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INTERACTIVE EFFECT OF ROOTING MEDIUM APPLICATION OF PHOSPHORUS AND NACL ON PLANT BIOMASS AND MINERAL NUTRIENTS OF RICE (*ORYZA SATIVA* L.)

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Abstract

A greenhouse experiment was conducted to assess the influence of rooting medium application of phosphorus on mineral nutrients status of rice (*Oryza sativa* L.) plants under normal or saline conditions. There were four levels (control, 20, 40 and 60 mmol kg^{-1}) of NaCl and three levels of phosphorus (control, 30 and 60 mg kg^{-1}) applied through rooting medium. Imposition of varying levels of salt stress markedly decreased the plant fresh and dry biomasses due to increase in the leaf, and root Na^+, and Cl^- concentrations. In contrast, a significant reduction in the leaf and root K^+, Ca^{2+} and root P was also observed in the rice plants. A non-significant effect of salt stress was observed on leaf P, and grain Cl^-, K^+, Ca^{2+} and P concentrations. Maximum increase or decrease in these nutrients was observed at 60 mmol NaCl/kg. Different levels of phosphorus applied through rooting medium inhibit the accumulation of Na^+ and Cl^- in leaf and roots, while enhance the leaf and root K^+, P alongwith Ca^{2+}. Among all levels, 60 mg kg^{-1} of phosphorous was observed most effective in relation to these nutrients studied in rice plants.

Introduction

Salinity is a major environmental constraint for crop production throughout the world (Ashraf, 2004; Flowers, 2004; Munns *et al*., 2006). Salt-affected soils occupy more than 7% of the earth land surface (Munns *et al*., 2006; FAO, 2008). Reduction in the osmotic potential and toxic effect of excessive Na^+ or $Cl^–$ on the plasma membrane are the direct effects of salts on plant growth that reduce the availability of water to plants. Now it is well known that salt stress causes a number of effects on plants such as osmotic effects, ion toxicity, hormonal imbalance, generation of reactive oxygen species and nutritional imbalance (Ashraf, 2004; Flowers & Flowers, 2005).

Different strategies have been listed by various plant scientists to improve crop salt tolerance that will result into enhanced productivity on salt affected soils. On the bases of these strategies it has been suggested that salt induced nutritional disorders can be alleviated by the addition of mineral nutrients in the growth medium. The deleterious effects of salt stress are associated with reduced uptake of K^+ and can be alleviated by the addition of K^+ to growth medium in many crops, e.g., tomato, maize, sunflower, beans etc. (Grattan & Grieve, 1999). In view of these reports, it is suggested that high level of macro-nutrients can be supplied exogenously so as to alleviate the adverse effects of salt stress on plant growth. Among various approaches in relevance to increase salt tolerance, application of plant nutrients to maintain nutritional imbalance has a great significance (Aslam *et al*., 1996).

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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. Phosphate compounds act as “energy currency” within plants (Tisdale *et al*., 1985; Russell, 1981). Plant-available P deficiency is common in most of our soils which hinders crop procuction (Memon, 1996).

Rice (*Oryza sativa* L.) is the second most important crop in the world after wheat, with more than 90% currently grown in Asia. It has been cultivated for more than 7000 years as a major crop and currently supported nearly one half of the world population (Mishra, 2004). It is a salt-sensitive crop, but it is the only cereal that has been recommended as a desalinization crop because of its ability to grow well under flooded conditions, and because the standing water in rice fields can help to leach the salts from the topsoil to a level low enough for subsequent crops. About 1.0 million hectares of salt-affected soils are under rice cultivation (Qureshi *et al*., 1991) and reduction in the paddy yield due to salinity has been estimated as 68% (Qayyum & Malik, 1988; Aslam *et al*., 1994). In view of effective role of P under salt stress, the present study was conducted to observe whether P alleviates the adverse effects of salinity on rice plants particularly in relation to mineral nutrients of rice.

