%), CAT (38.39%), APX (30.76%), DHAR (33.98%) and GR (15.48%) over the control plants and application of Spm (100 µM) to salt stressed plants maximally increased the activities of antioxidant enzymes by 52.16% for SOD, 26.16% for CAT, 54.37% for APX, 14.05% for DHAR and 41.90% for GR over the salt stressed plants (Figs. 5 and 6A-C). Salt treatment resulted in declined AsA production while as increased the GSH and tocopherol content over the control plants. Exogenous application of Spm at 100 µM increased AsA (13.67%), GSH (15.82%) and tocopherol (17.03%) content over the control, and attained maximal amelioration of 21.61% in AsA over NaCl stressed plants and maximal increase of 37.03% and 40.68% in GSH and tocopherol in NaCl + 100 µM Spm over the control (Fig. 6D–F).

3.5. Spm effects accumulation of osmolytes under salinity stress and relative water content

Application of Spm induced the accumulation of proline, glycine betaine and sugars under normal and salinity stress conditions with apparent enhancement at 100 µM Spm. Relative to control, increase in proline, glycine betaine and sugar content was 44.97, 42.21 and 49.33% respectively due to 100 µM Spm application. Salinity stress imparted significant enhancement in accumulation of proline, glycine betaine and sugar content over control and further increase of 36.46, 33.99 and 32.01% respectively due to Spm (100 µM) application was observed over the NaCl stressed plants (Fig. 7A–C). Salinity reduced RWC of leaf by 40.12% over the control, however was ameliorated by 30.41% in NaCl +100 µM Spm treated plants (Fig. 7D).

3.6. Effect of salinity and Spm application on secondary metabolites and PAL activity

Salinity stress imparted increased accumulation of total phenols and flavonoids, and the activity of PAL over the control plants. Salinity mediated increase was further enhanced by the application of Spm attaining a maximal increase of 56.22% for total phenol, 36.13% for flavonoids and 52.41% for PAL activity due NaCl +100 µM Spm treatment over the control plants. Under normal growth conditions 100 µM Spm imparted significant effect as compared to 50 µM (Fig. 8A–C).

3.7. Effect of Spm on NR activity and nitrogen content

Salinity stress declined the activity of NR (43.39%) and the content of N (48.37%) significantly over the control, however Spm (100 µM) application maximally ameliorated the decline by 42.58% (NR) and 37.28% (N) over the NaCl stressed plants. Under normal growth conditions activity of NR and the content of N were increased significantly due to application of 100 µM Spm (Fig. 9A and B).

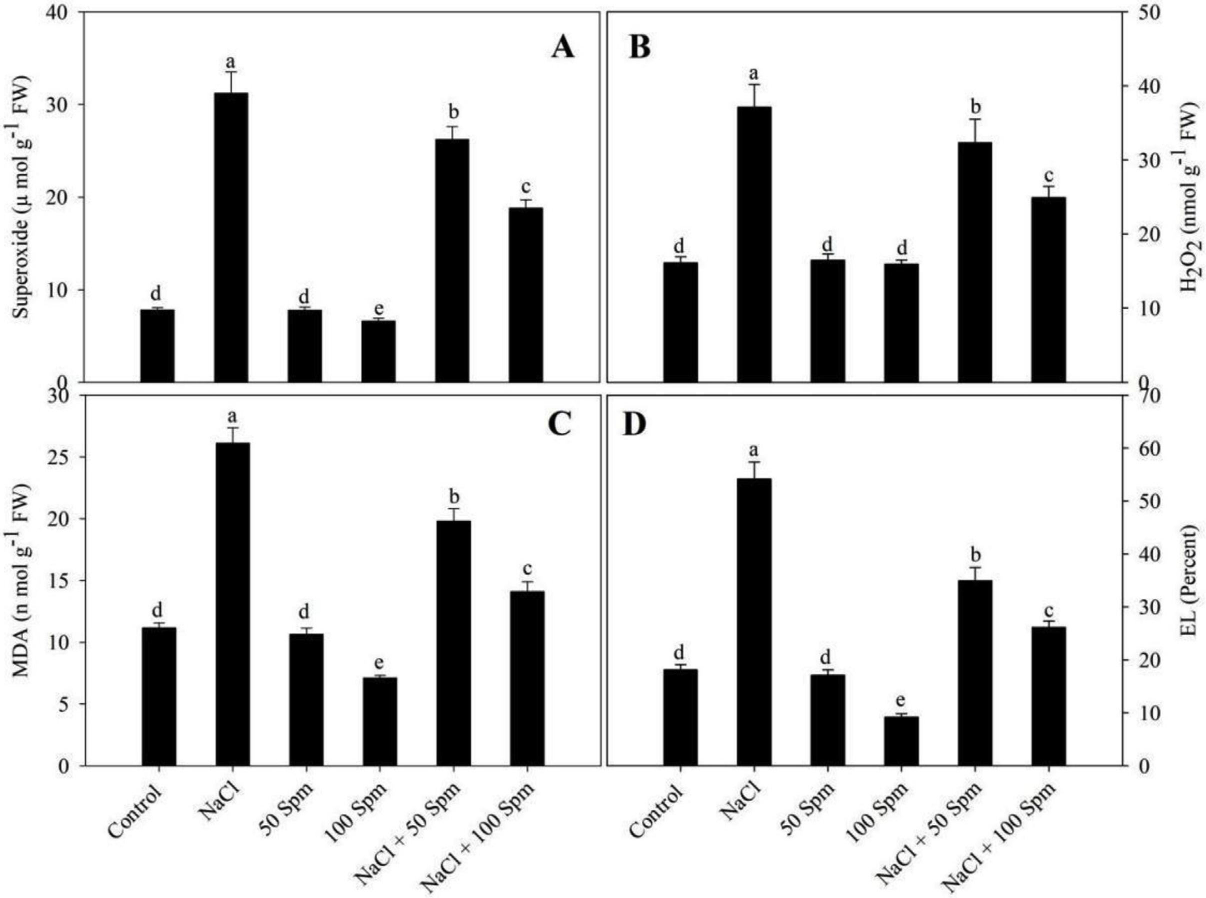

Fig. 3. Effect of Spm (50 and 100 µM) application on (A) superoxide, (B) hydrogen peroxide, (C) lipid peroxidation and (D) electrolyte leakage in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at P < 0.05.

3.8. Effect on the activity of polyamine metabolizing enzymes

Salinity stress enhanced the activities of PA biosynthesizing enzymes, ADC and ODC by 30.76 and 11.55%, and catabolizing enzyme, PAO by 13.32% over control. Exogenous application of Spm at both concentrations increased the activities of ADC, ODC and PAO. Maximal increase in ADC, ODC and PAO activities was 43.75, 41.13, 57.81% with NaCl +100 µM Spm over the control (Fig. 10).

3.9. Potassium and sodium content, and plant height and dry weight

Application of Spm significantly decreased the uptake and accumulation of Na while as increased the K content over the control as well as salinity stressed plants. Content of Na was observed to increase by 66.66% in NaCl treated seedlings over control and was declined by 33.12% due to application of 100 µM Spm to NaCl stressed counterparts (Fig. 11). K content was significantly enhanced due to Spm application however was declined by 51.48% due to NaCl treatment over the control (Fig. 11). Shoot length and dry weight was declined by 33.55 and 46.42% respectively due to NaCl stress over the control plants. Exogenous application of 100 µM Spm maximally enhanced the shoot length (23.03%) and plant dry weight (30.86%) and ameliorated the salinity induced decline by 27.19 and 38.77% over the NaCl stressed ones (Fig. 12).

