Effects of phosphorus on antioxidant system in pepper cultivars under saline conditions

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Abstract

Salinity is one of the most severe abiotic stresses in one-half of all irrigated lands, causing negative effects on physiological, biochemical, and molecular responses in plants. Application of chemical fertilizers, particularly phosphorus (P) fertilizers, may reduce harmful effects of salinity. In this study, an investigation was performed on the effects of exogenous application of different levels of phosphorous (0, 40, and 80 mg/kg soil as $CaHPO_4$) on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) generation, the content of soluble proteins, and activities of antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD), in shoots of two different cultivars of *Capsicum annuum* L. (cv DS 77-172 and cv Sera) under different concentrations of NaCl (0, 50, and 150 mM). Treatment of salt stressed grown seedling with $CaHPO_4$ increased activity of POD. Under salinity conditions, phosphorous treatment decreased contents of H_2O_2 and MDA. These results indicate salt-induced deleterious effects in both pepper cultivars alleviated by phosphorous treatment.

Key words: *Capsicum annuum*; catalase; peroxidase; phosphorus; salinity; superoxide dismutase

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Introduction

Abiotic stresses are the principal threat to plant growth and crop productivity all over the world (Khan et al., 2009). There are a number of abiotic stresses common in nature such as salinity, drought, heavy metals, extreme temperatures, moisture, light, mineral deficiencies or toxicity, pH, and pollutants which can diminish plant yield (Ozturket al., 2009). Of all these abiotic stresses, salinity can be disastrous as it affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High levels of salinity in the soil can upset the nutrient balance in the plant or interfere with the uptake of various biochemical compounds like nucleic acids, nucleotides, phospholipids, and phosphoproteins.

Phosphorus (P) is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars, starches, and nutrients (Blaylock, 1994). Nutritional disorder may result from the effect of salinity on nutrient availability, competitive uptake, transport and partitioning within the plant (Kaya and Higgs 2003; Omami,

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2005). After nitrogen, phosphorous is the second major nutrient for plant growth as it is an integral part of different biochemicals like nucleic acids, nucleotides, phospholipids and phosphoproteins. Phosphorus exists in two forms in soil, as organic and inorganic phosphate, and like other nutrient elements such as potassium, iron, zinc and copper, possesses limited mobility in the soil.

Interaction between salinity and P nutrition is complex and it depends on plant species or cultivar, stage of development, salt composition, and concentration, and the P level in the medium growth (Grattan and Grieve, 1993). In West Africa and in Nigeria in particular, *Capsicum annuum* is third among the cultivated vegetables being utilized in the dry state as a spice (Nwachkwu*et al*., 2007). It contains an alkaloid a digestive stimulant that is used in ointment for the relief of arthritic and neuropathic pains (Nwachkwuet al., 2007). C. annuum is eaten raw in salads (Remison, 2005). Capsaicin, the pungent constituents of capsicum pods is used in the manufacture of ginger ale and ginger beer (Remison, 2005). In this work, we studied the effect of supplementation of phosphorus on improvement of antioxidant system activity of two different cultivars of peppers influenced by salinity.

Material and Method

Plant material

The seeds of two different pepper cultivars *Capsicum annuum* L. cv. DS and *Capsicum annuum* L. cv. Sera were obtained from the Agricultural Research Center of Tabriz, Iran.

Growth conditions

The experiments were conducted hydroponically in a growth chamber with a temperature regime of 28/20° C day/night, 16/8 h light/dark period and relative humidity of 70%. Pepper seeds were planted in plastic pots containing a mixture of sand, clay, and peat with the ratio of (1: 1: 2), respectively. Treatments were investigated through a factorial design with 2 factors and 4 replications on 25-day-old plants. The first factor was salinity with 3 levels including 0, 50, and 150 M/l NaCl. The second factor was phosphorous fertilizer at 3 levels including 0, 40, and 80 kg/ha mono calcium phosphate chemical fertilizer. After 20 days of treatment, the plants were used for further analyses.

Enzyme assays

Fresh leaf samples were used for enzyme extraction and measurement of protein and metabolites. Leaf samples were ground at 4° C in extraction buffer. Each enzyme assay was tested for linearity between the volume of crude extract and the measured activity. Changes in the absorbance of substrates or products were measured using a spectrophotometer (Specord 200, Analytical Jena, Germany).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to Giannopolitis and Ries (1977). The enzyme was extracted in 25 mM HEPES (pH=7.8) and 0.1 mM EDTA, an centrifuged at 15000 g for 15 min. Test tubes containing 25 µl of enzyme extract, 25 µl extraction buffer and 450 µl of the reaction mixture were incubated at 22° C and the light intensity of 400 µM m-2 s^{-1}. The reaction mixture contained 25 mM HEPES (pH=7.6), 0.1 mM EDTA, 50 mM Na_2CO_3 (pH=10.2), 12 mM L-methionine, 75 µM NBT and 1 µM riboflavin. The reaction was started by removing a dark plastic foil from the surface of samples and continued for 10 min. one unit of SOD was defined as the amount of enzyme required to induce 50% inhibition of NBT reduction as measured at 560 nm, compared with control samples without enzyme aliquot.

