INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–999/2016/18–4–837–843
DOI: 10.17957/IJAB/15.0180
http://www.fspublishers.org

*Full Length Article*

# Effect of Manganese Application on PS-II Activity in Rice under Saline Conditions

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## Abstract

Salinity reduces the uptake of manganese (Mn) and induces its deficiency, which adversely affects photosynthesis-related photosystem-II activity, hence growth is hampered. The objective of the study was to investigate the effects of Mn application on the photosystem-II activity on the isolated thylakoid membranes of the rice. A hydroponic study was carried out in the glasshouse of National Agriculture Research Centre, Islamabad, Pakistan on two rice varieties, Pakhal and KS-282, using three replicates. Three salt concentrations, namely 0, 25, 50 mM NaCl were used. Manganese sulphate was used for foliar and root application with four concentrations (0, 2, 4, 8 µg Mn $mL^{-1}$). Salinity decreased the concentration of Mn, while the application of Mn, increased the contents of Mn in shoot and root. Increased salt concentration caused a decrease in the chlorophyll contents while Mn application increased chlorophyll contents across all the salinity levels, with maximum increase at 2 µg Mn $mL^{-1}$. Photosystem-II activity was inhibited by increasing salt concentration, while Mn treatment rescued this inhibitory affect, with maximum photosystem-II activity recovery at 2 µg Mn $mL^{-1}$ level in both varieties. Quite high concentration of Mn was observed in root application methods as compared to foliar but it failed to recover salinity impact on photosystem-II and photosynthesis. Applying Mn as foliar increased tolerance to salinity at 50 mM NaCl level while root application was found better at 25 mM NaCl concentration in culture solution. 

**Keywords:** Manganese content; PS-II activity; Chlorophyll content; Saline condition; Rice

## Introduction

During growth and development, plants are subjected to a variety of stresses such as heat, drought, cold, anaerobiosis and salt. Salt stress is major stress affecting agricultural productivity adversely especially in arid land, since major crop plants are relatively less salt tolerant (Greenway and Munns, 1980). Salinity is defined as the condition of soil with large concentrations of salts of cations (Na, K etc.) or also trace ions including B, Sr, Li, Rb, F, Mo, Ba and Al. Soil contains sufficient salts in root zone to impair growth of crop plant, as the level of salinity rises, plants extract water less easily (Tanji, 1990; Aslam *et al.*, 2015).

Crop performance may be adversely affected by salinity-induced nutritional disorders (Grattan and Grieve, 1999). Manganese (Mn) concentration in plant tissues is related to the growth rate but salinity reduces the uptake of Mn and induces its deficiency in shoots of plants, which diminishes photosynthetic-related parameters (Pandaya *et al*., 2004). Mn is an integral part of the catalase, manganese super oxide dimutase in the PS-II, which catalyzes the oxidation of two molecules of water by production of oxygen, four protons and equivalents of electrons (Yocum, 1996).

The electrons are transferred from water to $NADP^+$ within the chloroplast during the electron transport chain process (Govindjee and Coleman, 1990). This process is carried out by two photosystems, photosystem II (PS II), and photosystem I (PS I). PS II transfers electrons from water to plastoquinone, and PSI move electrons from plastocyanin to $NADP^+$ (Allakhverdiev *et al*., 1999).

Rice is considered as salt sensitive crop, and used as the cereal all over the world. Sensitivity of rice to salinity stress varies with the growth stage with more sensitive at young seedling stages than at reproduction. As Mn plays very important role in photosynthetic activity, hence the objective of the study was to measure the effect of Mn application methods on Photosystem-II activity of the isolated thylakoid membranes under saline conditions.

