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RESEARCH ARTICLE

ALTERATIONS IN ONION (*ALLIUM CEPA* L.) PROVOKED BY SALINITY AND ITS ALLEVIATION USING PHOSPHORUS

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ABSTRACT

A soil pot culture experiment was carried out in wire house condition to investigate the response of Onion (*Allium cepa* cv. N-53) grown in saline water and amended with supplementary Phosphorus (P). Plants were subjected to different electrical conductivity (EC) solution viz. 0, 2, 4, 6 and 8 dS/m prepared by mixing NaCl, Na_2SO_4, $CaCl_2$ and $MgCl_2$. For remediation approach two salinity levels (EC 6 and 8 dS/m) were subjected with combination of 40ppm Phosphorus. The results demonstrated that salt stress hampered the plant growth such as fresh and dry weight of plant. Increased accumulation of malondialdehyde content in the leaf of plant exposed to salty solution suggested that salinity promoted the oxidative stress. The other stress indicator parameters such as enzymatic and non enzymatic antioxidant were also analyzed. The non-protien thiol group was found to gradually enhance at lower doses and decreased at higher salinity levels (8EC) and cysteine increased at all salinity levels. Phosphorus application comparatively (salt treated plants) decreased the content of cysteine and NP-SH in the leaf of plant treated with salt +P as salinity level rose. Salt stress were also declined pigment contents like Chl T, Chl a, Chl b and Carotenoids and the activity of catalase (CAT) and peroxidase (POX) but P amendment increased their activity. Activity of ascorbate peroxidase (APX) and glutathione reductase (GR) were found to increase at lower levels of salinity while decreased at higher levels. In this study activity of superoxide dismutase (SOD) was increased at all tested salinity levels while P supply decreased activity. The results indicated that supplementary P can mitigate the negative influence of water salinity on plant growth and physiological development.

INTRODUCTION

Plants suffer from different environmental stresses like high temperature, cold, drought, salinity, UV and other biotic stresses. Amongst these stresses, salinity is considered the most limiting factor for productivity of agricultural crops. Salinity is one of the most prominent abiotic factors affecting crop yield in arid and semiarid regions (Subramanyam *et al*., 2010). According to the FAO Land and Nutrition Management Service (2008), over 6% of the world's land is affected by either salinity or sodicity which accounts for more than 800 million ha of land. A research by Central Soil Salinity Research Institute, India (CSSRI, 2010) estimate about 6.73 million hectares of agricultural land as salt affected, suffers huge economic losses. It has been estimated that with the expected increase in the population, an additional 40 million ha for fuel and 10 million ha for fodder and net sown area of 10 million ha for food are needed by the end of this century (Bhadauria *et al*., 2010). According to a standard definition, a soil is saline if the EC of the soil solution is greater than 4 dSm^{-1} (SSSA, 1997). Most of crop species i.e. beans, eggplant, onion, pepper, corn, sugarcane, potato and cabbage are sensitive to salinity (ECe 1.0-1.8 dSm^{-1}), which reduce crop productivity about 6-19 % (Chaum and Kirdmanee, 2009). Salinity stress caused generation of excessive reactive oxygen species (ROS), which leads to cell toxicity, membrane damage and cell death (Chookhampaeng, 2011).

To control the level of ROS and to protect the cells, plants possess low molecular weight antioxidants compounds and antioxidant enzymes such as CAT, POX and SOD which scavenge the ROS (Mishra *et al*., 2009). A high NaCl concentration causes reduction in growth parameters (Pessarakli and Touchane, 2006; Turhan *et al*., 2008) such as fresh and dry weight of leaves, shoots and roots along with a decrease in moisture content (Parvaiz and Riffat, 2005). The effectiveness of oxidative defense system in plants can be measured by the activities of antioxidant enzymes and levels of non-enzymatic antioxidants (Geebelen *et al*., 2002). Changes in activities of various antioxidant enzymes under salinity stress have been reported earlier (Dolatabadian & Saleh 2009). The negative effect of salinity is nutrient deficiency. As a result of decreasing water uptake from the soil, the entry of essential minerals such as phosphorus,