Materials and Methods

To assess the influence of different levels of phosphorous on nutrients status of rice (*Oryza sativa* L.) under saline or non-saline conditions, a pot experiment was conducted in the net house of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The soil used for the experiment was taken from field area of NIAB. Grains of a variety (Super Basmati) were taken from NIAB and sown in a normal soil free from salinity/sodicity. There were four levels of salt stress (control, 20, 40 and 60 m*M*/kg) and three of phosphorus (control, 30 and 40 mg P/kg) with four replicates. Five kilogram of air-dried soil previously passed through 2 mm sieve was filled in each pot. A basal dose of nitrogen (100 mg N/kg) as urea was applied in two equal splits; half at seedling transplantation and remaining half 15-days after seedling transplantation to meet the nutrient requirements and better growth of the rice seedlings. Eight seedlings of 30-day old were transplanted per pot and after a week thinning was done as to 5 seedlings per pot. The plants were allowed to establish for one week, before the start of salinity treatments. According to treatment, salinity was applied by adding NaCl and ECe was maintained in each pot. After 23 days salt stress treatment, one plant per pot was harvested for fresh biomass. Then, plants were oven dried at 65 °C for 72 h to constant dry weight and dry biomass was recorded, while remaining plants were used for attaining grain yield. Plant did not survive upto yield at highest level of salinity i.e., 60 m*M*/kg.

Determination of N, P, K^+, Ca^{2+}, Mg^{2+}, and Na^+: The dried ground leaf, root or grain material (0.1 g) was digested following Wolf (1982). Na^+, K^+, and Ca^{2+} in the digests were determined with a flame photometer (Jenway, PFP-7). Phosphorus (P) was determined spectrophotometrically following Jackson (1962). For the determination of Cl^- contents, the ground leaf, root or grain samples (100 mg) were extracted in 10 ml of distilled water, heated at 80 ^oC till the volume became almost half. The volume was again maintained to 10 mL with distilled deionized water. Cl^- content in the extracts was determined with a chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

Statistical analysis: Analysis of variance of data of all parameters was computed using the COSTAT computer package (CoHort software, Berkeley, USA). Mean values of each attribute were compared using the least significance difference test (LSD) at 5% probability following Snedecor & Cochran (1980).

Results

Salt stress significantly reduced the plant fresh and dry biomasses of rice plants. The most effective level was 60 m*M* NaCl/kg. However, application of different doses of phosphorous increased the plant fresh biomass while, did not show increasing or decreasing effect on plant dry biomass (Table 1; Fig. 1).

Leaf, root and grains accumulated significantly high concentration of Na$^+$ under salt stress. Maximum concentration was observed at highest level of salt stress i.e., 60 m*M* in case of leaf and root and 40 m*M* in grains. It was observed that Na$^+$ accumulation was decreased by phosphorus application through rooting medium. A remarked reduction in leaf or root K$^+$ was observed at all salt stress levels but grain K$^+$ was not affected by salt stress. Rooting medium application of P ameliorated the salt induced reduction and 30 or 60 mg P/kg was most effective at 20 or 40 m*M* of NaCl (Table 1; Fig. 1).

Table: 1 Mean squares from analyses of variance of data for plant biomass and nutrients concentrations of rice (*Oryza sativa* L.) when 30-day old plants were subjected for 23 days to varying levels of phosphorus under salt stress.

Source of variation	**Degrees of freedom**	**Plant fresh biomass**	**Plant dry biomass**	**Leaf Na$^+$**	**Root Na$^+$**
Salinity (S)	3	234.7***	4.335***	94.58***	95.46***
Phosphorus (P)	2	8.759*	1.653ns	10.64***	5.703ns
S x P	6	38.85**	0.763ns	7.104***	2.759ns
Error	36	3.239	0.205	0.798	1.903
		Leaf Cl$^-$	**Root Cl$^-$**	**Leaf K$^+$**	**Root K$^+$**
Salinity (S)	3	278.1***	79.15***	418.6***	105.9***
Phosphorus (P)	2	36.63*	5.426ns	59.45***	10.81ns
S x P	6	31.14*	20.03***	21.51**	4.423ns
Error	36	10.97	3.511	4.773	3.588
		Leaf P	**Root P**	**Leaf Ca^{2+}**	**Root Ca^{2+}**
Salinity (S)	3	408.1ns	1978.1*	17.52***	0.356***
Phosphorus (P)	2	2841.8**	7963.9***	22.59***	0.102*
S x P	6	825.8ns	2102.1**	3.699*	0.152***
Error	36	499.4	567.3	1.406	0.028
		Grain Na$^+$	**Grain Cl$^-$**	**Grain K$^+$**	**Grain P**
Salinity (S)	2	0.203***	0.264ns	0.061ns	1016.3ns
Phosphorus (P)	2	0.567***	0.150ns	1.929***	5505.8**
S x P	4	0.218***	0.335ns	0.207ns	940.7ns
Error	27	0.024	0.160	0.086	744.2
		Grain Ca^{2+}			
Salinity (S)	2	0.057ns			
Phosphorus (P)	2	7.79***			
S x P	4	10.64***			
Error	27	0.241			

*, **, *** = significant at 0.05, 0.01, 0.001 levels, respectively, ns = non-significant.

