

## ALLEVIATION OF THE ADVERSE EFFECTS OF SALT STRESS ON RICE (*ORYZA SATIVA L.*) BY PHOSPHORUS APPLIED THROUGH ROOTING MEDIUM: GROWTH AND GAS EXCHANGE CHARACTERISTICS

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### Abstract

A pot experiment was conducted to assess the effect of phosphorus on growth and gas exchange of rice (*Oryza sativa L.*) grown under salt stress. Three levels of phosphorus (control, 30 and 60 mg kg<sup>-1</sup> of phosphorus) and four treatments of salinity (0, 20, 40 and 60 mmol kg<sup>-1</sup> of NaCl) were applied through rooting medium. Shoot biomass, shoot length and gas exchange characteristics decreased with increase in salinity. In addition, application of P also decreased the growth of rice. Photosynthetic rate and stomatal conductance were significantly reduced under saline conditions, while with the addition of phosphorus in the rooting medium more reduction was observed. However, there was no change in sub-stomatal CO<sub>2</sub> concentration and Ci/Ca ratio with increase in rooting medium salinity or addition of phosphorus in both saline and non-saline media.

### Introduction

Salinity is one of the major factors causing reduction in growth and productivity of almost all the crops (Szabolcs, 1994). Soil salinity causes adverse effects on different physiological processes which are responsible for the reduction of growth of plants (Ashraf, 1994; 2004; Munns *et al.*, 2006). So, increase in salt tolerance of crops is necessary to sustain food production in different saline regions (Pitman & Lauchli, 2002). In view of current levels of the growing world population, it is estimated that there will be a need to increase food production upto 38 % by 2025 and 57 % by 2050 to maintain the food supply. The aim, therefore, should be to increase yield per unit of land rather than in the area cultivated (Wild, 2003).

Among cereal crops, rice is a major source of food after wheat for more than 2.7 billion people on a daily basis. It is planted on about one-tenth of the earth's arable land and is the single largest source of food energy to half of humanity. Of the 130 million hectares of land where rice is grown, about 30% contain levels of salt too high to allow normal rice yield (Mishra, 2004). According to Qayyum & Malik (1988) the reduction in yields of rice, wheat, cotton and sugarcane cultivated on such moderately salt-affected soils are 68, 64, 59 and 62%, respectively. Under saline conditions, growth of rice plants varies depending on the particular growth stage i.e., starting from germination and ending to maturation (Alam *et al.*, 2000).

Phosphorus deficiency in the soil reduces the rice yield (Wissuwa *et al.*, 1998). Supplementary phosphorus (P) has a role in alleviation of the adverse effects of high salinity on whole plant biomass for a variety of crop plants (Kaya *et al.*, 2003). However, role of P under saline conditions is crop specific. Substantial amount of P (in mM) is required for living cells, but plants have to face a severe problem for acquiring this level

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of P directly from their environment because inorganic phosphate (Pi- the form in which P is assimilable), is not uniformly distributed in soils, and most soil Pi is immobile and so not readily available to roots (Raghothama, 1999). Most of the soils of Pakistan are deficient in plant-available P (Memon, 1996) which becomes a major constraint for crop production under our soil conditions. The application of phosphorous has been shown to be beneficial for different crops under various soil conditions (Singh *et al.*, 1993; Shah *et al.*, 1996). So the primary objective of present study was to assess up to what level rooting medium phosphorus had any beneficial effect on growth of rice under both non-saline and saline conditions. Furthermore, what might be the status of gas exchange characteristics under saline conditions, when supplemented with phosphorus.

### Materials and Methods

A pot experiment was conducted to assess the effect of phosphorus on growth and gas exchange characteristics of rice (*Oryza sativa* L.) grown under salt stress. The experiment was conducted in the net house of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad.