4. Discussion

Salinity stress results in considerable damage to plant performance and productivity. Growing research evidences are available demonstrating the beneficial role of phytohormones supplied exogenously to plants either foliarly, priming or through root medium resulting in significant amelioration of deleterious effects of stress and have been considered to show promising role in global food security (Ahanger et al., 2018a,b; Ahmad et al., 2018). Therefore in present study we investigated the involvement of foliar application of polyamine (Spm) in alleviation of negative effects of salinity in *Solanum lycopersicum* L. Salinity stress inhibits root and shoot growth by inducing osmotic and ionic stress leading to reduced tissue water content and the mineral uptake and assimilation (Ahmad et al., 2018). Salinity stress reduced the uptake, accumulation and assimilation of key mineral ions including N and K with concomitant accumulation of Na thereby leading to significant inhibition of growth. Earlier several researchers have demonstrated significant decline in mineral uptake and growth due to salinity stress (Ahmad et al., 2012, 2018; Khan et al., 2014; Iqbal et al., 2015), however the role of Spm application in assuaging such deleterious effects are scanty. Excess availability of toxic ions like Na alters the functioning of transport proteins responsible for the efficient uptake of key mineral ions like N and K thereby rendering plant tissue deficient of these ions (Parihar et al., 2015; Ahanger et al., 2017a). Yao et al. (2008) have demonstrated reduced growth in tomato due to declined N uptake following NaCl mediated declined NR activity and the expression of nitrate transport protein. Reduced accumulation of Na with increase in beneficial ions like K and N is directly influenced by the functioning of transport proteins like SOS, NHX etc involved in compartmentation and exclusion of toxic ions from the cells thereby preventing the damage to plants (Roy and Chakraborty, 2018). Polyamines affect vacuolar and

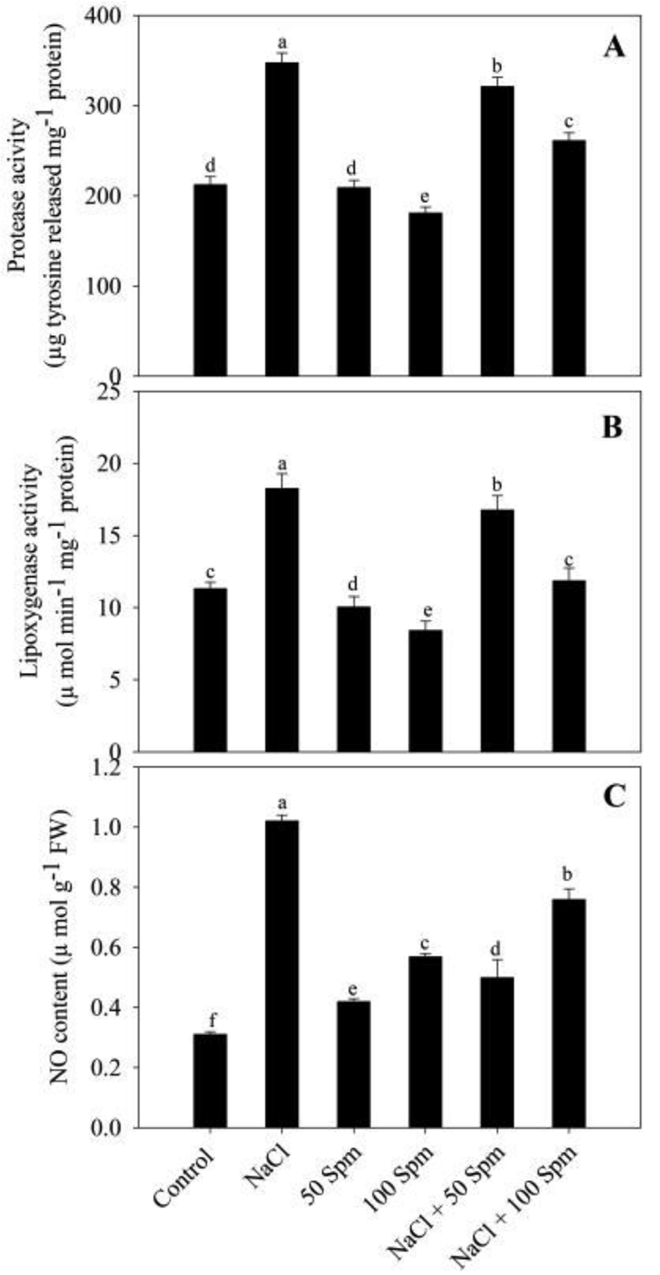

Fig. 4. Effect of Spm (50 and 100 µM) application on the activity of (A) protease, (B) lipoxygenase and (C) nitric oxide content in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

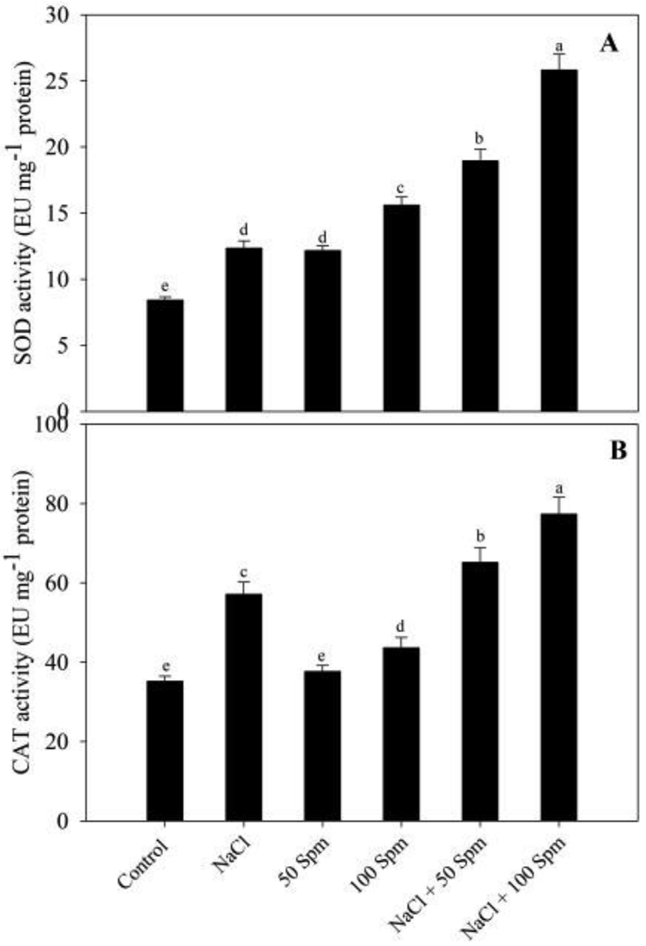

Fig. 5. Effect of Spm (50 and 100 µM) application on the (A) superoxide dismutase and (B) catalase activity in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

plasma membrane H^+ and Ca^{2+} pump (Pottosin and Shabala, 2014), in addition restrict the influx of NaCl (Shabala et al., 2007). It has been suggested that PAs provoke remodeling of the cationic and anionic conductance at the plasma membrane thereby affecting the Ca^{2+} signalling (Pottosin et al., 2014). Increased K and N uptake due to Spm application may have helped tomato plants to better endure the salinity damage by maintaining higher enzyme activities. Increased N (Iqbal et al., 2015) and K (Ahanger and Agarwal, 2017a) uptake have been reported to significantly influence the plant growth and stress tolerance by up-regulating the enzyme activity, chlorophyll synthesis and restricting Na uptake. Nitrate reductase catalyzes the rate limiting step in N assimilation, and improved NR activity benefits plant metabolism by improving the synthesis of amino acids (Ahanger et al., 2017b). Spm mediated enhancement in NR activity and N content may have improved the synthesis of amino acids and also maintained allocation of enough N for the synthesis of key proteins like Rubisco.

Exogenous application of Spm increased the synthesis of chlorophylls and carotenoids resulting in significant improvement in photosynthesis. Salinity stress hampers the chlorophyll synthesis by declining the activity of enzymes involved in chlorophyll biosynthesis and the synthesis of intermediates (Dalal and Tripathy, 2012). Similar to our study salinity stress has been reported to decline the synthesis of δ-ALA in rice (Turan and Tripathy, 2015). In addition it has been reported that stresses up-regulate chlorophyllase activity to trigger chlorophyll degradation (Szafranska et al., 2017). Application of Spm (100 µM) may have contributed to greater chlorophyll synthesis by improving expression of chlorophyll biosynthesizing enzymes leading to greater intermediate like δ-ALA synthesis. Further increased chlorophyll synthesis in Spm treated seedlings can be attributed to enhanced uptake of Mg ion, a key component of chlorophyll (Puyang et al., 2016). Supplementation of PAs have been demonstrated to mitigate the decline in chlorophyll synthesis under salinity (Roychoudhury et al., 2011), salinity-alkalinity (Li et al., 2015), and manganese (Hussain et al., 2019) stress. Maintaining relatively higher internal concentrations of CO_2 and the transpiration rate due to Spm treatment depicts the stomatal regulation of photosynthesis in addition of maintenance of temperature. Iqbal et al. (2015) have also demonstrated reduced CO_2 and stomatal conductance due to salinity stress. In present study photosynthetic regulation due to Spm under salinity may be attributed to increased N,