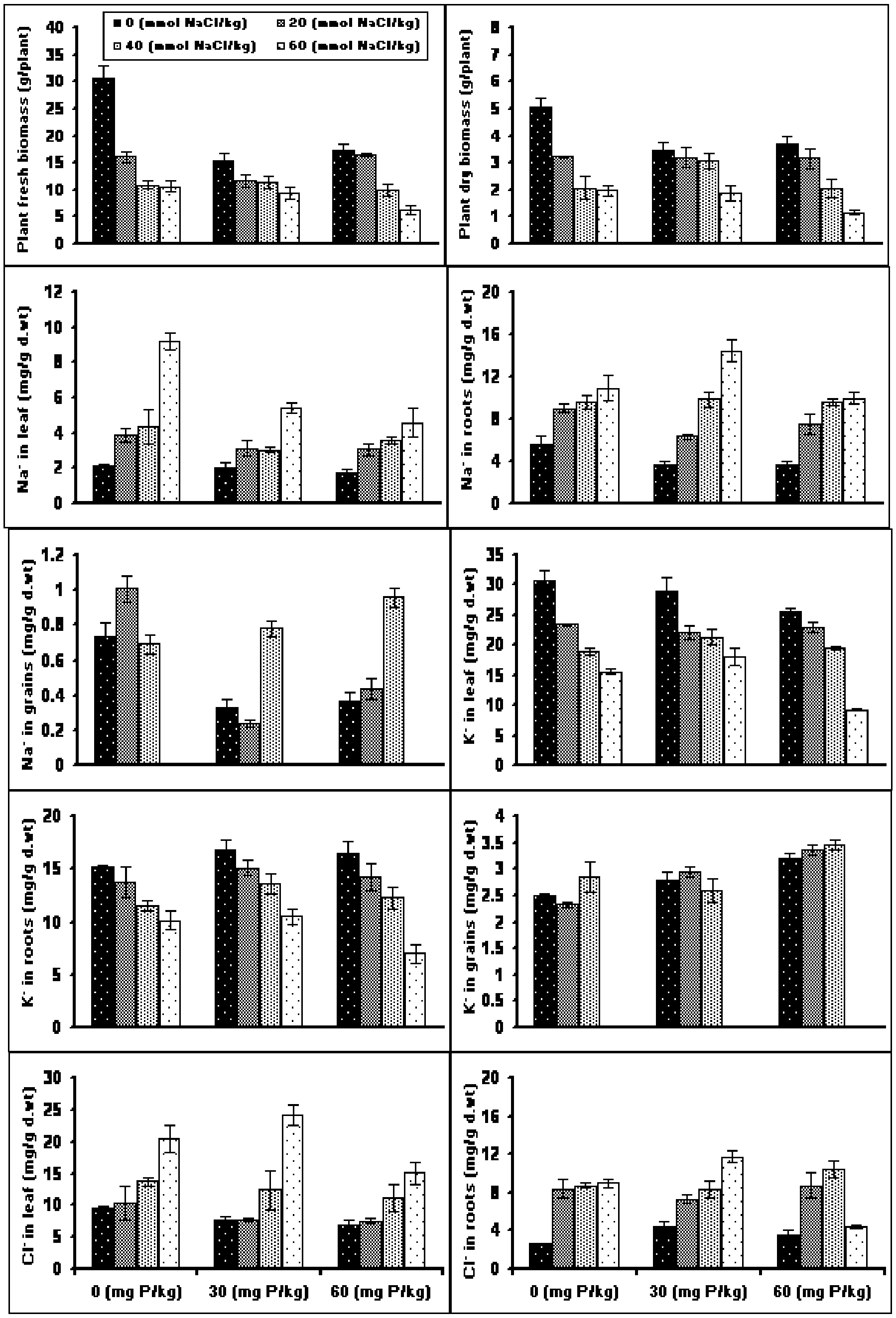

Fig. 1: Plant fresh and dry biomasses, leaf, root or grain Na^+, K^+ and Cl^- concentration of rice (*Oryza sativa* L.) plants when 30-day old plants were subjected for 23 days to different levels of phosphorus under salt stress.