Physical and chemical characteristics of original soil used are given below:

Characteristics	Values
Electrical conductivity (ECe) of soil saturation extract (dS m <sup>-1</sup> )	0.39
pH of soil saturated extract	8.01
Textural class	Loam soil
Saturation percentage	17
Phosphorous (mg P/kg)	6.7

Grains of a rice variety 'Super Basmati' were obtained from NIAB and sown in plastic pots containing sand supplemented with full strength Hoagland's nutrient solution. There were four salinity and three phosphorus treatments with four replicates. Salinity treatments were control (ECe 0.39 dS m<sup>-1</sup> control), 20, 40 and 60 mmol kg<sup>-1</sup> of NaCl, while phosphorous levels were control (without phosphorus), 30 and 40 mg P/kg. 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 seedling.

Eight seedlings of 30-days old were transplanted per pot. After a week thinning was done to 5 seedlings per pot. The plants were allowed to establish for one week and salinity was applied by adding NaCl and ECe was maintained in each pot according to the desired treatments. Two plants were harvested 23 days after transplanting. Plants were uprooted carefully and washed with distilled water. After measuring the fresh plant biomass and shoot length, plants were oven dried at 65 °C to constant dry weight, and then the dry biomass measured.

**Plant pigments:** The chlorophyll *a* and *b* were determined according to the method of Arnon (1949).

**Table 1.** Mean squares from analyses of variance of data for growth and physiological attributes of rice (*Oryza sativa* L.) when 30-day old plants were subjected for 23 days to soil containing varying levels of phosphorus under control or saline conditions.

Source of variation	Degrees of freedom	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Shoot length
Salinity (S)	3	234.7***	4.335***	3.405**	0.467**	219.5***
Phosphorus (P)	2	8.759ns	1.653ns	38.22***	0.300*	107.2**
S x P	6	38.85***	0.763***	13.84***	0.190ns	20.06ns
Error	36	3.239	0.205	0.617	0.085	15.63
		Chl. a	Chl. b	A	E	g <sub>s</sub>
Salinity (S)	3	0.042*	0.028ns	18.53***	2.408**	13250.7***
Phosphorus (P)	2	0.032ns	0.028ns	59.06***	9.232***	46369.4***
S x P	6	0.056*	0.035ns	6.663**	0.677ns	12740.7***
Error	36	0.071	0.041	1.480	0.310	1330.8
		C <sub>i</sub>	C <sub>i</sub> /C <sub>a</sub>	A/E		
Salinity (S)	3	111.4ns	170.5ns	0.254ns		
Phosphorus (P)	2	853.1ns	170.7ns	15.01***		
S x P	6	385.3ns	170.2ns	0.708*		
<b>Error</b>	<b>36</b>	<b>568.8</b>	<b>169.9</b>	<b>0.237</b>		

\*, \*\*, \*\*\* = Significant at 0.05, 0.01, 0.001 levels, respectively

ns = Non-significant.

A = Net CO<sub>2</sub> assimilation rate, E = Transpiration rate, g<sub>s</sub> = Stomatal conductance

C<sub>i</sub> = Intercellular CO<sub>2</sub> conc. C<sub>a</sub> = Ambient CO<sub>2</sub> conc. A/E = Water use efficiency

**Gas exchange characteristics:** Measurements of net CO<sub>2</sub> assimilation rate (A), transpiration rate (E), stomatal conductance (g<sub>s</sub>) and sub-stomatal CO<sub>2</sub> concentration (C<sub>i</sub>) were made on fully expanded youngest leaf of each plant using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Four weeks after the start of salinity treatment these measurements were made from 10:15 to 12:45 with the following specifications/adjustments: leaf surface area 11.35 cm<sup>2</sup>, ambient CO<sub>2</sub> concentration (C<sub>ref</sub>) 352 μmol mol<sup>-1</sup>, temperature of leaf chamber varied from 31.5 to 37.8 °C, leaf chamber gas flow rate (v) 251 μmol s<sup>-1</sup>, molar flow of air per unit leaf area (U<sub>s</sub>) 221.06 mol m<sup>-2</sup> s<sup>-1</sup>, ambient pressure 99.2 kPa, water vapor pressure into chamber ranged from 0.0006 to 0.00089 MPa, PAR (Q leaf) at the leaf surface was maximum up to 1048 μmol m<sup>-2</sup> s<sup>-1</sup>.