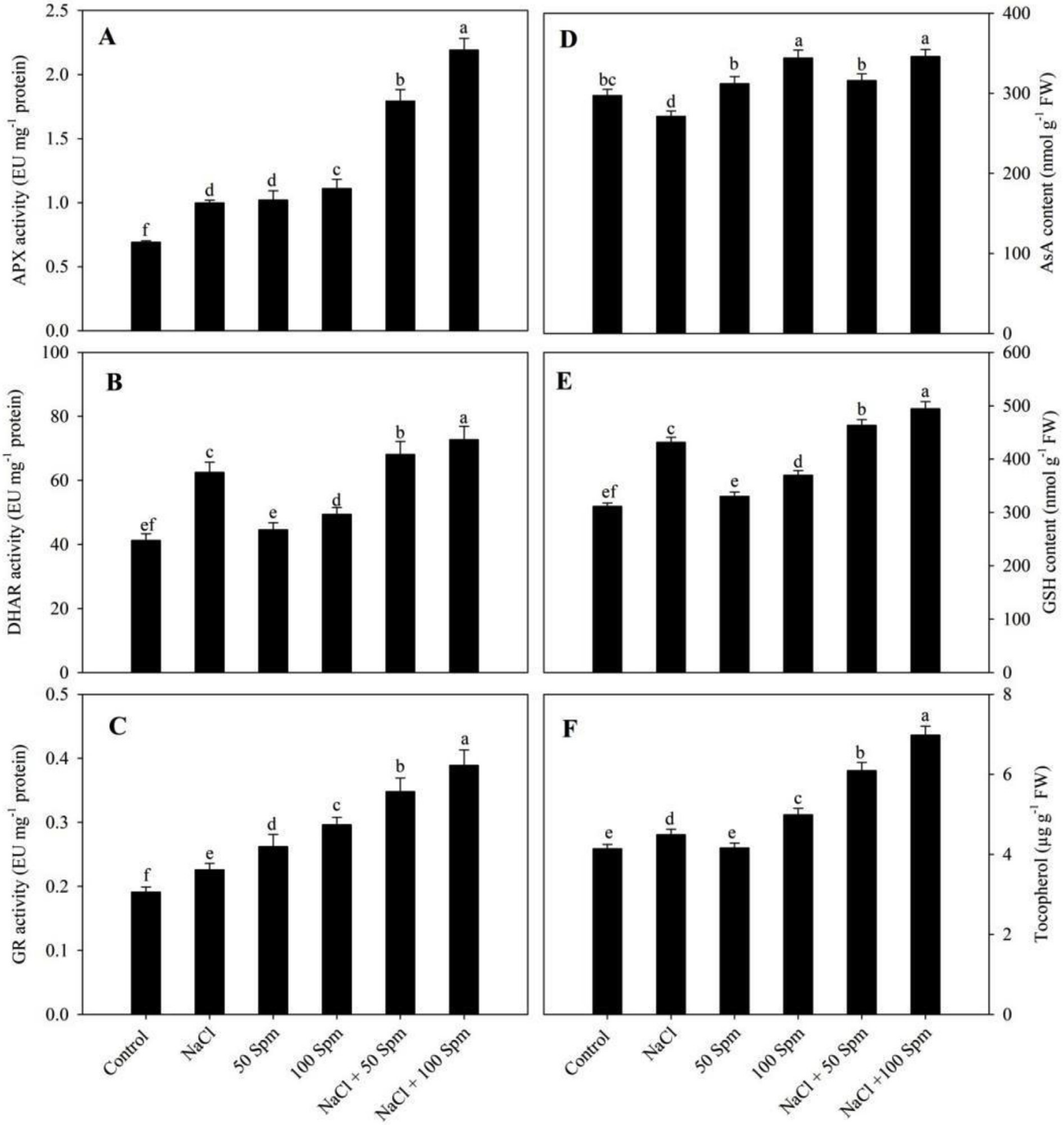

Fig. 6. Effect of Spm (50 and 100 µM) application on the activity of (A) ascorbate peroxidase, (B) dehydroascorbate reductase, (C) glutathione reductase activity and the content of (D) ascorbate, (E) reduced glutathione and (F) tocopherol in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

K uptake, δ-ALA synthesis, enhanced protection to D1 protein of PSII and antioxidant regulation (Li et al., 2015; Hu et al., 2016). Maintaining higher expression of PSII D1 protein coding genes influences the de novo synthesis of D1 and ultimately the repair of PSII photo damage (Hu et al., 2016), and which may be regulated by phytohormones including ABA (Wang et al., 2016), PAs (Hu et al., 2016) etc. In addition it has been reported that stomatal control mediated by PAs include NO production (Agurla et al., 2018). In present study also we observed increased NO production in Spm treated seedlings under normal as well as salt stressed conditions, however was significantly lower than salinity stressed plants. NO acts as signalling component in regulating the stomatal functioning (Agurla et al., 2014). Optimal NO has been reported to strengthen the salinity stress tolerance by improving cellular mechanisms to counter oxidative damage (Neill et al., 2008; Fatma et al., 2016).

Exogenous application of Spm affectively reduced the oxidative damage generated by salinity stress thereby preventing damage to membranes. Enhanced generation of ROS including H_2O_2 and O_2^- due to salinity stress results in greater lipoxygenase imparting direct deleterious effects on membrane functioning (Nahar et al., 2016). Similar to our results increased oxidative damage due to excessive generation of ROS has been earlier reported by several workers (Khan et al., 2014; Fatma et al., 2016; Ahanger et al., 2018b). Reports discussing the influence of foliar Spm on ROS accumulation and activity of protease and lipoxygenase under salinity are scanty. Increased protease (Ahanger and Agarwal, 2017a) and lipoxygenase (Zhang et al., 2013) activity has been reported in salt stressed wheat and rice respectively. PA treatment through root has been demonstrated to reduce the lipoxygenase activity considerably depicting lesser oxidative damage (Roychoudhury et al., 2011). Salinity stress induced increased lipoxygenase activity