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potassium, nitrate, and calcium, for plant growth is lowered (Xiong and Zhu, 2002). Phosphorus (P) is an important element of key molecules such as nucleic acids, phospholipids, and ATP. Increases in NaCl dosage led to a decrease in P concentration in plant tissues (Sharpley *et al*., 1992), possibly due to a fall in the P availability in the soil (Awad *et al*., 1990; Grattan and Grieve, 1994). Consequently, plants cannot grow without a reliable supply of this nutrient (Schachtman *et al*., 1998). Supplementary P has a role in alleviation of the adverse effects of salinity on plant biomass for a variety of crop plants (Kaya *et al*. 2003). A positive effect of P under saline conditions also has been reported in wheat (Abrol, 1968) and sorghum (Indulkar & More, 1985). In previous work on salinity stress done by other researchers has been focused on salinity impact on plant growth and yield, and management practices for better performance. But in this work the macronutrient phosphorus has been used to minimize the impact of salinity on growth, yield and activity of enzymatic and non-enzymatic antioxidants in onion plants.

MATERIALS AND METHODS

A soil culture experiment was carried out in wire house condition at the Department of Botany, University of Lucknow, Lucknow. Surface-sterilized (with 0.1% $HgCl_2$ solution for 5 min and washed thoroughly with distilled water) seeds were sown in earthen pots (320 cm^2) lined with polyethene bags and filled with a mixture of garden soil and farmyard manure (3:1). Sixty day's old plants were supplied with various salinity levels (0, 2, 4, 6 and 8 dSm^{-1}). The two higher salinity levels i.e. 6 and 8 dSm^{-1} was subjected to combination of 40 ppm of Phosphorus. Treatment was given weakly for two months. The saline solution was made using a mixture of NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$ in the eqimolar basis of various electrical conductivity levels. After two months of treatment, plants were harvested and used for analyzing various morphological, physiological and biochemical parameters.

Fresh and dry biomass

For determining fresh matter yield, the fresh plant samples were collected, thoroughly washed with running tap water and rinsed 2 to 3 times with distilled water. After rinsing the plant samples were blotted gently to wipe off the absorbed water and weighed for fresh matter yield. Now these samples were kept in an oven at 65±5ºC for 48 hours and weighted on an electronic balance to determine the effect on dry biomass production.

Photosynthetic pigments

Chlorophyll was estimated by the method of Arnon (1949). Leaves were plucked and washed with distilled water and blotted. 100 mg leaves were taken and ground in 10 ml chilled acetone (85% v/v). Extract was centrifuged at 2000 rpm for 10 minutes. The absorbance of supernatant was read at 663, 645, 510 and 480 nm using the double beam UV-VIS spectrophotometer UV5704SS. For calculation the following formulas were used and the content was expressed in mg g^{-1} fresh weight tissue.

Chl-a = [12.7 (A663) -2.69 (A645)] X v/1000Xw
Chl-b = [22.9 (A663) -4.78 (A645)] X v/1000Xw
Chl-T = [20.2 (A645) -8.02 (A663)] X v//1000Xw
Carotenoids = [7.60 (A663) -1.49 (A510)] X v/1000Xw

V= volume of initial samples in ml
W= weight of sample in mg

Lipid peroxidation

The level of lipid peroxidation in plant tissue was measured by the method of Heath and Packer (1971) in terms of malondialdehyde content, a product of lipid peroxidation determined by the thiobarbituric acid reaction. Fresh control and treated leaf tissue (0.3g) were homogenized in 3ml of 20% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3,500 rpm for 20 min. To 1ml of aliquot of the supernatant, 1ml of 20% TCA containing 0.5 %(w/v) TBA was added. The mixture was heated at 95^0C for 30 min and then quickly cooled in ice bath. The contents were centrifuged at 10,000 X g for 15 min and the absorbance was measured at 532 nm using double beam UV-VIS spectrophotometer UV5704SS. Value for non – specific absorbance at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient of 155Mm^{-1} cm^{-1}.

