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Original Research Article

Mitigatory role of phosphorus on radish (*Raphanus sativus* L.) exposed to salt stress

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Abstract: The aim of the current study was to elucidate the response of Radish (*Raphanus sativus* cv.chetkilong) grown under saline conditions and amended with supplementary Phosphorus (P). A soil pot culture experiment was accomplished in wire house. Plants were subjected to different electrical conductivity (EC) solutions viz. 0, 4, 6, 8 and 10 dSm^{-1} prepared by mixing NaCl, Na_2SO_4, $CaCl_2$ and $MgCl_2$. For remediation approach two salinity levels (EC 8 and 10 dSm^{-1}) were subjected with combination of 40ppm Phosphorus. The results uttered that salt stress hindered the plant growth such as fresh and dry weight and declined pigment contents like Chl T, Chl a and Chl b. Elevated level of malondialdehyde content and electrolyte leakage percentage in the leaves of plant exposed to salt solution suggested that salinity promoted the oxidative stress. Enzymatic and non enzymatic antioxidants also altered when plants were irrigated with saline solutions. The non-protien thiol group, proline and cysteine were found to gradually enhance 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. The activity of catalase (CAT) evoked at lower doses (4 and 6 dSm^{-1}) then reduced at 8 and 10dSm^{-1} while peroxidase (POX) aroused at all salinity levels but P amendment decreased its activity. In this study activity of superoxide dismutase (SOD) was decreased at all tested salinity levels while P supply increased the activity. The outcomes designated that supplementary P can ameliorate the damaging influence of salinity on the development of radish plants.

Key words: Amino acids, Antioxidative enzymes, Membrane damage, NP-SH, Phosphorus, Salinity

Introduction

Plant growth and productivity is adversely affected by nature's wrath in the form of various abiotic stress factors (Shahid *et al*., 2012). Plants are frequently exposed to a plethora of stress conditions such as salinity, drought, heat, flooding and heavy metal toxicity among others, where the various anthropogenic activities have accentuated the existing stress factors (Allakhverdiev *et al*., 2000; Siringam *et al*., 2012). Among these stresses, salinity is a serious problem in worldwide agriculture areas because it limits plant growth and productivity (Yildirim *et al*., 2009; Qin *et al*., 2010). A recent report of Central Soil Salinity Research Institute, India (CSSRI, 2010) estimates about 6.73 million hectares of agricultural land as salt affected. In Uttar Pradesh alone about 1.3 million ha of land is under salt affected soils (Source: CSSRI). Salt condition under irrigation water affects physiological process negatively including water relations and gas exchange attributes (Maeda and Nakazawa, 2008), nutritional imbalance (Yang *et al*., 2008), and disturbing the stability of membranes (Dogan *et al.*, 2010). Salts induce the ionic and osmotic stress which alters the morpho-physiological and biochemical processes at tissue and cell level (Murphy and Durako, 2003). Excessive salts in soil solution lower the soil water potential as compared to the potential within plant and this difference in water potential between soil and plant prevents the root to absorb water (Lloyd *et al*., 1989). This hindrance in the absorption of available water under saline condition causes the cell dehydration which ultimately leads to cell death. An excessive amount of toxic ions (Na^+ and Cl^-) in plant tissues unstable the cellular membranes by displacing the K^+ and Ca^{2+} (Grattan and Grieve, 1992) and affect their permeability. Salinity can also reduce photosynthetic activity by affecting the non stomatal attributes such as destruction of green pigments, lowering the leaf area or by decreasing the activity of photosynthetic enzymes in calvin cycle (Misra *et al*., 1997). The photosynthetic activity declination by salt stress is associated with an increase in

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reactive oxygen species (ROS). These ROS are highly reactive and may disrupt growth physiology through oxidative damage to protein, nucleic acids, and lipids (Namjooyan *et al*., 2012). Salt stress can break down chlorophyll (Chl), the effect ascribed to increased level of the toxic cation, Na^+ (Pinheiro *et al.,* 2008, Li *et al*., 2010, Yang *et al*., 2011). To alleviate the stress induced oxidative effects, plants generate different kinds of antioxidants like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Shahid *et al*., 2011). Plants synthesize and accumulate organic compounds in the cytosol and organelles for osmotic balance when they are exposed to salt stress (Ashraf and Harris 2004; Bartels and Sunkar 2005; Sairam *et al*., 2006). Osmotic adjustment is the improvement in cell water balance due to the accumulation of inorganic and organic osmolytes such as proline, betaines and/or sugars. Phosphorus plays a role in an array of processes, including energy generation, nucleic acid synthesis, photosynthesis etc. 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 (Grattan and Grieve, 1994). In agronomic terms P supply is important for new growth, flowering, fruiting and seed production. The principal objective of this study was to test whether the application of phosphorus could reduce salinity stress in radish. This study also aimed to investigate the results of the interactive effects of P and salinity on morphology, antioxidant systems, and amino acids in the leaves of radish plants.