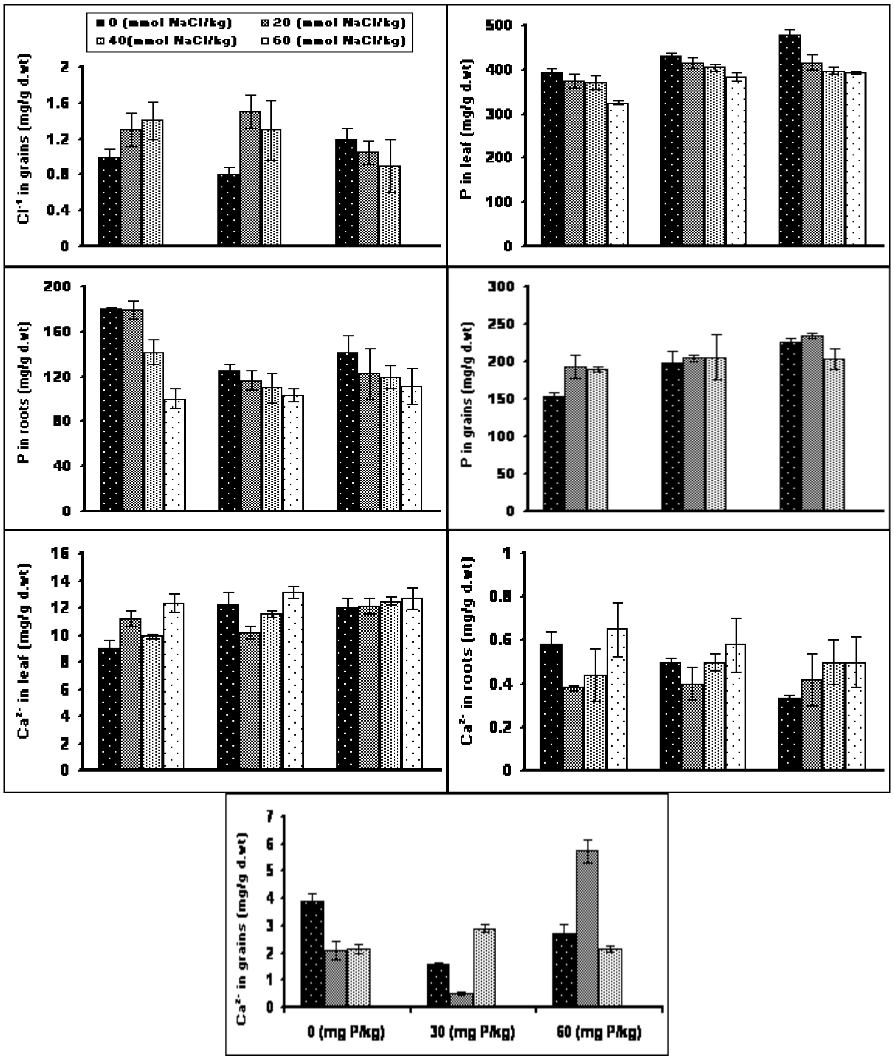

Fig. 2: Leaf, root or grain P and Ca^{2+} concentration of rice (*Oryza sativa* L.) when 30-day old plants were subjected for 23 days to different levels of phosphorus under salt stress.

Increase in leaf and root Cl$^-$ in rice plants was observed while, application of phosphorous showed consistent results for this element in all three parts of rice plant. Non-significant effect of salt stress was observed on leaf and grain phosphorus while a slight reduction was observed in root phosphorus concentration. At all salinity levels phosphorus was increased by increasing P levels. Varying levels of salt stress enhanced the leaf and root Ca^{2+} accumulation while, a non significant effect of salt stress was observed on Ca^{2+} in grains. Rooting medium application accelerated the leaf, root and grain Ca^{2+} concentration significantly and the most effective level was 60 mg P/kg (Table 1; Fig. 2).