**Statistical analysis:** Analysis of variance of the data for each attribute was carried out following Steel & Torrie (1980). The mean values were compared with the least significance difference test (LSD) following Snedecor & Cochran (1980).

## Results

Mean squares from analysis of variance of data for shoot fresh and dry weights of rice when 30-day old plants were subjected for 23 days to varying levels of phosphorus under control or saline conditions showed that both shoot fresh and dry weights decreased significantly with increase in salt concentration, whereas phosphorus application in rooting medium did not alter the above mentioned attributes. However, under saline conditions maximum reduction was at 60 mg P/kg under highest level of salinity i.e. 60 mM of NaCl (Table 1; Fig. 1). Root fresh and dry weights were affected due to the imposition of P in the rooting medium, while salinity reduced both roots fresh and dry weights effectively. High levels of salinity i.e. 40 and 60 mM of NaCl were effective in reducing shoot length. Only high level of P i.e. 60 mg P/kg reduced the shoot length while the remaining levels proved to be non-effective under both saline and non-saline conditions (Table 1; Fig. 1).

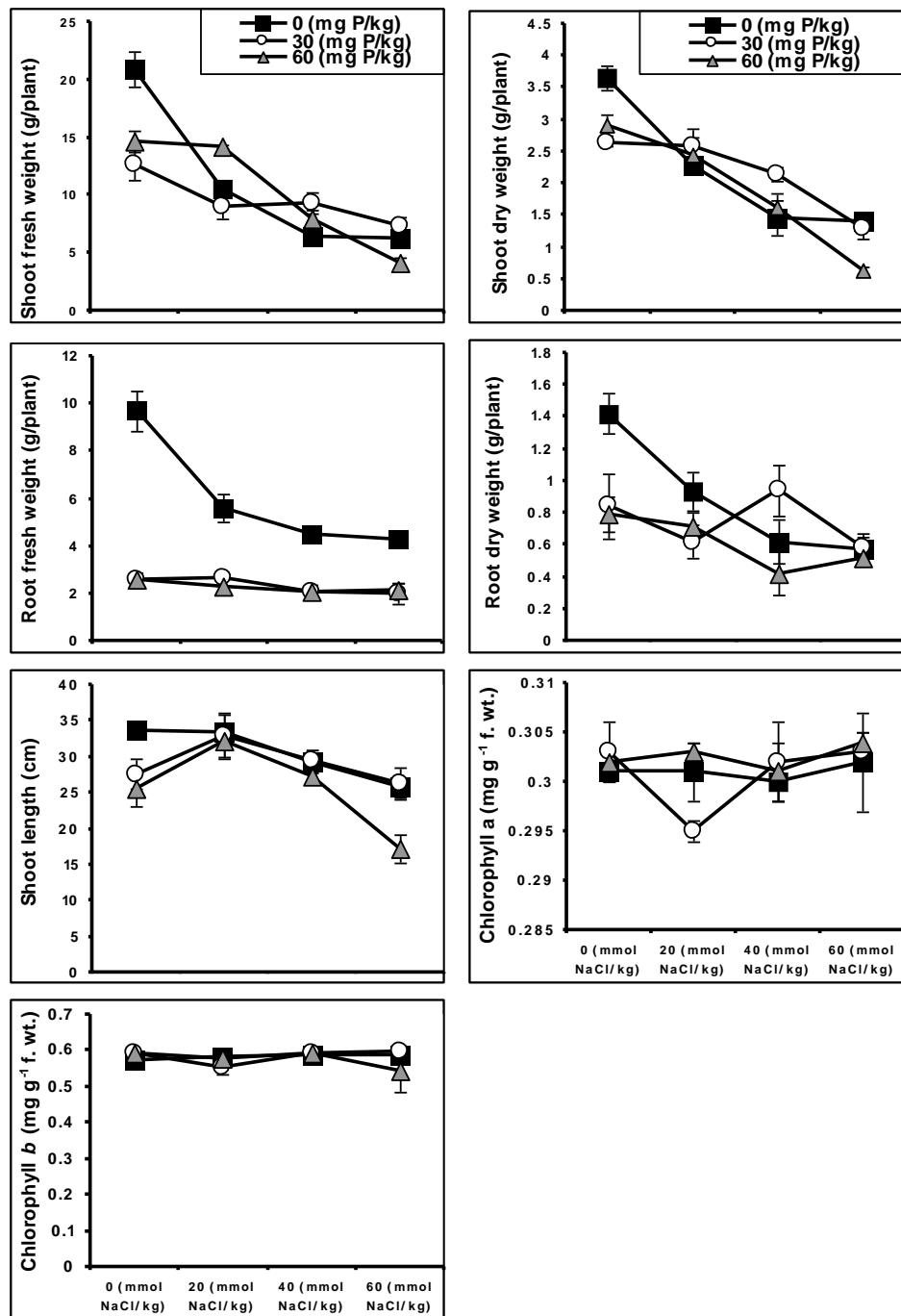


Fig. 1. Plant biomass and chlorophyll pigments of rice (*Oryza sativa* L.) when 30-day old plants were subjected for 23 days to soil containing different levels of phosphorus under control or saline conditions.

Pattern of increase or decrease of chlorophyll *a* pigment was not consistent under saline and non-saline conditions but it was low at 30 mg P/kg and at 20 mM of NaCl. Application of both P and salts in the rooting medium had non-significant effect on chlorophyll *b* pigments (Table 1; Fig. 1).