Catalase

Catalase activity was assayed by the method of Bisht (1972), a modified method of Euler and Josephson 1927. The reaction mixture for catalase containing 0.01 mM phosphate buffer (pH 7.0) and 0.5mM H_2O_2 in 10 ml was incubated with suitable aliquot from the extract. The reaction was run for 5 minutes at room temperature (25^oC) and was stopped by the addition of 5ml 2N H_2SO_4, Corresponding zero hour blanks with added H_2SO_4 was also run. The mixture was titrated against 0.1 N $KMnO_4$ and the activity of catalase were expressed as µmol H_2O_2 decomposed / 100 mg fresh weight tissue.

Peroxidase

The peroxidase activity was determined as per the method of Luck (1963). The assay system for peroxidase contained 0.5 mM phosphate buffer (pH 6.0), 0.01% (v/v) H_2O_2, 5 mg p-phenylenediamine and extract in 8 ml. The reaction was run at 25^0C for 5 minute and stopped with 2ml 5N H_2SO_4. Blanks with added H_2SO_4 were also taken. After centrifugation, OD was measured at 485 nm on double beam UV-VIS spectrophotometer UV5704SS.

Superoxide dismutase

The activity of SOD was assayed by the method of Beauchamp and Fridovich (1971) by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazoliun (NBT). The reaction mixture (3ml) contained 40mM phosphate buffer (PH 7.8), 13mM methionine, 75µM NBT, 2µM riboflavin, 0.1mM EDTA and a suitable aliquot of enzyme extract. Riboflavin was added in the end. The test tubes were shaken and reaction

The reduction in NBT was followed by reading the absorbance min^{-1} g^{-1} fresh weight.

Table 1. Effects of different salinity levels and their interaction with Phosphorus on shoot length, fresh and dry weight of shoot and bulb of onion plants observed at 120 days

Parameters	Treatment							LSD
	Control	2EC	4EC	6EC	8EC	6EC+P	8EC+P	***
Shoot length (cm Plant^{-1})	58.0±1.25	49.0±1.42*	44.3±2.23**	37.3±1.44**	35.0±3.30**	44.7±0.86**	42.1±0.95**	7.66 11.6
Shoot FW (g Plant^{-1})	16.2±1.58	14.7±1.11	9.13±062**	8.62±0.98**	5.21±0.21**	10.78±0.51*	9.69±0.36**	3.75 5.69
Bulb FW (g Plant^{-1})	18.5±0.69	13.7±0.68*	10.0±1.43**	7.6±0.36**	6.6±0.16**	11.3±0.48**	9.8±0.49**	4.03 6.10
Shoot DW (g Plant^{-1})	1.44±0.08	0.88±0.007*	0.72±.009**	0.45±0.02**	0.25±0.02**	0.74±0.03**	0.61±0.009**	0.37 0.57
Bulb DW (g Plant^{-1})	13.1±0.13	9.8±0.32	6.9±01.4**	4.0±0.75**	2.46±0.75**	7.2±0.50**	6.1±0.42**	3.54 5.37

± shows S.E. value (n=3); Parenthesis indicate percentage increase (+) or decrease (-) over control. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Table 2. Effects of different salinity levels and their interaction with Phosphorus on total chlorophyll, chlorophyll a, chlorophyll b and carotenoids of onion plants observed at 120 days

Parameters	Treatment							LSD
	Control	2EC	4EC	6EC	8EC	6EC+P	8EC+P	***
Chlorophyll t (mg g^{-1} FW tissue)	2.70±0.09	2.62±0.06	2.56±0.07	2.33±0.16	1.57±0.09**	2.75±0.10	2.15±0.10*	0.41 0.63
Chlorophyll a (mg g^{-1} FW tissue)	1.65±0.02	1.56±0.03	1.48±0.01	1.22±0.03** -	0.90±0.02**	1.53±0.05	1.18±0.08**	0.26 0.40
Chlorophyll b (mg g^{-1} FW tissue)	1.32±0.07	1.30±0.03	1.22±0.04	1.08±0.009	0.58±0.02**	1.13±0.007	0.83±0.07**	0.27 0.41
Carotenoids (mg g^{-1} FW tissue)	0.90±0.009	0.82±0.014	0.76±.009*	0.70±0.009**	0.61±0.017**	0.85±0.024	0.70±0.014**	0.10 0.15