**To cite this paper:** Tabassam, T., S. Kanwal, S.M.S. Naqvi, A. Ali, B.U.Z. Zaman and M.E. Akhter, 2016. Effect of manganese application on PS-II activity in rice under saline conditions. *Int. J. Agric. Biol.*, 18: 837–843

## Materials and Methods

### Plants and Stress Conditions

A hydroponic experiment was conducted at Land Resources Research Institute, NARC, Islamabad Pakistan to explore the effect of Mn on Photosystem II activity of the isolated thylakoid membranes of rice under saline conditions. Seeds of rice varieties Pakhal and KS-282 were surface-sterilized with 0.1% sodium hypochloride for five min, washed thoroughly with distilled water. The seeds were soaked for 24 h in a beaker of distilled water and then spread on trays containing sand. Two weeks old, four seedlings per pot, in triplicate were transplanted to 2 cm plugged holes in black painted pots containing nutrient solution without any sodium and manganese contents. All the pots and solution culture studies were placed in a glass house having exhaust fans and no other environmental control. Inside the glass house, maximum temperature ranged from 35–45ºC, minimum temperature 15–20ºC and bright sunlight, with active photoperiod of 7–9 h. Yoshida nutrient solution (Yoshida *et al.*, 1976) at pH 5.0 was used and manganese levels were maintained at 0, 2, 4, 8 µg Mn $mL^{-1}$ solution as $MnS0_4$ through root application and foliar spray. The pH was adjusted every second day with 1 N KOH or 1 N HCl and nutrient solution was replaced once a week. After two weeks, salt stress was applied at the rate of 0, 25, 50 mM NaCl with three increments. The seedlings were harvested after five weeks of transplanting. Oven dried plant samples were digested with nitric perchloric acid (2:1) mixture (Ryan *et al.,* 2001). Digested samples were used for determination of Mn using Atomic Absorption Spectrophotometer (Perkin-Elmer, 4000).

### Chlorophyll Determination

The chlorophyll content of samples was measured according to dimethyl sulphoxide (DMSO) chlorophyll extraction technique of Hiscox and Israelstam (1979). For the extraction, glass centrifuge vials containing 7 mL DMSO were preheated to $65^0$C in a water bath. Chlorophyll was extracted from 0.5 g of each leaf sample at 65ºC for 30 minutes. When the extraction was completed, samples were removed from the water bath and each graduated vial was topped up to exactly 10 mL with DMSO. The spectrophotometer (Genesys 5) was calibrated to zero absorbance using a blank of pure DMSO. Absorbance of both blank and samples were measured at 645 and 663 nm. The total chlorophyll (mg $g^{-1}$) was calculated using Arnon's equation: $C= (0.0202xA_{645} + 0.00802xA_{663})$ x dilution factor.

### PS-II Activity Measurement

PS-II activity measurement was carried on the thylakoid membranes. The thylakoid membranes with photosynthetic activity were isolated from the crude extract of the shoot tissue from control and treated plants according to (Ozalp *et al*., 2000). 500 mg tissue was weighed and homogenized with 4 mL of ice-cold grinding medium by mixer. All the steps were executed at 4ºC. The grinding medium consists of 0.33 M Sorbitol, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.1% BSA (Bovine Serum Albumin) as cold protectant of proteins and 50 mM HEPES-KOH (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer at pH 7.0. The homogenate was filtered. The filtrate was centrifuged at 1.300-x-g for six minutes. The supernatant was discarded and pellet was solubilized in 3 mL of suspension medium for washing. The pellet again was taken for centrifugation at 12.000-x-g for 10 min. The pellet at the end was solubilized in 200 µL of suspension medium (0.1 M Sorbitol, 50 mM $MgCl_2$, 50 mM HEPES-KOH buffer of pH 7.0). The assay of PS-II activity was carried on an equal quantity of control and treated samples. The amount of the thylakoid membranes was measured on the basis of chlorophyll amount. The PS-II activity of the isolated thalakoid membranes was determined according to the modified method of Chetti and Nobel (1987). Equal amount of thalakoid membranes based on the chlorophyll contents were assayed by using DCPIP (Dicholorophenol Indophenol) as an electron accepter of PS-II photosynthetic complex. 50~mg of chlorophyll containing thylakoid membranes were mixed in 2 mL reaction medium containing 40 mM Tris KOH buffer of pH=8.0, 5 mM $K_2HPO_4$, 10 mm KCl and 0.01% DCPIP. Illuminating the mixture with a 40 W incandescent lamp at 10 cm distance started the reaction. The spectrophotometric measurement at wavelength of 590 nm was taken after 20 s illumination for at least 3 min. The rate of PS-II activity was calculated as $\Delta A_{590}$/sec.