Exogenous application of P in the rooting medium slightly improved photosynthetic rate at 30 mg P/kg, however, high level of P i.e. 60 mg P/kg caused a significant reduction in photosynthetic rate of rice under both non-saline and saline conditions. Addition of P caused a significant decrease in stomatal conductance under both non-saline and saline conditions. In comparison with control, pattern of stomatal reduction with increase in salt stress was prominent at 30 mg P/kg level (Table 1; Fig. 2). Application of external P and salinity regimes had a significant increasing effect on transpiration rate when P was applied @ 30 mg P/kg, while 60 mg P/kg decreased transpiration only under saline conditions while under non-saline conditions it was high as compared to plants which were without P (Table 1; Fig. 2). Both salinity and phosphorus did not have any beneficial or toxic effect on both sub-stomatal CO<sub>2</sub> concentration and *Ci/Ca* ratio. Both of these attributes remained unaffected by increasing salinity or P levels (Table 1; Fig. 2). Rooting medium P had a significant reducing effect on water use efficiency, while the effect of salinity was not prominent (Table 1; Fig. 2). Maximum water use efficiency was found in control condition i.e. without P, but it was reduced consistently with the application of P i.e., 30 or 60 mg P kg<sup>-1</sup>. Among all the treatments, more appropriate level for better water use efficiency was control i.e., without salt and phosphorus.