Peroxidase (POD, EC 1.11.1.7) activity was determined using the guaiacol test (Chance and Maehly, 1955). The enzyme was extracted by 10 mM phosphate buffer (pH=7.0) and assayed in a solution containing 10 mM phosphate buffer 5 mM H_2O_2 and 4 mM guaiacol. The reaction started by addition of the enzyme extract at 25 °C. The increase in the absorbance at 470 nm was followed for 2 min. The enzyme activity unit was calculated as amount of protein required for the formation of 1 µM tetraguaiacol for 1 min.

Other assays

Table 1
Effect of phosphorus (P) on shoot fresh and dry weights in two pepper cultivars under saline and non –saline conditions
Each value represented as mean ± SE (n=4), mean values followed by the same letter(s) are not significantly different (p<0.05).

Cultivar	NaCl (mM)	P (mg/kg)	Fresh weight (g)	Dry weight (g)
DS	0	0	1.75c±0.005	0.174b±0.004
	50	0	1.46f±0.005	0.147cd±0.004
	150	0	0.923i±0.24	0.101e±0.004
	0	40	2.09a±0.005	0.202a±0.004
	50	40	1.72d±0.005	0.17b±0.004
	150	40	1.15h±0.25	0.152c±0.013
	0	80	1.93b±0.005	0.195a±0.004
	50	80	1.66e±0.005	0.143d±0.021
	150	80	1.26g±0.20	0.133d±0.004
Sera	0	0	5.35a±0.14	0.47a±0.013
	50	0	2.92d±0.07	0.22de±0.005
	150	0	1.84f±1.02	0.19e±0.016
	0	40	5.28a±0.12	0.48a±0.009
	50	40	4.31b±0.05	0.39b±0.004
	150	40	1.89f±1.07	0.24cd±0.048
	0	80	3.55c±0.51	0.27cd±0.038
	50	80	3.85c±0.09	0.36b±0.008
	150	80	2.33e±1.24	0.29c±0.038

Soluble proteins were determined as described by Bradford (1976) using a commercial reagent and BSA as standard (Bradford, 1976).

The Hydrogen peroxide content was estimated according to the Harinasut et al. (2003). Samples were homogenized with 0.1% (w/v) Trichloracetic acid (TCA). The mixture was centrifuged at 12000 g for 15 min. To 0.5 ml of the supernatant, 0.5 ml of 10 mM phosphate buffer (pH=7.0) and 1 ml of 1 M potassium iodide (KI) was added. The mixture was incubated at 25° C for 15 min. The absorbance was measured at 390 nm. The H_2O_2 content was calculated from a standard curve prepared in a similar way.

Lipid peroxidation was estimated from the amount of Malondialdehyde (MDA) formed in a reaction mixture (Heath and Packer, 1968). Leaf tissues were homogenized in 0.1% (w/v) (TCA). The homogenate was centrifuged at 10,000 g for 5 min. To 1 ml of the supernatant, 4 ml of 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was incubated at 95° C in a water bath for 30 min, and then quickly cooled on ice. The mixture was centrifuged at 10000 g for 15 min and the absorbance measured at 532 nm. MDA levels were calculated from 1,1′3,3,′-tetraethoxypropan standard curve.

All chemicals and reagents used in this experiment were purchased from Sigma Aldrich (Steinheim, Germany), Fluka (Steinheim, Germany) and Merck (Darmstadt, Germany).

Statistical analysis

Experiments were conducted in complete randomized block design with 4 replications. Results were tabulated as mean values ± SD. Differences between control and treated seedlings were analyzed by SPSS16 with Duncan's Multiple Range Test ($P<0.05$).