### Statistical Analysis

The data recorded for various parameters were analyzed with completely randomized design (CRD) three factor factorial analyses of variances technique using Statistix 8.1 software. For significant F-value, LSD was used for means comparison at 5% level (Steel *et al.,* 1997).

## Results

### Effects of Salinity on Manganese Concentration in Shoot and Root of Rice Cultivars

Mn concentration in shoot of rice varieties in relation to NaCl and Mn application showed significant difference with increasing NaCl levels, which decreased but increasing the Mn application had opposite effect and increase in the shoot Mn contents was observed (Fig. 1). Maximum Mn concentration was found at 8 µg Mn $mL^{-1}$ treatment at all the salinity levels. Both varieties showed same response.

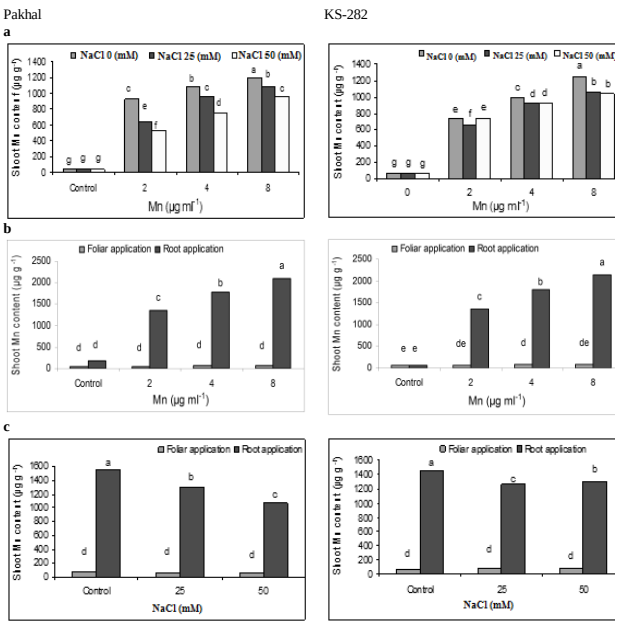


**Fig. 1:** Shoot Mn contents in relation to (a) NaCl and Mn treatment (b) method of application and Mn treatment (c) method of Mn application and NaCl in rice varieties Pakhal and KS-282. Means with different letters differs significantly ($p \leq 0.05$)

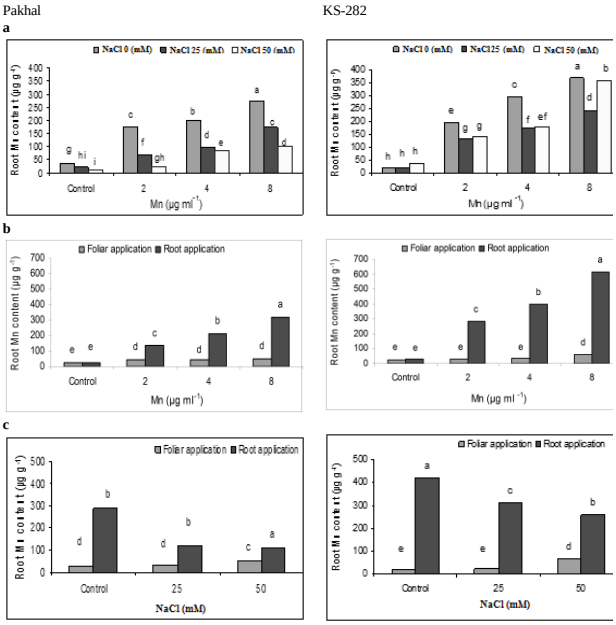


**Fig. 2:** Root Mn contents in relation to (a) NaCl and Mn treatment (b) method of application and Mn treatment (c) method of Mn application and NaCl in rice varieties Pakhal and KS-282. Means with different letters differs significantly ($p \leq 0.05$)