## Discussion

Thirty days old plants of rice were subjected for 23 days to varying levels of phosphorus under control or saline conditions. Various growth, biochemical and photosynthetic attributes were studied. Salinity had an inhibitory effect on all growth parameters of rice e.g., shoot length, shoot and root dry and fresh weights etc. Salinity affects the growth of rice in varying degrees at all stages of its life cycle starting from germination and ending to maturation (Alam *et al.*, 2000). Salinity inhibits the plant growth and it has been extensively studied in many plants e.g., in rice (Alam *et al.*, 2004), corn (Bar-Tal *et al.*, 1991), tomato (Satti & Al-Yahyai, 1995), spinach, cucumber and pepper (Kaya *et al.*, 2001a), and cotton (Leidi & Saiz, 1997). Biomass production of rice decreased with increasing salinity over a range of even 0.5 to 4 dS m<sup>-1</sup> (Shannon *et al.*, 1998). This reduction in plant biomass might have been due to limited supply of metabolites to young growing tissues (Mass & Nieman, 1978). Application of P reduces plant growth i.e., shoot and root fresh and dry weights etc., this might be due to Zn deficiency in soil where P is applied because high rates of P application leads to Zn deficiency (Probna *et al.*, 1976). However, in contrast, Vega *et al.*, (1986) observed the reverse role of P in the Zn availability and observed the improved plant growth under saline conditions. A positive effect of P under saline conditions also has been reported in wheat (Abrol, 1968) and sorghum (Indulkar & More, 1985). Low level of salinity i.e., 20 mM of NaCl did not alter the shoot length because low concentrations of salts actually increase turgor pressure, cell wall synthesis, cell enlargement and it may result in faster growth (Orden, 1960). Inhibited vegetative growth in highly saline medium is due to reduced cell division, cell enlargement and cell wall expansion (Greenway, 1973).