± shows S.E. value (n=3); Parenthesis indicate percentage increase (+) or decrease (-) over control. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

at 560nm using double beam UV-VIS spectrophotometer UV5704SS. Blanks were run in the same way but without illlumination.

Ascorbate peroxidase

The activity of APX was measured according to the Nakano and Asada (1981) by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM^{-1} cm^{-1}). The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm and enzymes activity was expressed as µmol of ascorbate oxidized min^{-1} g^{-1}.

Glutathione reductase

The GR activity was assayed by the method of Smith *et al*. (1988). The reaction mixture contained 1. ml of 0.2 M potassium phosphate buffer (pH 7.5) containing 1mM EDTA, 0.5 ml 3 mM 5, 5'- dithiobis (2-nitrobenzoic acid) in 0.01M phosphate buffer (pH 7.5), 0.25 ml H_2O, 0.1 ml 2 mM NADPH, 0.05 ml enzyme extract and 0.1 ml 20 mM GSSG. The components were added in order of listed above directly to a cuvette and the reaction was started by the addition of GSSG. The increase in absorbance was monitored for 5 minute at 412 nm. The rate of enzymes activity was calculated using standard curve prepared by known amount of GR (Sigma, USA).

Cysteine

Cysteine content in control and metal exposed plants was estimated following the method of Gaitonde (1967). Plant material (500mg) was homogenized in 5% chilled perchloric acid and centrifuged at 10,000×g for 10 min at 4 ^{0}C. Cysteine content was measured in supernatant using acid- ninhydrin regent. For preparation of every 10 ml of acid – ninhydrin reagent, 250mg of nihydrin was dissolved in 6 ml glacial acetic acid and 4 ml HCl. Reaction mixture (3ml) contained one ml each of supernatant, glacial acetic acid and acid nin-hydrin reagent. Mixture was heated for 15min at 95 ^{0}C, and then cooled rapidly to room temperature and absorbance was recorded at 560 nm. Cysteine content was calculated from the standard curve prepared using known concentration of cysteine (L-cysteine hydrochloride, sigma) and is expressed as nmol g^{-1} fw.

Non-protein thiol

Non protein thiol group was estimated by the method of Ellman (1959). Leaves were plucked and washed with distilled water then blotted.700mg plant tissue grind in 3ml of 6.67% sulphosalicylic acid. Extract was centrifuged at 13,000 rpm for 10 minute at 4oC. Reaction mixture contains 5mM of EDTA, 0.6 mM of DTNB and 120mM of phosphate buffer. For the absorbance of supernatant was read at 412nm using double beam UV-VIS spectrophotometer UV5704SS.

Statistical analysis

least significant difference (LSD) to compare means of different treatments that have an equal number of replications. All statistical test were performed with analysis tool from Microsoft office excel 2007.

RESULTS AND DISCUSSION

A decrease in whole plant FW and DW was noted due to salinity stress (Table-1). The shoot FW was significantly retarded by 9.45, 43.8, 46.9 and 67.8 % under salinity treatments of 2, 4, 6 and 8EC levels, respectively. Whereas, Bulb FW was significantly ($P<0.05$ and $P<0.01$) declined by 25.5, 45.8, 58.9 and 64.2% at 2, 4, 6 and 8EC levels respectively. Aghaleh and Niknam (2009) also has reported decrease in the growth parameters of soyabean plants under high salinity levels (100, 150 and 200 mM NaCl). Decrease in growth parameter can be due to disturbance in mineral uptake, photosynthesis, water potential, defense system and specific ion toxicity arising from higher concentration of Na^+ and Cl^- (Khan and Ashraf, 1988 and Marschner, 1995). Phosphorus application increased the FW of shoot by 85 and 35.9% and by 48 and 42% of bulb at 6 and 8EC levels. Salinity levels of 2, 4, 6 and 8EC caused 38.6, 50, 68.5 and 82.7 and 25, 46.8, 69.2 and 81% reduction in shoot and bulb DW respectively while application of P increased the shoot DW at 6 and 8EC levels by 144 and 64% and bulb DW by 147 and 80 % respectively. This protective role of Phosphorus may ascribe to its role in controlling the uptake of Na^+ and other toxic ions.