Mn contents in shoot in relation to methods of Mn application and treatment showed significant difference and higher accumulation of Mn was seen with increasing Mn with root application method, but very low and non-significant difference of Mn contents was seen in foliar applied method. The similar trend was observed in both varieties (Fig. 1b). Mn contents in relation to methods of Mn application and NaCl showed significant difference between two methods of Mn application (Fig. 1c). In root application method, increasing NaCl level significantly decreased Mn concentration in shoot but in KS-282 at 50 mM NaCl level there was a little increase in Mn concentration as compared to 25 mM NaCl. In foliar application, non-significant difference in Mn concentration was observed in both varieties.

Interactive effect of Pakhal and Mn application increased the Mn concentration with foliar application but was non-significant. At 0 mM NaCl level, maximum Mn i.e., 81.53 µg $g^{-1}$ was estimated for 8 mg Mn $L^{-1}$ level. For root application, the trend was much clear, increasing salinity decreased Mn and increasing Mn level. In root application method, at 0 mM NaCl level, the maximum Mn i.e., 2334.13 µg $g^{-1}$ was observed at 8 mg Mn $L^{-1}$ treatment. Almost same trend was observed in KS-282, where foliar application at 25 mM NaCl level, increased Mn concentration to 107.32 µg $g^{-1}$ at 4 µg Mn $mL^{-1}$ treatment, while with root application at 0 mM salinity level, the maximum Mn concentration was 2415.85 µg $g^{-1}$ at 8 µg Mn $mL^{-1}$ treatment (Table 1).

Significant difference of Mn concentration in the root of rice, in relation to salinity and Mn treatment showed that increasing NaCl inhibited the Mn uptake, however, increasing Mn level increased the Mn contents (Fig. 2a). At 50 mM NaCl, the maximum increase was observed at 8 µg Mn $mL^{-1}$ treatment. KS-282 as well as Pakhal showed same behavior. Significant difference of Mn concentration in roots of rice in relation to methods of Mn applied and Mn treatment was found (Fig. 2b). Increasing Mn application through roots up to 8 µg Mn $mL^{-1}$ increased Mn concentration, however by foliar application, increase in Mn concentration was very low and non-significant. Same trend was observed in both varieties (Fig. 2c).

Significant interactive effect of application methods, NaCl and Mn treatment on the Mn contents in root of two rice varieties is presented in Table 2. In case of Pakhal, for foliar application, the trend was significant but not uniform. At 50 mM NaCl level, maximum concentration i.e., 62.13 µg $g^{-1}$ was observed for 8 µg Mn $mL^{-1}$ treatment. In root application method, increasing NaCl level decreased Mn concentration however increasing Mn level increased Mn concentration in the root tissue. At 50 mM NaCl level, the maximum concentration i.e., 487.2 µg $g^{-1}$ was observed in 8 µg Mn $mL^{-1}$ treatment. KS-282 exhibited same trend for root application with increasing NaCl level decreased Mn contents but increasing Mn application increased Mn concentration. At 0 mM NaCl level maximum concentration was 487.2 µg $g^{-1}$ at 8 µg Mn $mL^{-1}$ treatment.