Results

Salt stress significantly ($p<0.05$) decreased fresh and dry weights of shoots in both cultivars. In non-saline condition, application of 40 and 80 mg/kg, and 40 mg/kg P increased shoot fresh and dry weights of DS cultivar, respectively. Application of 40 mg/kg P for 50 mM NaCl-treated plants increased shoot fresh weight in both cultivars. Usage of 40 and 80 mg/kg P + 150 mM NaCl increased the shoot dry weight in DS and Sear cultivars (Table 1). In Sera cultivar application of P at two levels under both levels of salinity increased shoot dry weight (Table 1). The activity of antioxidant enzymes SOD (Fig. I) and POD (Fig. II) significantly increased ($p<0.05$) in both cultivars due to salt stress. Concentration of 50 mM NaCl increased shoot SOD and POD activity up to 1.7 and 2.5 times in DS and up to 1.2 and 4.1 times in Sera cultivar, respectively. Concentration of 150 mM NaCl increased shoot SOD and POD activities up to 1.8 and 3.6 times in DS and up to 1.3 and 8.5 times in Sera cultivar, respectively. Under the non-saline condition, application of 40 mg/kg P had no significant effect on the activities of both POD and SOD. In saline-condition application of 40 mg/kg P with 50 mM NaCl increased the activities of both SOD and POD. However, treatment of 150 mM NaCl increased the activity of SOD in the cultivars under study.

Application of 80 mg/kg P in plants treated with 50 mM NaCl decreased the activity of SOD in both cultivars but POD activity was affected slightly. Application of 80 mg/kg P with 150 mM NaCl decreased SOD activity.

Salt stress led to reduction of leaf soluble protein, while, application of P under saline condition recovered significantly leaf soluble proteins in both cultivars (Fig. III). The content of H_2O_2 (Fig. IV) and MDA (Fig. V) in shoots of both cultivars increased significantly in 50 and 150 mM salinity. In non-saline condition application of two levels of P had no effect on H_2O_2 and MDA content of both cultivars. Under salinity conditions, application of 40 and 80 mg/kg P reduced H_2O_2 and MDA content compared to control in both cultivars.

Discussion

Plant growth and productivity is adversely affected by various abiotic stress factors (Shahidet al., 2012). In this study, both pepper cultivars showed sensitivity to 50 and 150 mM NaCl. Changes in plant metabolism in response to salinity could be responsible for the

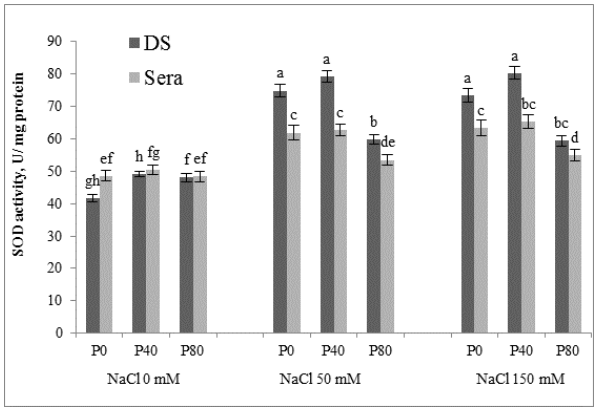

Fig. I. Effects of NaCl and phosphorus on SOD activitiy in two pepper cultivars.

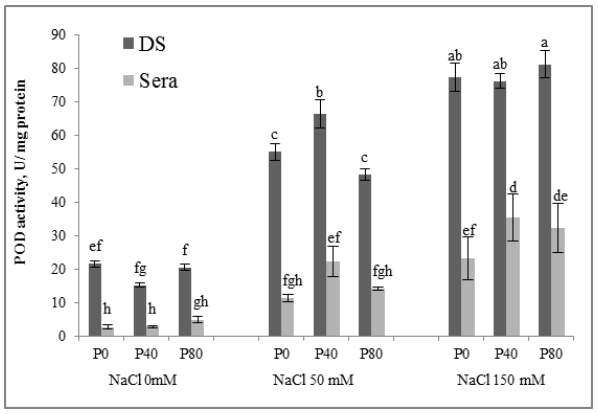

Fig. II. Effects of NaCl and phosphorus on POD activitiy in two pepper cultivars.

diminished growth of plants under high NaCl concentration (Erdalet al., 2011). The role of P application on mitigating detrimental effects of salinity in plants which was seen in this study, also, reported by several authors (Kaya et al., 2001; Khalil et al., 1967; Awadet al., 1995; Bernstein and Francois Clark, 1974). Phosphorus plays an important role in carbohydrate metabolism and also regulation of ion accumulation and compartmentalization in the cell (Gibson, 1988).

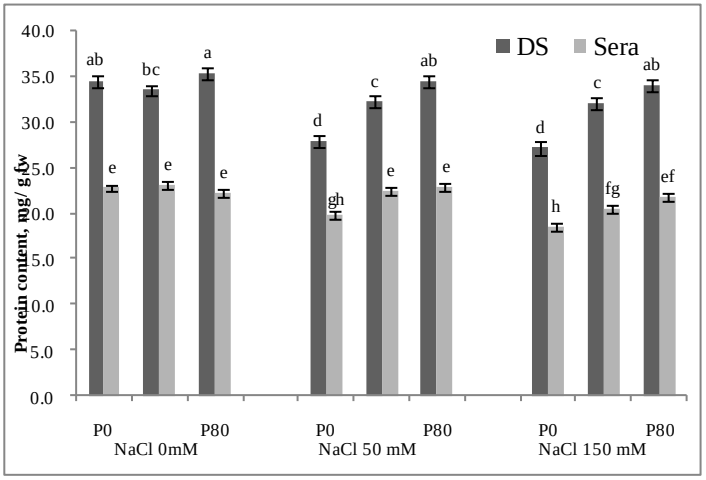

Fig. III. Effects of NaCl and phosphorus on protein in two pepper cultivars.