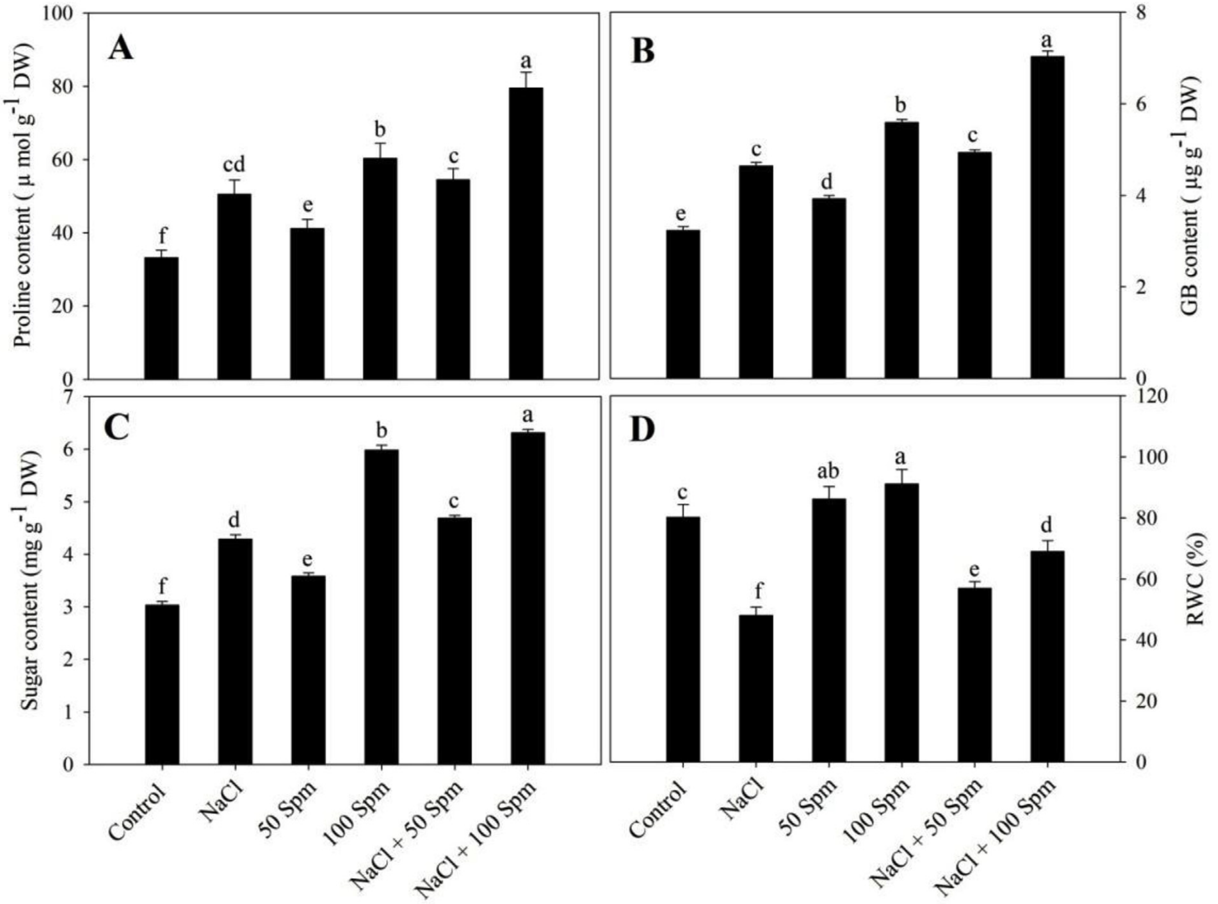

Fig. 7. Effect of Spm (50 and 100 μM) on the content of (A) free proline, (B) glycine betaine, (C) sugars, and (D) relative water content in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

influences the membrane functioning by reducing the polyunsaturated fatty acids (Zhang et al., 2013). PAs form complex with Fe^{2+} and bind with polar head groups of phospholipids thereby restricting the autoxidation and hence protecting membranes from oxidative effects of ROS (Velikova et al., 2000). Reduced lipoxygenase activity under stress conditions following exogenous treatment of PA has been considered as important stress tolerance adaptation (Nahar et al., 2016). Spm mediated decline in lipoxygenase and protease activity under salinity and normal conditions reflects the increased protein and lipid stability. Proteases hydrolyze peptides and cleave substrate proteins however at their optimal functioning they have important role in bulk degradation and signalling (Stael et al., 2019).

Accumulation of compatible osmolytes is considered as an important tolerance strategy to avert the deleterious effects of stresses (Ahanger et al., 2014). In present study salinity stress triggered the accumulation of osmolytes including proline, glycine betaine and soluble sugars. Foliar application of Spm enhanced the synthesis of osmolytes thereby further improving their accumulation when applied to salt stressed plants with effect much apparent in 100 μM Spm treated seedlings. Proline, glycine betaine and sugars protect cellular structures and functioning by maintaining the water content and scavenging ROS (Hayat et al., 2012; Ahanger et al., 2014). PAs have been reported to reprogram the oxidative and nitrosative status for improved salt tolerance in citrus plants (Tanou et al., 2014). Increased proline accumulation due to PA application is directly related to the gene expression of its biosynthesizing genes (Tanou et al., 2014). Increased proline, glycine betaine and sugar accumulation due to salinity has been reported in several crops (Khan et al., 2014; Ahanger and Agarwal, 2017a; Ahanger et al., 2018b). Sugars act as substrates in carbon and energy metabolism, and in polymer biosynthesis. In addition sugars show hormone-like functions as primary messengers in signal transduction (Rolland et al., 2002), therefore Spm mediated enhancement in their accumulation may have coordinated the internal regulators and environmental cues governing growth and development (Sheen et al., 1999; Smeekens, 2000; Smeekens and Hellmann, 2014). Increased accumulation of proline and glycine betaine in Spm supplemented seedlings may have contributed to photosynthetic regulation by protecting the carboxylase activity besides control on the stomatal attributes. Earlier proline, sugars (Sivakumar et al., 2000, 2002) and glycine betaine (Incharoensakdi et al., 1986) has been reported to protect the carboxylase activity of Rubisco in several crop species.

Exogenous application of Spm maintained differential effect on the activities of antioxidant enzymes assayed. The activities of SOD, APX and GR were up-regulated more in Spm treated seedlings as compared to control and NaCl stressed ones while as CAT and DHAR exhibited maximal increment due to salinity stress. Exogenous Spm application further increased the activities under salinity stress conditions. Earlier increased activities of antioxidant enzymes due to application PAs (spermidine) has been reported by Roychoudhury et al. (2011) and Li et al. (2015) in rice and tomato respectively. Increased activities of antioxidant enzymes due to exogenous application of Spm significantly reduced the oxidative effects of ROS on membranes. SOD scavenges superoxide radical preventing the formation of hydroxyl radicals (Ahmad et al., 2010; Ahanger et al., 2017a). Hydrogen peroxide generated after SOD mediated superoxide detoxification or from the other production sites is eliminated by CAT individually or APX in intriguing AsA-GSH cycle (Ahanger et al., 2017a). Up-regulation of the enzymatic components of AsA-GSH components due to foliar Spm application

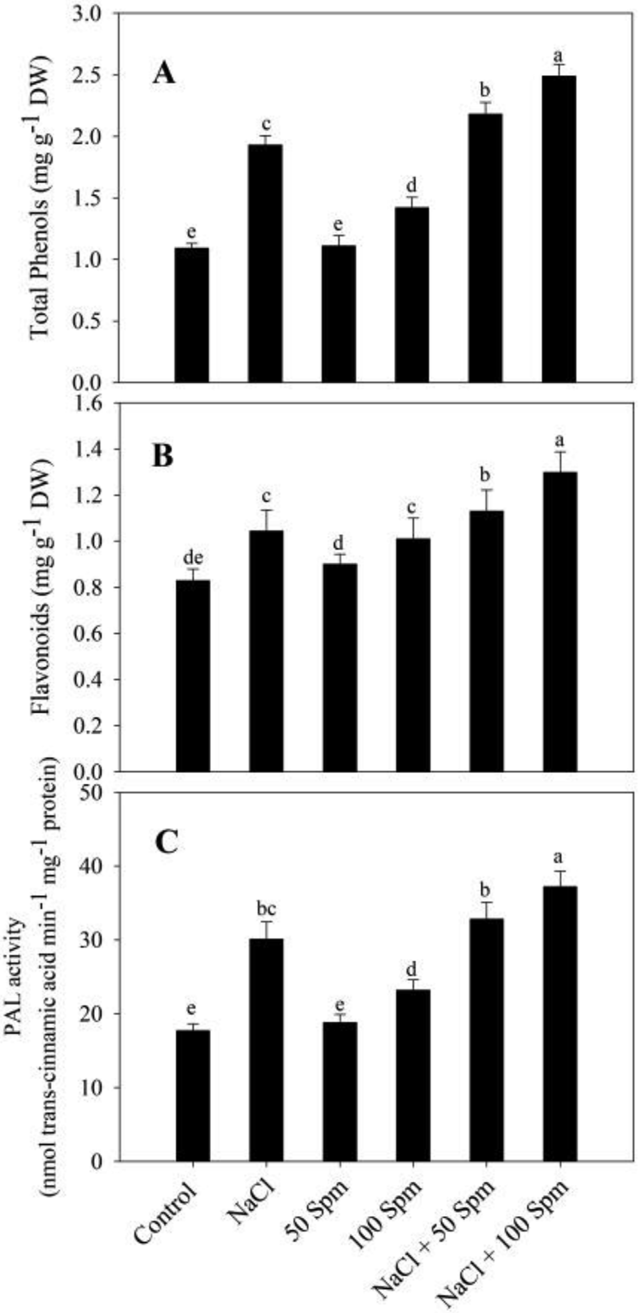

Fig. 8. Effect of Spm (50 and 100 µM) application on the content of (A) total phenols, (B) flavonoids, and the activity of (C) phenylalanine ammonia lyase in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