The application of P fertilizer can improve plant growth considerably under drought conditions (Ackerson, 1985; Studer, 1993; Garg *et al*., 2004). The positive effects of P on plant growth under drought have been attributed to an increase in water-use efficiency, stomatal conductance (Brück *et al*., 2000), and photosynthesis (Ackerson, 1985), to higher cell-membrane stability, and to effects on water relations (Sawwan *et al*., 2000). The content of Chl T, Chl a, Chl b and carotenoids were dropped at all EC levels (Table 2). This work is supported by Chaum and Kirdmanee (2009) in salt-stressed seedlings of two maize cultivars viz. Saccharata and Ceratina. However researchers summarized the results by showing that reduction in chlorophyll may be due to variation in its synthesis between the plant species or variation in specific enzymes under saline conditions (Keutgen and Pawelzik, 2007). In Onion, phosphorus application significantly ($P<0.01$) increased the content of Chl T up to 36.7% at 8EC Similarly, Phosphorus interaction raised Chl a significantly of about 31 and 25.1% at 6 and 8EC. Chl b was significantly increased of about 36.8% and 13.8% at 6 as well as 8EC respectively .Carotenoids revealed significant data of about 14% at 8EC in onion using Phosphorus.

The present study showed that application of phosphorus under salt stress condition enhanced the content of Chl a, Chl b, Chl T and carotenoids in onion. Malondialdehyde content (MDA) which can be used as an indicator to assess the tolerance of plants against oxidative damage and sensitivity of plant to salinity stress. MDA content in shoot and bulb was significantly ($P<0.05$ and $P<0.01$) higher under all salinity levels compared with control plant (Fig-1). Increase in MDA Ìsmail, 2005), alfalfa (Wang and Han, 2007), cotton (Diego *et al*., 2003) and wheat (Sairam and Srivastava, 2002). The higher level of MDA under salinity stress suggests that onion plant is sensitive to salt stress. The cysteine content was significantly enhanced in shoot and bulb of salt stressed onion plants. A more pronounced increment was observed under 8EC level in shoot and bulb (Fig-1). The present observation revealed that the increased level of cysteine mitigates toxicity imposed by salt stress. However, P application decreased cysteine content by 28 and 15.7% at 6 and 8EC in shoot and 16 and 21% at 6 and 8 EC respectively, in bulb. Since P application minimizes the synthesis of cysteine that suggests P prevents the excessive uptake of ions. The level of non protein thiol was significantly ($P<0.05$ and $P<0.01$) stimulated at all salinity levels in shoot, similar trend was observed in bulb. (Fig.1). It could be due to its role against salt-induced oxidative stress. The results show conformity to the finding of Mishra *et al*. (2006) in Bacopa monnieri. The antioxidant property of NP-SH depends on the oxidation of -SH group of the tripeptide to disulfide form (Noctor and Foyer, 1998; Sanita di Toppi and Gabbrielli, 1999).