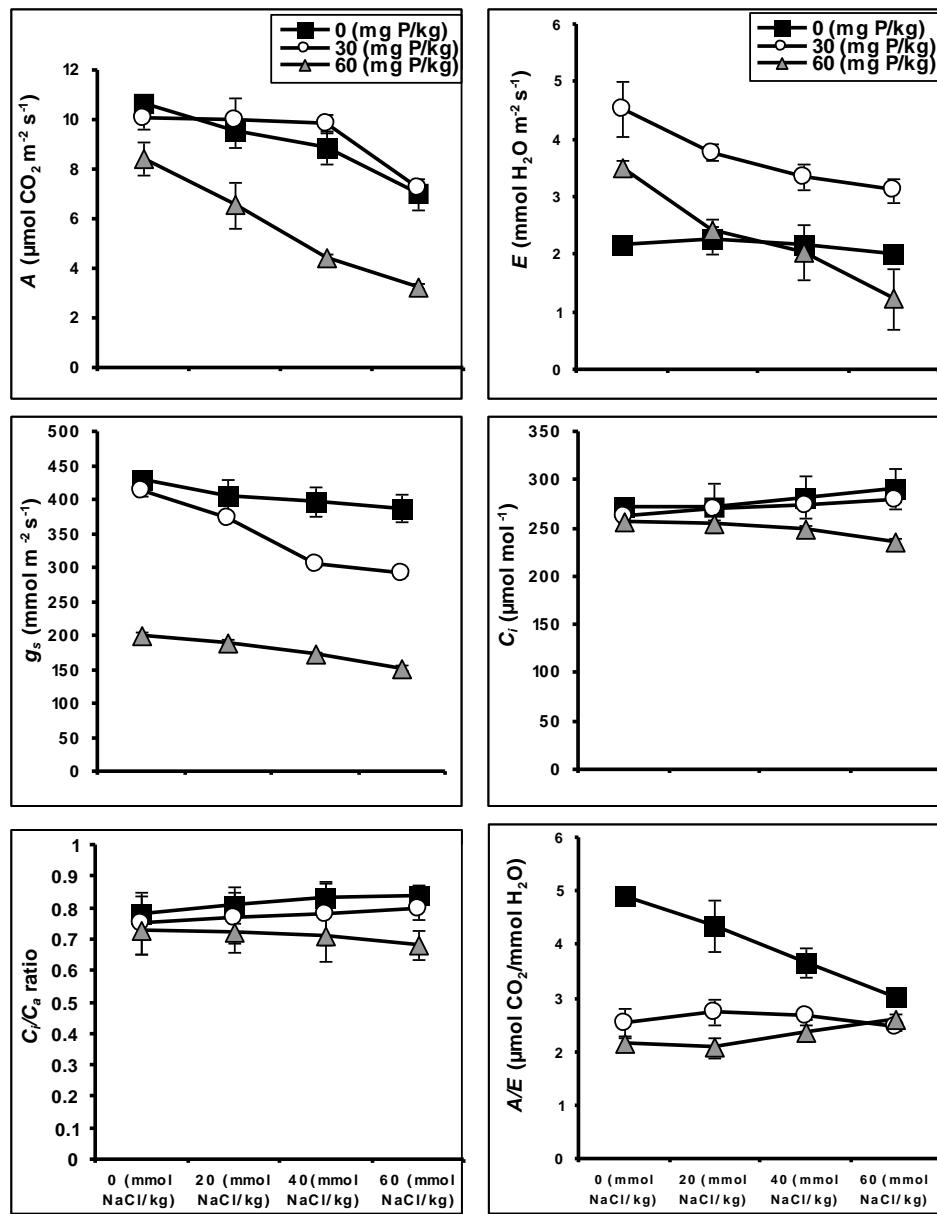


Fig. 2. Gas exchange characteristics of rice (*Oryza sativa* L.) when 30-day old plants were subjected for 23 days to soil containing different levels of phosphorus under control or saline conditions.

Photosynthesis has an effective role in plant growth. However, salinity affects the photosynthetic rate adversely. This adverse effect could be due to reduction in leaf area per plant. According to Cramer *et al.*, (1994), Munns *et al.*, (1982) and Fricke *et al.*, (1996), after 2-3 weeks of salinization, photosynthetic activity per unit may be little

affected but overall rates of photosynthesis were reduced as a result of reduction in photosynthetically active leaf area. Therefore, it seems to be true that reduction in photosynthesis for per unit leaf area leads to reduced shoot growth (Alam *et al.*, 2004). Water use efficiency of spinach decreased by adding 60 mmol kg<sup>-1</sup> NaCl into the nutrient solution (Kaya *et al.*, 2001c). Reduction in water use efficiency was also observed in tomato (Kaya *et al.*, 2001b), cucumber and pepper (Kaya *et al.*, 2001a). Maximum water use efficiency was observed in control condition, but it was reduced consistently with the application of P alone and with salinity.

Relationship between stomatal conductance and leaf water potential have been shown in many studies (Ashraf *et al.*, 2003). It is now generally known that severe plant water deficits either due to drought or salt stress are correlated with suppression in stomatal conductance. By the application of P stomatal conductance was reduced at the highest level of P i.e., 60 mg kg<sup>-1</sup>. However, low value of stomatal conductance was recorded under control or saline conditions. Low stomatal conductance could have been due to high solute concentration in the rooting medium and in turn due to reduced water availability to the plants.

It is possible that decrease in the shoot and root growth in salinized plants could be due to several reasons. One possibility is that salinity reduces photosynthesis, which in turn limits the supply of carbohydrates needed for growth (Alam *et al.*, 2004). A second possibility is that salinity reduces shoot and root growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium (Alam *et al.*, 2004). A third possibility is that the root response to salinity was to down-regulate shoot growth (and root also) *via* a long distance signal (Alam *et al.*, 2004). Fourth, a disturbance in mineral supply, either on excess or deficiency, induced by changes in concentrations of specific ions the growth medium might have directly affected growth (Lazof & Bernstein, 1998). There are many contradictions about the effect of P on shoot length. Some are of the view that P has no effect on shoot length (Bhatti & Khattak, 1985), but others say that shoot length increases with the addition of P (Abid *et al.*, 2002).

In conclusion, growth of rice plants was reduced with increase in salt levels. Application of phosphorus through rooting could not ameliorate the adverse effects of salt stress on rice in terms of growth and gas exchange characteristics measured.

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