Materials and Methods

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). Twenty five day's old plants were supplied with various salinity levels (0, 4, 6, 8 and 10 dSm^{-1}). The two higher salinity levels i.e. 8 and 10 dSm^{-1} was subjected to combination of 40 ppm of P. Treatment was given weakly for fifteen days. The saline solution was made using a mixture of NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$ in the equimolar basis of various electrical conductivity levels. After fifteen days of treatment, plants were harvested and used for analyzing various morphological, physiological and biochemical parameters. Chlorophyll was estimated by the method of Arnon (1949), amended by Lichtenthaler (1987). The level of lipid peroxidation in plant tissue was measured as thiobarbituric acid reaction by the method of Heath and Packer (1971) in terms of malondialdehyde content. Sullivan and Ross (1979) method was used for determination of electrolyte leakage percentage (ELP). CAT activity was assayed by the modified method of Euler and Josephson (1927). The POX activity was determined by the method of Luck (1963). 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). Non protein thiol group was estimated by the method of Ellman (1959). Cysteine content was estimated following the method of (Gaitonde, 1967). The determination of proline was performed according to the method of Bates *et al*., 1973.

Statistical analysis

The experimental data were tested for significance by using 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

Table 1 shows the changes in the morphological parameters in radish plants. As it is evident from the data, plant height showed a remarkable decrease with increasing salinity levels as compared to control. FW(fresh weight) of shoot and root were declined in all increasing salinity levels and P supply improved FW of plants treated with 8 and 10 dSm^{-1} saline solution by 160 and 400% in shoot and 83 and 50% in root respectively. Likewise DW (dry weight) of shoot and root was reduced at all salinity levels but P application increased DW of shoot and root by 75 and 58 % and 166 and 66% respectively. Number of leaf was also significantly ($P<0.05$ and $P<0.01$) decreased at all salinity levels. A decrease in the total chlorophyll content was found at all salinity levels except 4EC in radish leaves. At higher salinity level (10EC) it was declined by 25.2%. The total chlorophyll content significantly enhanced of about 38.8% and 33.8% at 8EC and 10EC with P application. The Chl a content in radish plant was

significantly (P<0.01) decreased with increasing salinity levels except 4EC. Contents of Chl a, for control was 1.36 which was reduced to 1.44, 0.96, 0.79 and 0.15 mg g-1 tissue in the leaves treated with 4, 6, 8 and 10EC. But P application at two salinity levels (8EC and 10EC) increased the Chl a by 37.9 and 30.4 % respectively. Contents of Chl b, for control radish leaves was 0.83 mg g-1 tissue which was reduced to 0.79, 0.74 and 0.63 at 6, 8 and 10EC except 4EC which was enhanced by 0.93 mg g^{-1} tissue in the leaves. On rectification with P at 8 and 10EC, Chl b increases and value became 1.02 and 0.92 mg g^{-1} respectively compared to control plants (table 2). The MDA content in radish plant was significantly (P<0.01) increased with increasing salt concentration. Contents of MDA, for control was 42.4 nmol g^{-1} which increased to 50.2, 75.6, 90.6 and 104.7 nmol g^{-1} tissue in the radish leaves treated with 4, 6, 8 and 10EC of saline solution. On rectification with Phosphorus at 8 and 10EC saline solution, MDA content was significantly (P<0.01) decreased by 7.39 % and 0.96%.The ELP in the radish leaves increased significantly (P<0.05 and P<0.01) by 17.9, 35, 43 and 52.6 % at 4, 6, 8 and 10EC. Phosphorus application at 8 and 10EC, it was decreased by 17.7 and 32.7% (table 2). The activity of CAT in radish leaves for control plants was 1090 which was increased by 4.89, 7.64% at 4 and 6EC then decreased significantly (P<0.05 and P<0.01) by 15.9 and 44.9% by 8 and 10EC. However, remediation with P showed significant (P<0.01) increase at 10EC in CAT activity by 33.3 %. (table 3). In radish leaves, activity of POX for control was 1.4 which was significantly (p<0.05 and P<0.01) enhanced by 229.5, 296.6, 390 and 474.5% for the plant exposed to 4, 6, 8, and 10. Application of P at 8EC, POX activity was significantly (P<0.05) increased by 21%. SOD activity was declined significantly (P<0.01) by 22.1, 33.8, 45.3 and 56.7% at 4, 6, 8 and 10EC respectively. But P application significantly increased the activity of SOD about 32.6 and 39% at 8 and 10EC. The NP-SH content significantly increased with increasing salinity levels except 4EC (table 4). Contents of NP-SH, for control was 0.36 mM g^{-1} FW tissue which was increased to 0.42, 0.44, 0.48 and 0.52 mM g^{-1} tissue in the leaves treated with 4, 6, 8 and 10EC. On rectification with P, NP-SH synthesis was found to decrease by 25 and 12.5% at 8 and 10EC. Cysteine content increased by 4.16, 12.5, 20.83 and 25% for 4, 6, 8 and 10EC. Phosphorus supply at 8 and 10 dSm^{-1}, cysteine content reduced by 22.2% and 33.3%. Proline content for control was 0.29 which was significantly (P<0.05 and P<0.01) enhanced to 86.2, 113.7, 142.5 and166.6 % for the plant exposed to 4, 6, 8 and 10 dSm^{-1}. Application of P at 8 and 10 dSm^{-1} significantly (P<0.05) decreased by 17 and 16.8%.