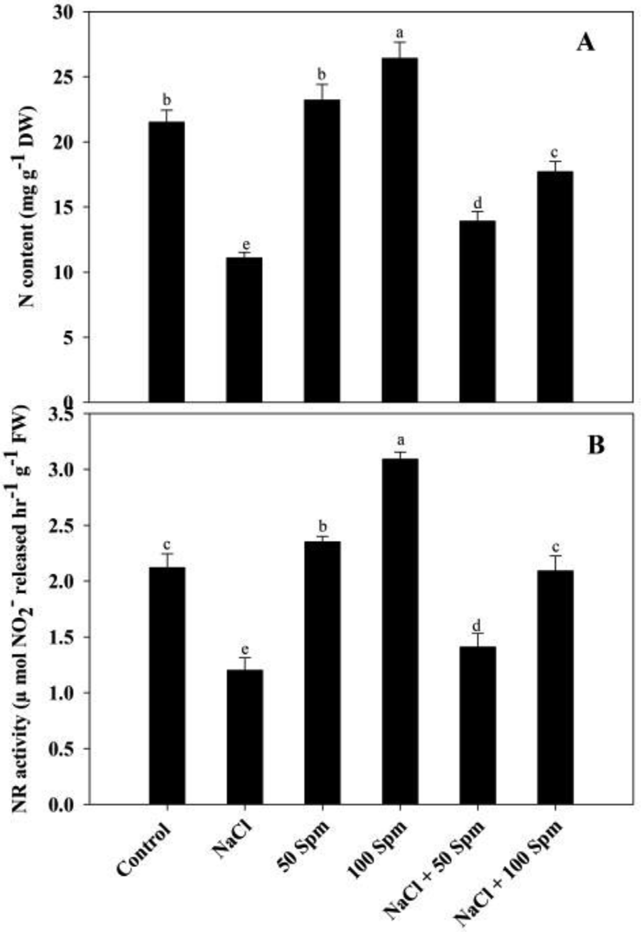

Fig. 9. Effect of Spm (50 and 100 µM) application on the activity of (A) nitrate reductase and (B) nitrogen content in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

assisted the tomato plants to (a) neutralize excess H_2O_2, (b) maintain the ratio of NADP/NADPH for optimal functioning of photosynthetic electron transport subsequently preventing the generation of superoxide and (c) maintaining the redox homeostasis by generating GSH and AsA content. Recently, in *Brassica juncea* Hussain et al. (2019) have also demonstrated significant up-regulation of the activities of SOD, CAT and peroxidase due to foliar application of spermidine resulting in reduced oxidative damage to membranes by reducing the accumulation of H_2O_2 and O_2^{-}. Plants over-expressing antioxidant genes have been reported to exhibit increased tolerance to variety of stresses (Kornyeyev et al., 2003; Al-Taweel et al., 2007; Prashanth et al., 2008; Kim et al., 2008). Besides this the redox homeostasis and the antioxidant system was strengthened by Spm application by improving the tocopherol content in them. Tocopherols are key liposoluble redox buffers and majorily scavenge singlet oxygen in addition of other ROS (Foyer and Noctor, 2005; Kapoor et al., 2015) thereby protecting the major cellular processes in plants. Redox buffers like AsA, tocopherol and GSH act as enzyme cofactors and regulate defense, cell proliferation and death (Tokunaga et al., 2005; Kapoor et al., 2015). Increased accumulation of AsA and GSH prevents salinity induced oxidative effects in *Triticum aestivum* significantly (Ahanger and Agarwal, 2017a).

Exogenous application of Spm resulted in significant enhancement in the accumulation of secondary metabolites including phenols and flavonoids. Secondary metabolites form important component of antioxidant system in plants and their accumulation has been earlier reported by several workers (Agati et al., 2011; Ahanger and Agarwal, 2017a, b; Astaneh et al., 2018), however reports on PAs (Spm) mediated modulations in their accumulation are not available. Toxic free radicals that are diffused from chloroplast and transported into vacuole are believed to get neutralised by flavonoids (Fini et al., 2011) and also lessen their generation by acting at their production sites (Agati et al., 2012, 2013). The plant secondary metabolites have been reported to come into function when enzymatic ROS scavenging system weakens (Ahanger and Ahmad, 2019) and Spm induced enhancement in phenol and flavonoids may have influenced ROS accumulation significantly thereby preventing damage to major cellular structures and their functioning. Flavonoids with antioxidant activity existing in chloroplast scavenge singlet oxygen thereby preventing the photoinhibition and

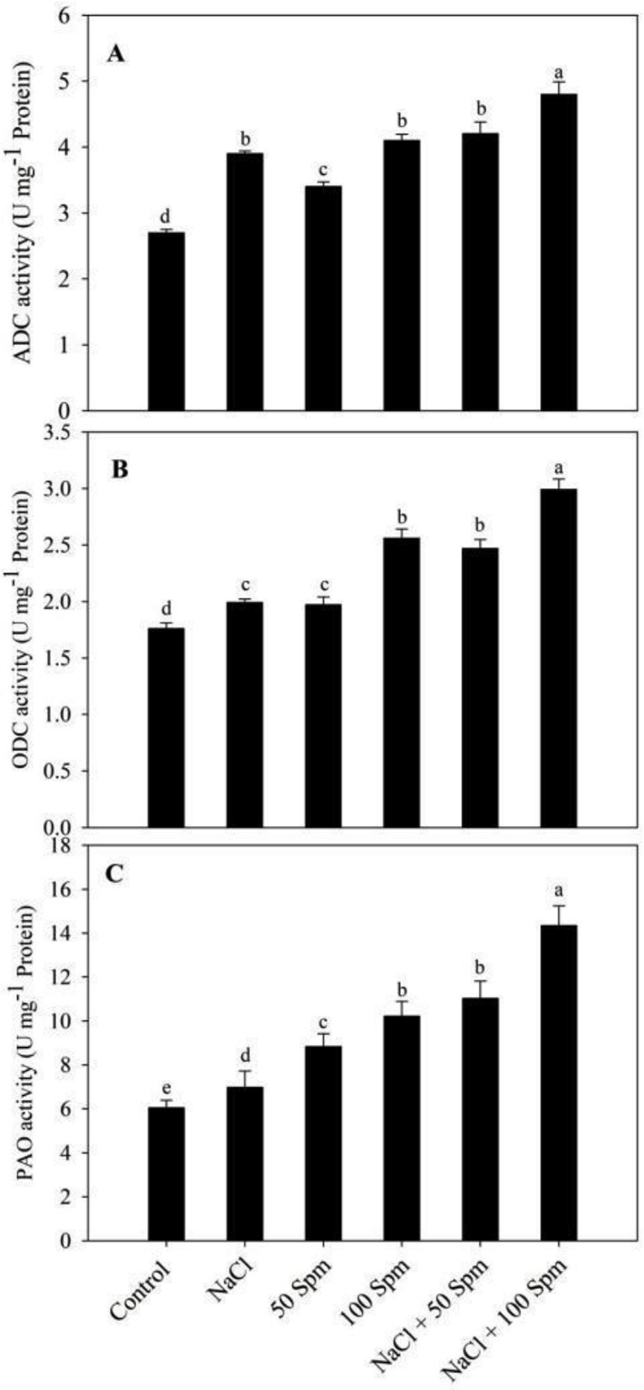

Fig. 10. Effect of Spm (50 and 100 μM) application on the activity of (A) arginine decarboxylase, (B) ornithine decarboxylase and (C) polyamine oxidase in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at P < 0.05.

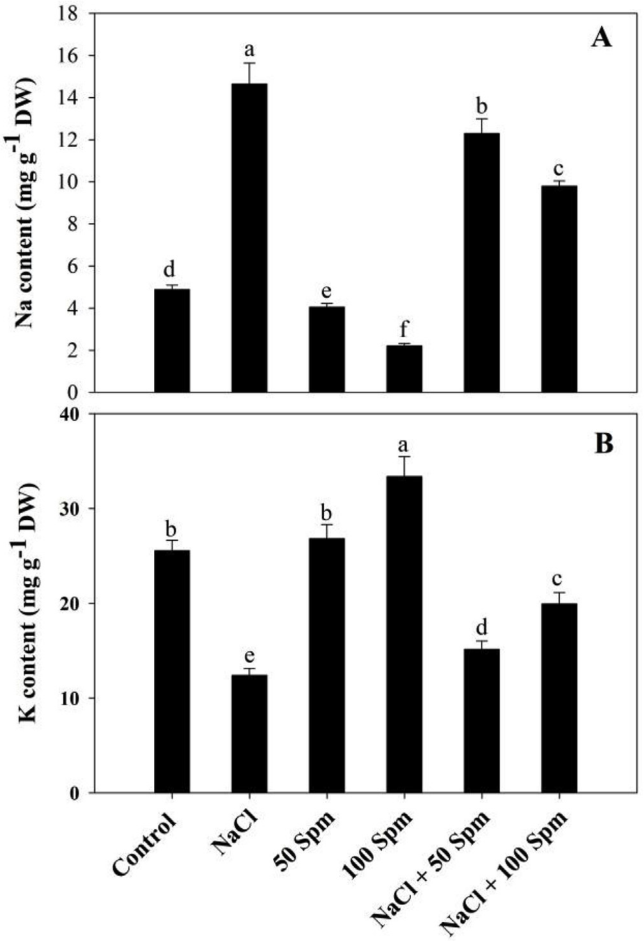

Fig. 11. Effect of Spm (50 and 100 μM) application on the content of (A) sodium and (B) potassium in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at P < 0.05.

also stabilizing the chloroplast membranes, and also prevent ROS generation by making complexes with Fe and Cu (Agati et al., 2012). It has been reported that accumulation of secondary metabolites co-relates with DPPH radical scavenging activity (Ahanger and Agarwal, 2017a, b). Up-regulation of the PAL activity due to salinity stress has been demonstrated earlier by Astaneh et al. (2018). In present study further increment in salinity induced accumulation of metabolites due to Spm treatment depicts the further improvement in the stress withstanding potential. Increase in phenols due to putrescine treatment has been reported in okra (Phornvillay et al., 2019).