The increased level of NP-SH may also be due to stimulation of sulphate reduction pathway such as APS reductase and serine acetyltransferase (Noctor and Foyer, 1998). P addition significantly ($P<0.01$) minimized the NP-SH level in shoot at 8EC by 39.3% while it was significantly declined 15.9% at 6EC level in bulb. Thus, this result suggested that salinity reduced oxidative stress which was controlled efficiently by P supplementation. Activity of CAT and POX were decreased in both shoot and bulb as salinity level increased (Fig-1). However, CAT activity was significantly increased at 6 and 8EC levels by 45 and 275% in shoot and 90 and 38% in bulb with the use of P. CAT activity in maize (Azevedo *et al*., 2006) and *Sesamum indicum* (Koca *et al*., 2007) is found to be differing under the influence of salt. Decrease in CAT activity under salt stress has also been observed by Saha and Gupta (1997) in sunflower and Bishnoi and Singh (1997) in cluster bean. The decrease in CAT activity suggests a greater accumulation of H_2O_2 which often generated in stressed plants (Elstner, 1982). This finding suggested that Onion is very sensitive to salt stress and P plays mitigatory role in maintaining the defense system of Onion plant. POX activity in shoot and bulb was significantly ($P<0.05$ and $P<0.01$) reduced (Fig-1).

This decrease in POX activity suggested that Onion was sensitive plant in response to salinity. A gradual decline in POX activity is observed by Saha and Gupta (1997) in salt stressed sunflower seedlings. The reduced POX activity indicated that it had a lower capacity for the decomposition of H_2O_2 generated by SOD. But with the application of P, it was raised at 8EC by 129% in shoot and 14 and 27% at 6 and 8EC % in bulb. But enhancement in POX activity has also been observed in rice (Lee *et al*., 2001), pea (Shahid *et al*., 2011) and mulberry (Harinasut *et al*., 2003). Under stress condition enhanced activity of POX are related to tolerance while in current study it was observed that salt stress reduced the activity of the POX in Onion that suggested Onion is very sensitive to salt stress. When P was supplied to salt stressed