Table 1: Effect of different concentration of salt and its interaction with phosphorus on root and shoot length, fresh and dry weight of root and shoot of Radish plants observed at 40 days.

Treatments	Parameters						
	Root length (cm $Plant^{-1}$)	Shoot length (cm $Plant^{-1}$)	Root FW (g $Plant^{-1}$)	Shoot FW (g $Plant^{-1}$)	Root DW (g $Plant^{-1}$)	Shoot DW (g $Plant^{-1}$)	No. of leaf
0.0EC	10.7±0.54	17.8±0.59	0.26±0.02	4.4±0.40	0.08±0.01	0.38±0.01	6.3±0.27
4EC	9.0±0.47*	15.0±0.94*	0.23±0.01	3.4±0.64*	0.05±0.01	0.25±0.02	5.0±047
6EC	8.0±0.47**	11.0±0.47**	0.18±0.01	2.1±0.16**	0.04±0.01	0.18±0.01	4.3±0.27*
8EC	6.7±0.27**	8.7±0.72**	0.12±0.01	1.5±0.20**	0.03±0.01	0.16±0.02	3.7±0.27**
10EC	4.5±0.24**	6.2±0.72**	0.08±0.01*	0.6±0.08**	0.02±0.00*	0.12±0.02	3.3±0.27**
8EC+P	11.3±0.72	14.7±1.09*	0.22±0.02	3.9±0.33	0.08±0.01	0.28±0.02	7.0±0.47
10EC+P	8.3±0.54**	10.7±0.98**	0.12±0.01	3.0±0.41**	0.05±0.00	0.19±0.01	6.0±0.54
*	1.27	2.18	0.18	0.80	0.06	0.28	1.75
**	1.93	3.30	0.31	1.21	0.10	0.43	2.50

± Represent SE. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Table 2: Effect of different concentration of salt and its interaction with phosphorus on total chlorophyll, chlorophyll a, chlorophyll b, MDA and Electrolyte leakage percent in leaves of Radish plant observed at 40 days.

Treatments	Parameters				
	Total chlorophyll (mg g^{-1} FW tissue)	Chlorophyll, a (mg g^{-1} FW tissue)	Chlorophyll, b (mg g^{-1} FW tissue)	MDA (nmol g^{-1} FW)	ELP
0.0EC	2.25±0.06	1.36±0.02	0.83±0.01	42.4±0.33	48.7±1.47
4EC	2.38±0.05	1.44±0.02	0.93±0.01	50.2±0.14**	57.5±2.06
6EC	2.00±0.04	0.96±0.26**	0.79±0.02	75.6±1.07**	65.8±3.04*
8EC	1.93±0.02	0.79±0.26**	0.74±0.02	90.6±1.05**	69.7±1.19*
10EC	1.80±0.02*	0.15±0.01**	0.63±0.01*	104.7±1.97**	74.3±1.19**
8EC+P	2.68±0.02*	2.47±0.36**	1.02±0.02*	83.9±1.42**	57.3±1.44
10EC+P	2.41±0.02	1.50±0.02	0.92±0.01	103.2±2.58**	50.0±2.36
*	0.35	0.24	0.14	3.21	16.2
**	0.53	0.37	0.21	4.86	25.3

± Represent SE. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Table 3: Effect of different concentration of salt and its interaction with phosphorus on activity of catalase, peroxidase and superoxide dismutase in leaves of Radish plant observed at 40 days.