Salinity stress up-regulated the activities of PA metabolizing enzymes like ADC, ODC and PAO. Exogenous application imparted significant effect on their activities which may have contributed to further enhancement in PA concentrations. Increased synthesis of PAs will increase their availability for conjugation with phenols and other key macromolecules like proteins and nucleic acids (Kaur-Sawhney et al., 2003). Exogenous application of PAs have been reported to improve the endogenous PA concentration by up-regulating the activities of enzymes involved (Hu et al., 2012; Puyang et al., 2016).

5. Conclusion

Salinity stress affected growth considerably by altering the physiological and biochemical parameters. Application of Spm prevented the salinity triggered oxidative damage by up-regulating the ROS scavenging mechanisms including antioxidant system and secondary metabolite accumulation. At 100 μM concentrations, Spm affectively improved the tissue water content, photosynthetic pigment synthesis and photosynthesis. Increased osmolyte accumulation due to Spm may have strengthened the ROS scavenging system, protected enzyme activity and maintained cellular redox state besides their beneficial role in preventing salinity induced osmotic and ionic stress. Maintenance of nitric oxide concentrations due to Spm may have helped in integrating the signalling events for efficient salinity tolerance, however further studies are required in this direction.

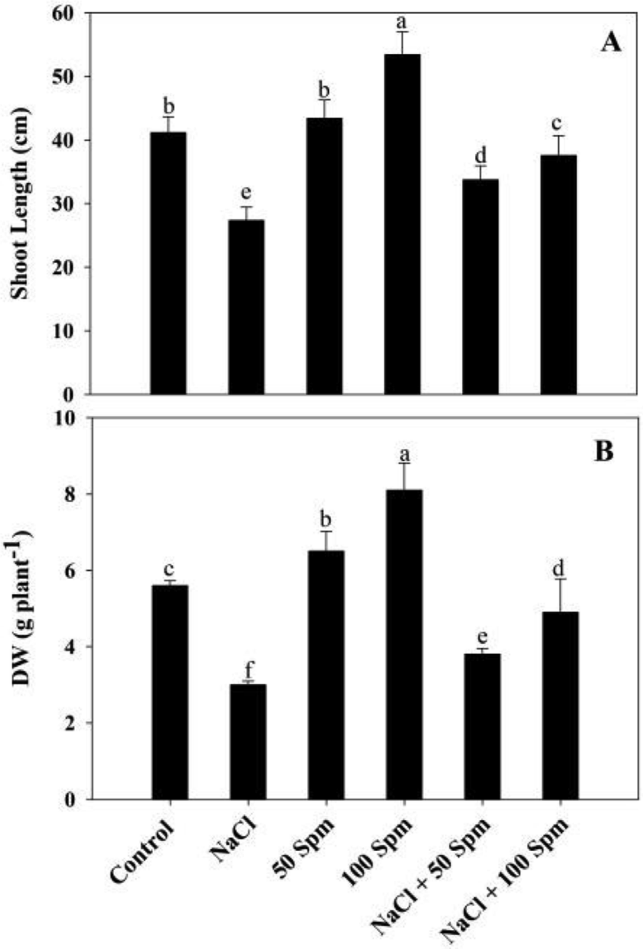

Fig. 12. Effect of Spm (50 and 100 μM) application on (A) shoot length and (B) plant dry weight in *Solanum lycopersicum* L. cultivar Huange 108 subjected to salinity stress. Data is mean (± SE) of three replicates and different letters on bars denote significant difference at $P < 0.05$.