suggested P helps in the mitigation of oxidative damage. SOD

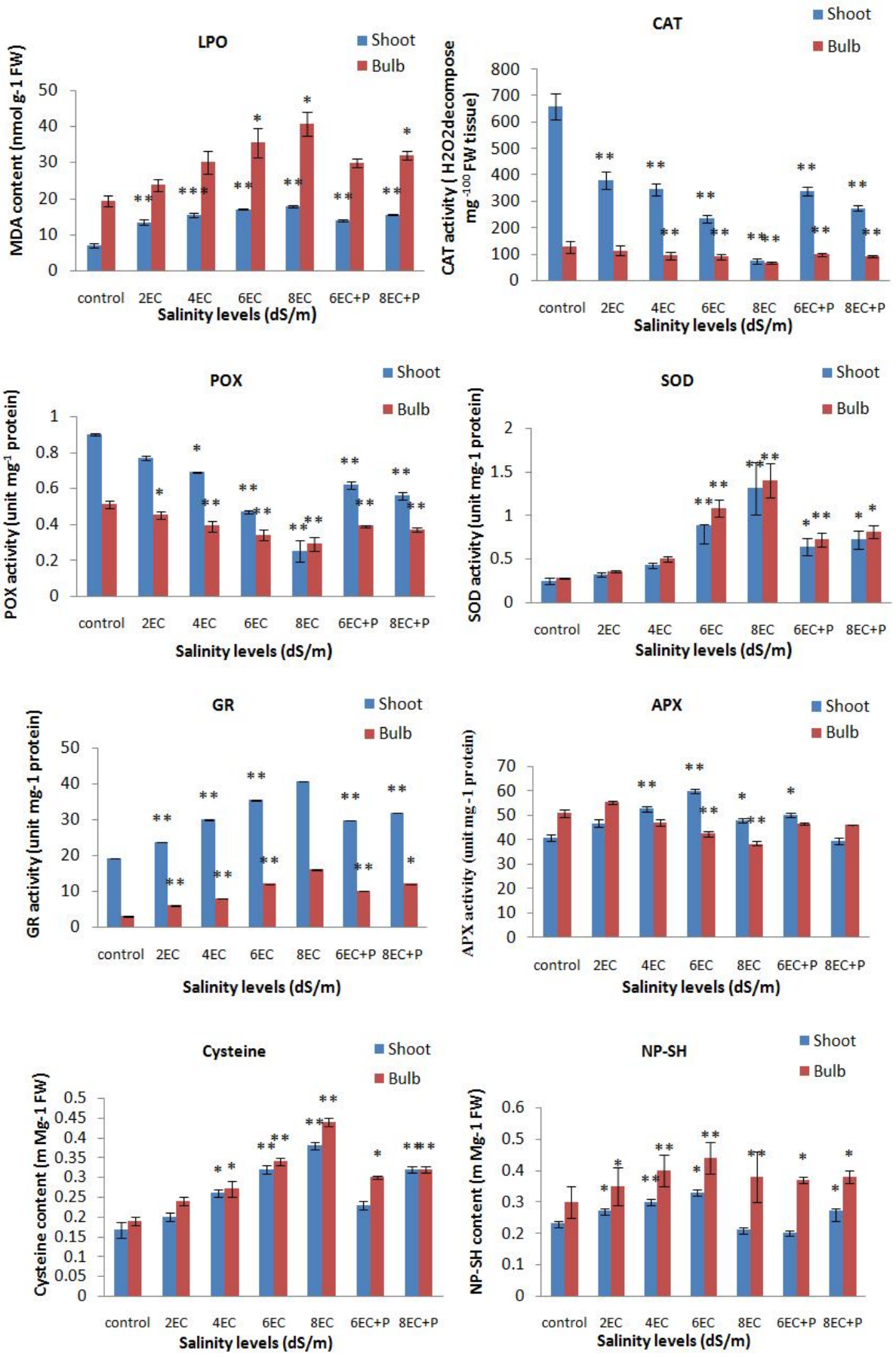

Figure 1. Effects of different salinity levels and their interaction with Phosphorus on MDA content, CAT, POX, SOD, GR, APX, Cysteine and NP-SH of onion plants observed at 120 days

is one of that enzyme catalyze the disproportionation of two O_2^- radicals to H_2O_2 and O_2. The SOD activity was significantly ($P<0.01$) elevated in shoot and bulb at all salinity levels. Whereas P application on plants exposed to salinity stress, significantly minimized SOD activity was noticed in shoot at 6 and 8EC by 28 and 45% and it was 33 and 42% in bulb. P application reduced the SOD activity in salt stressed plants. Reduction in SOD synthesis suggested reduction in the O_2^- means P helps in minimizing the production of O_2^- in plants exposed to salt stress. APX activity was significantly ($P<0.05$ and $P<0.01$) increased in shoot up to 6 EC level while at 8EC level it was decreased by 17.9%. In case of bulb, it was enhanced at 2EC by 8.4% then progressively declined at all salinity levels. Since, Results showed that decrease in the activity of the POX was found to be compensated by the APX activity. P addition significantly minimized the activity of APX at 6 and 8EC by 16.6% and 18.7% in shoot and in bulb it was improved by 11 and 20.7% at 6 and 8EC levels respectively. This result revealed that decreased activity of APX with P plays an important role in ameliorating oxidative damage by quenching hydrogen peroxide provoked by salinity stress. The activity of GR was significantly increased in shoot upto 4EC by 157% then reduced upto 8EC by 42.8%. Similar trend is obtained in bulb. Increased activity of GR has been reported to play a role in tolerance to salt stress in pea (Hernandez *et al*., 1999), egg plant (Yasar 2003), maize (Zacchini *et al*., 2003) and rice (Maribel and Tobita 2003). P supply significantly ($P<0.01$) improved the activity of GR in shoot at 8EC levels by 35% whereas; it was significantly increased in bulb only at 8EC by 44%. Similarly, in this study GR activity was moderately increased. Elevated activity results in accumulation of GSH levels and ultimately confers tolerance of plant (Sheela and Robina 2007).

Conclusion

This investigation highlighted that, plants irrigated with saline solution adversely affect growth and biochemical attributes in onion plants. Findings of this experiment lead to the conclusion that possible salt toxicity, especially in Phosphorus deficient soil, could be alleviated by P treatment and thus it increases tolerance against salinity stress in sensitive plant like onion.

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