Treatments	Parameters		
	Catalase (H_2O_2 decompose mg^{-100} FW tissue)	Peroxidase (activity mg^{-100} FW tissue)	SOD (activity mg^{-100} FW tissue)
0.0EC	1090±37.5	1.4±0.02	9.5±0.17
4EC	1143±22.3	4.6±0.53	7.4±0.17**
6EC	1173±21.8	5.6±0.24*	6.3±0.22**
8EC	917±49.1*	6.9±0.38**	5.2±0.15**
10EC	600±47.2**	8.0±0.09*	4.1±0.06**
8EC+P	1013±56.9	5.4±0.15*	6.9±0.05**
10EC+P	800±47.2**	5.0±0.47	5.7±0.13**
*	133.0	3.80	0.93
**	201.5	5.54	1.83

± Represent SE. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Table 4: Effect of different concentration of salt and its interaction with phosphorus on activity of Proline, Cysteine and NP-SH in leaves of Radish plants observed at 40 days.

Treatments	Parameters		
	Proline (µ mol g^{-1} FW)	Cysteine (mM g^{-1}FW)	NP-SH (mM g^{-10}0 FW)
0.0EC	0.29±17.02	0.48±0.05	0.36±1.8
4EC	0.54±23.75**	0.50±0.12	0.42±1.20
6EC	0.62±54.70*	0.54±0.24	0.44±1.52*
8EC	0.70±44.86**	0.58±0.13	0.48±2.63**
10EC	0.77±56.90**	0.60±0.05	0.52±1.35**
8EC+P	0.58±51.77*	0.42±0.18	0.36±1.25
10EC+P	0.64±7.33*	0.40±0.05	0.42±0.78
*	0.23	0.29	0.07
**	0.38	0.49	0.11

± Represent SE. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Discussion

Salinity stress may cause decreased plant growth (Ephron *et al*., 1999). Reduction in shoot weight, plant height and root length under salt stress were also reported by Parida and Das, 2005. Salinity stress may induce damage to the photosynthetic apparatus (Yasseen, 1983). Reduction in photosynthetic pigments, such as Chl *a* and *b* has been reported in some earlier studies on different crops, *e.g*., sunflower, *Heliantus annuus* (Akram and Ashraf, 2011). Salinity stress leads to irreversible damage of the cell membrane (Mundree *et al.,* 2002).). Increase in MDA contents under salt stress was also found in rice (Tijen and Ìsmail, 2005). Membrane injury due to salt stress may be related to increased production of ROS (Shalata *et al.,* 2001), and highly toxic ROS must be scavenged in order to maintain normal plant growth (Demiral and Turkan 2004). Plants detoxify the ROX species by maintaining the high activities of antioxidant

enzymes i.e. SOD, POD and CAT (Sekmen *et al*., 2012). Literature depicts that salt tolerance potential is highly linked with the maximum ratios of antioxidant enzymes (Shahid *et al*., 2011; Balal *et al*., 2012. Reduced activity of SOD suggested that plant was salt sensitive. Increased electrolyte leakage observed in the present study at salinity stress could be partly due to decreased chlorophyll concentration (Kaya *et al.,* 2001). Electrolyte leakage from cells exposed to oxidative stress may occur not only as a result of nonspecific oxidation of the plasma membrane phospholipids; it may result from the direct activation of ion-permeable channels by ROS species (Demidchik *et al*., 2010). The activity of antioxidant enzymes has been reported to increase under saline conditions in the case of salt tolerant cotton (Meloni *et al*., 2003) and pea (Hernandez *et al*., 1999). One of the responses of plants to salinity stress is to accumulate proline, and this has been shown by many researchers. At the cellular level, accumulation of proline may regulate osmotic adjustment (Perez-Alfocea *et al*., 1993). Proline acts as a major supply of energy, which helps to improve salinity tolerance (Chandrasekhar and Sandhyarani, 1996). The present observation revealed that the increased level of cysteine mitigates toxicity imposed by salt stress. P application minimized the synthesis of cysteine that suggested P may prevent the excessive uptake of ions. 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 supply reduced the level of NP-SH in salt stressed plants. Hence, P may act as ameliorative agent when plants were treated with saline water.

Conclusion

In the view of this experiment, it can be concluded that growth, physiological and biochemical parameters of radish plants alter due to salt stress. Observations of this experiment depicted that P treatment can overcome the detrimental impact of salinity, especially in Phosphorus deficient soil and enhances tolerance against salt stress.

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