Author contribution

Conceived and designed by MAA. Experimentation was carried by MAA, CQ, QM and XXD. MAA and XXD wrote the manuscript. PA did statistical analysis and LZ supervised the work. EFAA helped in revision.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Agati, G., Azzarello, E., Pollastri, S., Tattini, M., 2012. Flavonoids as antioxidants in plants: location and functional significance. Plant Sci. 196, 67–76.
Agati, G., Biricolti, S., Guidi, L., Ferrini, F., Fini, A., Tattini, M., 2011. The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in *L. vulgare* leaves. J. Plant Physiol. 168 (3), 204–212.
Agati, G., Brunetti, C., Di Ferdinando, M., Ferrini, F., Pollastri, S., Tattini, M., 2013. Functional roles of flavonoids in photoprotection: new evidence, lessons from the past. Plant Physiol. Biochem. 72, 35–45.
Agurla, S., Gayatri, G., Raghavendra, A.S., 2014. Nitric oxide as a secondary messenger during stomatal closure as a part of plant immunity response against pathogens. Nitric Oxide 43, 89–96.
Agurla, S., Gayatri, G., Raghavendra, A.S., 2018. Polyamines increase nitric oxide and reactive oxygen species in guard cells of *Arabidopsis thaliana* during stomatal closure. Protoplasma 255 (1), 153–162.
Ahanger, M.A., Agarwal, R.M., 2017a. Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L) as influenced by potassium supplementation. Plant Physiol. Biochem. 115, 449–460.
Ahanger, M.A., Agarwal, R.M., 2017b. Potassium up-regulates antioxidant metabolism and alleviates growth inhibition under water and osmotic stress in wheat (*Triticum aestivum* L). Protoplasma 254 (4), 1471–1486.
Ahanger, M.A., Agarwal, R.M., Tomar, N.S., Shrivastava, M., 2015. Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (*Avena sativa* L cultivar Kent). J. Plant Interact. 10 (1), 211–223.
Ahanger, M.A., Ahmad, P., 2019. Role of mineral nutrients in abiotic stress tolerance – revisiting the associated signalling mechanisms. In: Khan, M.I.R., Reddy, P.S., Ferrante, A., Khan, N.A. (Eds.), Plant Signalling Molecules. Elsevier Academic Press, USA, pp. 269–285.
Ahanger, M.A., Alyemeni, M.N., Wijaya, L., Alamri, S.A., Alam, P., Ashraf, M., Ahmad, P., 2018b. Potential of exogenously sourced kinetin in protecting *Solanum lycopersicum* from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate-glutathione cycle and glyoxalase system. PLoS One 13 (9), e0202175. https://doi.org/10.1371/journal.pone.0202175.
Ahanger, M.A., Gul, F., Ahmad, P., Akram, N.A., 2018a. Environmental stresses and metabolomics - deciphering the role of stress responsive metabolites. In: Plant Metabolites and Regulation under Environmental Stress, pp. 53–62.
Ahanger, M.A., Tittal, M., Mir, R.A., Agarwal, R.M., 2017b. Alleviation of water and osmotic stress-induced changes in nitrogen metabolizing enzymes in *Triticum aestivum* L. cultivars by potassium. Protoplasma 254 (5), 1953–1963.
Ahanger, M.A., Tomar, N.S., Tittal, M., Argal, S., Agarwal, R.M., 2017a. Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. Physiol. Mol. Biol. Plants 23, 731–744. 4. https://link.springer.com/journal/12298/23/4/page/1.
Ahanger, M.A., Tyagi, S.R., Wani, M.R., Ahmad, P., 2014. Drought tolerance: roles of organic osmolytes, growth regulators and mineral nutrients. In: Ahmad, P., Wani, M.R. (Eds.), "physiological Mechanisms and Adaptation Strategies in Plants under Changing Environment" Volume Ist. Springer Science + Business media, inc, pp. 25–56.
Ahmad, P., Ahanger, M.A., Alyemeni, M.N., Wijaya, L., Alam, P., Ashraf, M., 2018. Mitigation of sodium chloride toxicity in *Solanum lycopersicum* L. by supplementation of jasmonic acid and nitric oxide. J. Plant Interact. 13 (1), 64–72.
Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G., Sharma, S., 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Crit. Rev. Biotechnol. 30, 161–175.
Ahmad, P., Kumar, A., Gupta, A., Hu, X., Hakeem, K.R., Azooz, M.M., Sharma, S., 2012. Polyamines: role in plants under abiotic stress. In: Ashraf, M. (Ed.), Crop Production for Agricultural Improvement. Springer Science + Business Media. https://doi.org/10.1007/978-94-007-4116-4_19.
Alcazar, R., Altabella, T., Marco, F., Bortolotti, C., Reymond, M., Koncz, C., Carrasco, P., Tiburcio, A.F., 2010. Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Planta 231, 1237–1249.
Alcazar, R., Cuevas, J.C., Planas, J., Zarza, X., Bortolotti, C., Carrasco, P., Salinas, J., Tiburcio, A.F., Altabella, T., 2011. Integration of polyamines in the cold acclimation response. Plant Sci. 180, 31–38.
Al-Taweel, K., Iwaki, T., Yabuta, Y., Shigeoka, S., Murata, N., Wadano, A., 2007. A bacterial transgene for catalase protects translation of d1 protein during exposure of salt-stressed tobacco leaves to strong light. Plant Physiol. 145, 258–265.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplast polyphenol oxidase in Beta vulgaris. Plant Physiol. 24, 1–15.
Astaneh, R.K., Bolandnazar, S., Nahandi, F.Z., Oustan, S., 2018. Effect of selenium application on phenylalanine ammonia-lyase (PAL) activity, phenol leakage and total phenolic content in garlic (*Allium sativum* L.) under NaCl stress. Inf. Process. Agric. 5 (3), 339–344.
Backer, H., Frank, O., de Angells, B., Feingold, S., 1980. Plasma tocopherol in man at various times after ingesting free or ocetylaned tocopherol. Nutr. Rep. Int. 21, 531–536.
Bagni, N., Tassoni, A., 2001. Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants. Amino Acids 20, 301–317.
Bates, L.S., Waldre, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. Plant Sci. 39, 205–207.
Bayer, W.F., Fridovich, J.L., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161, 559–566.
Bose, J., Rodrigo-Moreno, A., Shabala, S., 2014. ROS homeostasis in halophytes in the context of salinity stress tolerance. J. Exp. Bot. 65 (5), 1241–1257.
Dalal, V.K., Tripathy, B.C., 2012. Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis. Plant Cell Environ. 35, 1685–1703.
Demidchik, V., Straltsova, V., Medvedev, S.S., Pozhvanov, G.A., Sokolik, A., Yurin, V., 2014. Stress-induced electrolyte leakage: the role of K^+ permeable channels and involvement in programmed cell death and metabolic adjustment. J. Exp. Bot. 65, 1259–1270.
Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9.
Doderer, A., Kokkelink, I., van der Veen, S., Valk, B., Schram, A., Douma, A., 1992. Purification and characterization of two lipoxygenase isoenzymes from germinating barley. Biochim. Biophys. Acta 112, 97–104.
Ellman, G.L., 1959. Tissue sulphydryl groups. Arch. Biochem. Biophys. 82, 70–77.
Farooq, M., Wahid, A., Lee, D.J., 2009. Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiol. Plant. 31, 937–945.
Fatma, M., Masood, A., Per, T.S., Khan, N.A., 2016. Nitric oxide alleviates salt stress inhibited photosynthetic performance by interacting with sulfur assimilation in

mustard. Front. Plant Sci. 7, 521. https://doi.org/10.3389/fpls.2016.00521.

Fini, A., Brunetti, C., Di ferdinando, M., Ferrini, F., Tattini, M., 2011. Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. Plant Signal. Behav. 6 (5), 709–711.

Fong, J., Schaffer, F.L., Kirk, P.L., 1953. The ultramicrodetermination of glycogen in liver. A comparison of the anthrone and reducing-sugar methods. Arch. Biochem. Biophys. 45 (2), 319–326.

Foyer, C.H., Halliwell, B., 1976. The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. Planta 133, 21–25.

Foyer, C.H., Noctor, G., 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17, 1866–1875.

Green, N.M., Neurath, H., 1954. Proteolytic enzymes. In: In: Neurath, H., Vailey, K. (Eds.), The Proteins, vol. II. Part B, Academic Press, New York, pp. 1057–1198.

Grieve, C.M., Grattan, S.R., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70, 303–307.

Harel, E., Klein, S., 1972. Light dependent formation of 5-aminolevulinic acid in etiolated leaves of higher plants. Biochem. Biophys. Res. Commun. 49, 364–370.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A., 2012. Role of proline under changing environments: a review. Plant Signal. Behav. 7, 1456–1466.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.

Hernandez, I., Alegre, L., Van Breusegem, F., Munne-Bosch, S., 2009. How relevant are flavonoids as antioxidants in plants? Trends Plant Sci. 14 (3), 125–132.

Hu, L., Xiang, L., Li, S., Zou, Z., Hu, X.H., 2016. Beneficial role of spermidine in chlorophyll metabolism and D1 protein content in tomato seedlings under salinity–alkalinity stress. Physiol. Plant. 156, 468–477.

Hu, X.H., Zhang, Y., Shi, Y., Zhang, Z., Zou, Z.R., Zhang, H., Zhao, J.Z., 2012. Effect of exogenous spermidine on polyamine content and metabolism in tomato exposed to salinity–alkalinity mixed stress. Plant Physiol. Biochem. 57, 200–209.

Hussain, A., Nazir, F., Fariduddin, Q., 2019. 24-epibrassinolide and spermidine alleviate Mn stress via the modulation of root morphology, stomatal behavior,photosynthetic attributes and antioxidant defense in *Brassica juncea*. Physiol. Mol. Biol. Plants 25 (4), 905–919.

Incharoensakdi, A., Takabe, T., Akazawa, T., 1986. Effect of betaine on enzyme activity and subunit interaction of ribulose-1,5-bisphosphate carboxylase/oxygenase from *Aphanothece halophytical*. Plant Physiol. 81, 1044–1049.

Iqbal, N., Umar, S., Khan, N.A., 2015. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). J. Plant Physiol. 178, 84–91.

Iswaran, V., Marwaha, T.S., 1980. A modified rapid kjeldahl method for determination of total nitrogen in agricultural and biological materials. Geobios 7, 281e282.

Jackson, M.L., 1973. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd, New Delhi.

Jain, V.K., Guruprasad, K.N., 1989. Effect of chlorocholin chloride and gibberellic acid on the anthocyanin synthesis in radish seedlings. Plant Physiol. 75, 233–236.

Kapoor, D., Sharma, R., Handa, N., Kaur, H., Rattan, A., Yadav, P., Gautam, V., Kaur, R., Bhardwaj, R., 2015. Redox homeostasis in plants under abiotic stress: role of electron carriers, energy metabolism mediators and proteinaceous thiols. Front. Environ. Sci. 04. https://doi.org/10.3389/fenvs.2015.00013.

Kaur-Sawhney, R., Tiburcio, A.F., Altabella, T., Galston, A.W., 2003. Polyamines in plants: an overview. J. Cell Mol. Biol. 2, 1–12.

Khan, M.I.R., Asgher, M., Khan, N.A., 2014. Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycine betaine and ethylene in mungbean (*Vigna radiata* L.). Plant Physiol. Biochem. 80, 67–74.

Kim, Y.H., Kim, C.Y., Song, W.K., Park, D.S., Kwon, S.Y., Lee, H.S., Bang, J.W., Kwak, S.S., 2008. Over-expression of sweet potato swpa4 peroxidase results in increased hydrogen peroxide production and enhances stress tolerance in tobacco. Planta 227, 867–881.

Kornyeyev, D., Logan, B.A., Payton, P., Allen, R.D., Holaday, A.S., 2003. Elevated chloroplastic glutathione reductase activities decrease chilling-induced photoinhibition by increasing rates of photochemistry, but not thermal energy dissipation, in transgenic cotton. Funct. Plant Biol. 30, 101–110.