Discussion

Plants differ greatly in their degree of salt stress tolerance (Ashraf *et al.*, 2006; Ulfat *et al.*, 2007) and of the cereal crops, rice (*Oryza sativa*) is the most sensitive under salt stress (Munns *et al.*, 2006; Naheed *et al.*, 2007; Munns & Tester, 2008). Generally, optimal concentration of any nutrient is required for normal plant growth and below it growth is reduced. Nutrients activity or availability is reduced under salt stress conditions, thus optimal concentration of essential nutrients by the plant is increased (Marschner, 1995). Optimal concentration of a nutrient varies depending upon the type of species or cultivar, level of salt stress, or environmental conditions (Grattan & Grieve, 1999). It is generally believed that high amount of Na^+ reduces the nutrient uptake (Marschner, 1995; Grattan & Grieve, 1999; Ashraf, 2004; Flowers & Flowers, 2005). In the present study varying levels of salt stress considerably reduce the plant fresh and dry biomasses. Soil salinity affects plant growth (Naheed *et al.*, 2007) and development due to osmotic stress, toxicity of Na^+ and $Cl^–$ ions and nutrients imbalance caused by excess of Na^+ and $Cl^–$ ions (Sairam & Tyagi, 2004).

Varying levels of NaCl significantly increase the leaf, root and grains Na^+. This increase may be due to increasing concentration of Na^+ in the root medium which ultimately resulted in the increased uptake of sodium by plant (Munns, 2002; Munns *et al.*, 2006; Munns & Tester, 2008). Ionic or nutritional imbalance results in salt stressed plants due to competition of salt ions with nutrients (Ashraf *et al.*, 1992; Ashraf & Sarwar, 2002). The accumulation of Na^+ in roots provides a mechanism for rice to cope with salinity in the rooting medium and/or may indicate the existence of an inhibition mechanism of Na^+ transport to shoot. These results are in agreement with the results of Kaya *et al.* (2001b) for spinach. Application of external P has significant reducing effects on Na^+ concentration in shoot and grains. It has already been observed that P fertilization reduced the concentration of Na^+ in shoots, resulting in better survival, growth and yield of rice (Qadar, 1998) and wheat (Salim *et al.*, 1999). However, by increasing the supply of P to the saline medium tended to decrease the concentration of Na^+ in rice (Aslam *et al.*, 1996; Asch *et al.*, 1999), sunflower (Malik *et al.*, 1999) spinach (Kaya *et al.*, 2001a) and wheat (Abid *et al.*, 2002).

Salinity has significant reducing effect on shoot and root K^+ concentration. The decrease in K^+ concentration due to salinity might be related to the competition and resultant selective uptake between K^+ and Na^+ which causes an increase in uptake of Na^+ at the cost of K^+ (Kuiper, 1984) or decline in K^+ content occurs due to decrease in sink size with the higher concentrations of NaCl, which strongly inhibited shoot and root growth (Ashrafuzzaman *et al.*, 2000). Malik *et al.* (1999) reported that synergistic relationship between P and other beneficial elements like K^+, Ca^{2+} and Mg^{2+} might have initiated the osmotic effect and thus can be held responsible for salt tolerance to some degree. It has previously been reported that K^+ concentration is lowered by increasing NaCl concentration in nutrient solution or in the soil as observed in rice (Hussain *et al.*, 2003). Like Na^+, increase in Cl^- is also observed in shoots and roots of rice which are in agreement with the results obtained from rice (Aslam, 1992; Aslam *et al.*, 1996) and maize (Zalba & Peinemann, 1998).

According to Grattan & Grieve (1999) common mechanism by which salinity disrupts the mineral relations of plants is by reduction of nutrient availability by competition with major ions (Na^+ and Cl^-) in the substrate. These interactions often led to

Na-induced Ca^{2+} or K^+ deficiencies. Application of P had significant positive reducing effect on shoot Cl^- concentration but has non significant effect on root and grain Cl^- concentration. Aslam *et al.* (1996) also observed lower shoot Cl^- when rice plants exposed to salt stress in the presence of P. Salinity had no effect on shoot and grain P but has significantly reducing effect on root P. These results are in contrast with the results of Aslam (1992) who reported that P concentration in rice plants is increased with increasing salinity. The P contents increases at higher P rates in roots and grains might be due to better supply of P in growing medium (Niazi *et al.*, 1992). However, concentration of P deceased in the presence of salinity in spinach (Kaya *et al.*, 2001b).

Overall, salt stress reduced the plant fresh and dry biomass due to increase in the accumulation of Na^+ and Cl^- in all parts of rice plants. However, rooting medium application of phosphorus reduces the accumulation of Na^+ and Cl^- and enhances the K^+, Ca^{2+} and P.

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