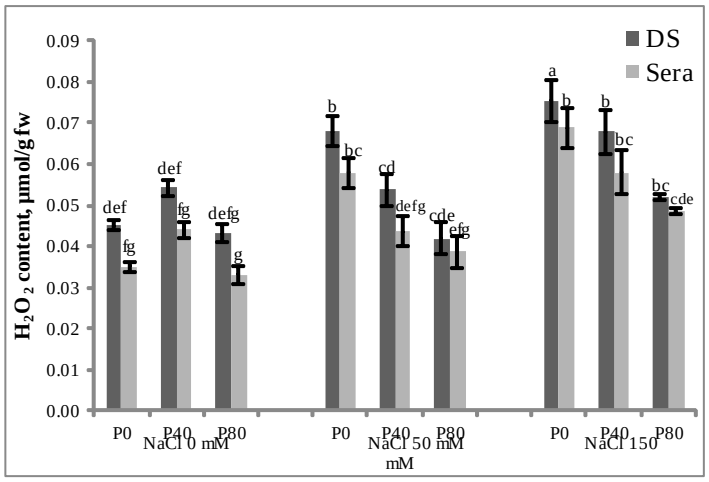

Fig. IV. Effects of NaCl and phosphorus on H2O2 contents in two pepper cultivars.

The increase in the activities of antioxidant enzymes SOD and POD in both cultivars were seen in salt-stressed plants that did not receive P in this study. This increase indicates a build-up of protective mechanism that reduce oxidative damages induced by stresses (Chawlaet al., 2013; Harinasutet al., 2003). It seems that compared to Sera, DS produces high amount of H_2O_2 which is removed effectively by POD and SOD at salinity conditions. According to our results, application of P in these plants could result in increased SOD activity. This increase in antioxidant enzymes activities causes the control of H_2O_2 and MDA contents. In the present study the activity of SOD had different patterns under salinity in respect P level.

The contents of H_2O_2 and MDA of pepper cultivars increased with NaCl concentration. Salinity stress can lead to oxidative stress (Raslein, 1992) by stomatal closure, which reduces CO_2 availability for carbon fixation. These conditions can expose chloroplasts to excessive excitation energy which in turn increase the generation of reactive oxygen species. The increased reactive oxygen species levels in plants

can cause damage to biomolecules such as lipids, thus increasing the MDA content as the decomposition product of polyunsaturated fatty acids of membranes (Panda et al., 2003). When applied exogenously at suitable concentration, P is found to alleviate the oxidative stress generated by salt stress. Under salinity condition,

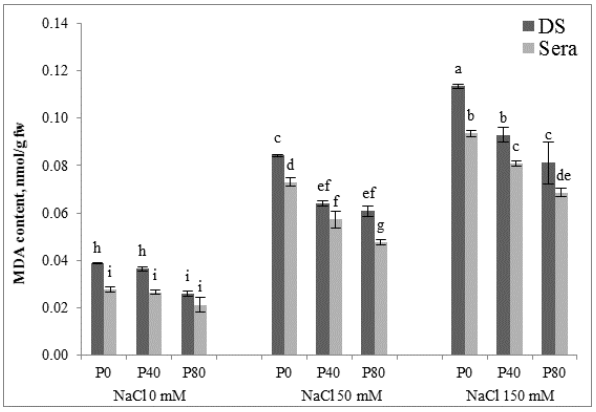

Fig. V. Effects of NaCl and phosphorus on MDA contents in two pepper cultivars.

a decrease in H_2O_2 and MDA contents occurred in plants treated with P levels. In other words, the salt induced deleterious effects were significantly alleviated by the P treatment.

The activation of pepper plants growth by P under salinity stress was associated with enhanced levels of soluble proteins (Fig 3). Reactive oxygen species are highly reactive and may cause oxidation of proteins. Changes in gene expression, protein relative abundance and activity are controlled by signaling events induced by salinity stress. Soluble proteins are important in osmotic adjustment under salinity stress. Increase in soluble protein content by P treatment under salinity may be the result of enhanced synthesis of specific stress-related proteins.

In conclusion, this study showed that pepper plants are sensitive to salinity. The supply of plants with P is effective in mitigating salt stress effects to protect plants against oxidative injury caused by salt stress.

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