Li, J., Hu, L., Zhang, L., Pan, X., Hu, X., 2015. Exogenous spermidine is enhancing tomato tolerance to salinity–alkalinity stress by regulating chloroplast antioxidant system and chlorophyll metabolism. BMC Plant Biol. 15, 303. https://doi.org/10.1186/s12870-015-0699-7.

Malick, C.P., Singh, M.B., 1980. Plant Enzymology and Histo-Enzymology. Kalyani Publishers, New Delhi, pp. 286 1980.

Mukherjee, S.P., Choudhuri, M.A., 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plant. 58, 166–170.

Nahar, K., Hasanuzzaman, M., Alam, M.M., Rahman, A., Suzuki, T., Fujita, M., 2016. Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in mung bean plants through up-regulating the metal detoxification, antioxidant defense andmethylglyoxal detoxification systems. Ecotoxicol. Environ. Saf. 126, 245–255.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.

Nazar, R., Iqbal, n., Syeed, S., Khan, N.A., 2011. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. J. Plant Physiol. 168, 807–815.

Neill, S., Barros, R., Bright, J., Desikan, R., Hancock, J., Harrison, J., Morris, P., Ribeiro, D., Wilson, I., 2008. Nitric oxide, stomatal closure, and abiotic stress. J. Exp. Bot. 59, 165–176.

Parihar, P., Singh, S., Singh, R., Singh, V.P., Prasad, S.M., 2015. Effect of salinity stress on plants and its tolerance strategies: a review. Environ. Sci. Pollut. Res. 22 (6), 4056–4075.

Phornvillay, S., Pongprasert, N., Wongs-Aree, C., Uthairatanakij, A., Srilaong, V., 2019. Exogenous putrescine treatment delays chilling injury in okra pod (*Abelmoschus esculentus*) stored at low storage temperature. Sci. Hortic. (Amst.) 256, 108–550.

Pottosin, I., Shabala, S., 2014. Polyamines control of cation transport across plant membranes: implications for ion homeostasis and abiotic stress signaling. Front. Plant Sci. 5, 154. https://doi.org/10.3389/fpls.2014.00154.

Pottosin, I., Velarde-Buendía, A.M., Bose, J., Zepeda-Jazo, I., Shabala, S., Dobrovinskay, O., 2014. Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: implications for plant adaptive responses. J. Exp. Bot. 65 (5), 1271–1283.

Prashanth, S.R., Sadhasivam, V., Parida, A., 2008. Over expression of cytosolic copper/zinc superoxide dismutase from a mangrove plant *Avicennia marina* in indica rice var Pusa Basmati-1 confers abiotic stress tolerance. Transgenic Res. 17, 281–291.

Puyang, X., An, M., Xu, L., Han, L., Zhang, X., 2016. Protective effect of exogenous spermidine on ion and polyamine metabolism in Kentucky bluegrass under salinity stress. Hortic. Environ. Biotechnol. 57 (1), 11–19.

Rolland, F., Moore, B., Shee, J., 2002. Sugar sensing and signaling in plants. Plant Cell S185–S205.

Roy, S., Chakraborty, U., 2018. Role of sodium ion transporters and osmotic adjustments in stress alleviation of *Cynodon dactylon* under NaCl treatment: a parallel investigation with rice. Protoplasma 25 (1), 175–191.

Roychoudhury, A., Basu, S., Sengupta, D.N., 2011. Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of indica rice differing in their level of salt tolerance. J. Plant Physiol. 168, 317–328.

Shabala, S., Cuin, T.A., Pottosin, I.I., 2007. Polyamines prevent NaCl induced K^+ efflux from pea mesophyll by blocking non-selective cation channels. FEBS Lett. 581, 1993–1999.

Sheen, J., Zhou, L., Jang, J.C., 1999. Sugars as signaling molecules. Curr. Opin. Plant Biol. 2, 410–418.

Sivakumar, P., Sharmila, P., Jain, V., Saradhi, P.P., 2002. Sugars have potential to curtail oxygenase activity of Rubisco. Biochem. Biophys. Res. Commun. 298 (2), 247–250.

Sivakumar, P., Sharmila, P., Saradhi, P.P., 2000. Proline alleviates salt stress-induced enhancement in ribulose-1, 5-bisphosphate oxygenase activity. Biochem. Biophys. Res. Commun. 279, 512–515.

Smart, R.E., Bihgham, G.E., 1974. Rapid estimates of relative water content. Plant Physiol. 53, 258–260.

Smeekens, S., Hellmann, H.A., 2014. Sugar sensing and signaling in plants. Front. Plant Sci. 5, 113. https://doi.org/10.3389/fpls.2014.00113.

Smeekens, S., 2000. Sugar-induced signal transduction in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51, 49–81.

Srivastava, H.S., 1974. In vivo activity of nitrate reductase in maize seedlings. Indian J. Biochem. Biophys. 11, 230–232.

Stael, S., Van Breusegem, F., Gevaert, K., Nowack, M.K., 2019. Plant proteases and programmed cell death. J. Exp. Bot. 70 (7), 1991–1995.

Su, G.X., An, Z.F., Zhang, W.H., Liu, Y.L., 2005. Light promotes the synthesis of lignin through the production of H_2O_2 mediated by diamine oxidases in soybean hypocotyls. J. Plant Physiol. 162, 1297–1303.

Szafranska, K., Reiter, R.J., Posmyk, M.M., 2017. Melatonin improves the photosynthetic apparatus in pea leaves stressed by paraquat via chlorophyll breakdown regulation and its accelerated *de novo* synthesis. Front. Plant Sci. 8, 878. https://doi.org/10.3389/fpls.2017.00878.

Tanou, G., Ziogas, V., Belghazi, M., Christou, A., Filippou, P., Job, D., Fotopoulos, V., Molassiotis, A., 2014. Polyamines reprogram oxidative and nitrosative status and the proteome of citrus plants exposed to salinity stress. Plant Cell Environ. 37, 864–885.

Tokunaga, T., Miyahara, K., Tabata, K., Esaka, M., 2005. Generation and properties of ascorbic acid-overproducing transgenic tobacco cells expressing sense RNA for L-galactono-1, 4-lactone dehydrogenase. Planta 220, 854–863.

Turan, S., Tripathy, B.C., 2015. Salt-stress induced modulation of chlorophyll biosynthesis during de-etiolation of rice seedlings. Physiol. Plant. 153, 477–491.

Turkan, I., Demiral, T., 2009. Recent developments in understanding salinity tolerance. Environ. Exp. Bot. 67, 2–9.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain treated bean plants, protective role of exogenous polyamines. Plant Sci. 151, 59–66.

Wang, F., Liu, J., Chen, M., Zhou, L., Li, Z., Zhao, Q., Pan, G., Zaidi, S.H.R., Cheng, F., 2016. Involvement of abscisic acid in PSII photodamage and d1 protein turnover for light-induced premature senescence of rice flag leaves. PLoS One 11 (8), e0161203.

Yang, H., Wu, F., Cheng, J., 2011. Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. Food Chem. 127, 1237–1242.

Yao, J., Shi, W.M., Xu, W.F., 2008. Effects of salt stress on expression of nitrate transporter and assimilation-related genes in tomato roots. Russ. J. Plant Physiol. 55 (2), 232–240.

Zhang, L., Zhang, G., Wang, Y., Zhou, Z., Meng, Y., Chen, B., 2013. Effect of soil salinity on physiological characteristics of functional leaves of cotton plants. J. Plant Res. 126 (2), 293–304.

Zhang, R.H., Li, J., Guo, S.R., Tezuka, T., 2009. Effects of exogenous putrescine on gas-exchange characteristics and chlorophyll fluorescence of NaCl-stressed cucumber seedlings. Photosynth. Res. 100, 155–162.

Zhao, F.G., Sun, C., Liu, Y.L., Zhang, W.H., 2003. Relationship between polyamine metabolism in roots and salt tolerance of barley seedlings. Acta Bot. Sin. 45, 295–300.

Zhishen, J., Mengcheng, T., Jianming, W., 1999a. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555–559.

Zhou, B., Guo, Z., Xing, J., Huang, B., 2005. Nitric oxide is involved in abscisic acid-induced antioxidant activities in *Stylosanthes guianensis*. J. Exp. Bot. 56, 3223–3228.

Zucker, M., 1965. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiol. 40, 779–784.