**Table 1:** Interactive effect of application methods, NaCl levels and Mn treatment on the Mn content (µg $g^{-1}$) in shoot of rice varieties

| Mn treatment (µg $mL^{-1}$) | Foliar application | | | Root application | | | Mean |
|---|---|---|---|---|---|---|---|
| | --------- NaCl level (mM)--------- | | | -------- NaCl level (mM)-------- | | | |
| | 0 | 25 | 50 | 0 | 25 | 50 | |
| Pakhal | | | | | | | |
| 0 | 43.23 g | 43.73 g | 38.80 g | 42.73 g | 42.43 g | 38.70 g | 41.40 D |
| 2 | 54.87 g | 65.70 g | 54.47 g | 1800.97 c | 1238.40 e | 982.13 f | 695.80 C |
| 4 | 72.00 g | 76.80 g | 71.60 g | 2084.87 b | 1834.27 c | 1446.77 d | 929.20 B |
| 8 | 81.53 g | 42.47 g | 71.93 g | 2334.13 a | 2106.80 b | 1838.13 c | 1084.90 A |
| Mean | 59.80 | | | 1315.90 | | | |
| *LSD (≤ 0.05) = Treatment., 20.57; Application, 14.54; NaCl level, 17.81; Treatment * Application* NaCl level, 50.38 | | | | | | | |
| *SE = Treatment., 10.22; Application, 7.22; NaCl level, 8.85; Treatment * Application* NaCl level, 25.03 | | | | | | | |
| KS-282 | | | | | | | |
| 0 | 61.43 j | 66.92 j | 66.76 j | 61.89 j | 65.83 j | 73.36 ij | 66.00 D |
| 2 | 66.10 j | 76.13 h-j | 77.43 h-j | 1411.73 e | 1220.06 f | 1408.64 e | 710.00 C |
| 4 | 71.22 ij | 107.32 g | 102.18 gh | 1903.29 c | 1730.10 d | 1753.25 d | 944.60 B |
| 8 | 76.33 h-j | 96.32 g-i | 74.83 h-j | 2415.85 a | 2027.09 b | 2001.97 b | 1115.40 A |
| Mean | 76.60 | | | 1339.4 | | | |
| *LSD (≤ 0.05) = Treatment., 11.58; Application, 8.19; NaCl level, 10.03; Treatment * Application* NaCl level, 28.38 | | | | | | | |
| *SE = Treatment., 5.75; Application, 4.07; NaCl level, 4.98; Treatment * Application* NaCl level, 14.10 | | | | | | | |

**Table 2:** Interactive effect of application methods, NaCl levels and Mn treatment on the Mn content (µg $g^{-1}$) in root of rice varieties

| Mn treatment (µg $mL^{-1}$) | Foliar application | | | Root application | | | Mean |
|---|---|---|---|---|---|---|---|
| | --------- NaCl level (mM)--------- | | | -------- NaCl level (mM)-------- | | | |
| | 0 | 25 | 50 | 0 | 25 | 50 | |
| Pakhal | | | | | | | |
| 0 | 11.58 k | 21.32 jk | 36.87 h-j | 11.78 k | 20.96 jk | 36.60 h-j | 23.19 D |
| 2 | 21.97 jk | 47.10 g-i | 59.93 g | 30.80 ij | 89.73 f | 292.05 c | 90.26 C |
| 4 | 39.53 hi | 30.61 ij | 52.83 gh | 340.47 b | 170.73 d | 161.70 d | 132.64 B |
| 8 | 49.77 gh | 31.53 ij | 62.13 g | 487.20 a | 298.37 c | 131.30 e | 176.72 A |
| Mean | 38.76 | | | 172.64 | | | |
| *LSD (≤ 0.05 ) = Treatment., 4.62; Application, 3.27; NaCl level, 4.00; Treatment * Application* NaCl level, 11.32 | | | | | | | |
| *SE = Treatment., 2.30; Application, 1.62; NaCl level, 1.99; Treatment * Application* NaCl level, 5.63 | | | | | | | |
| KS-282 | | | | | | | |
| 0 | 17.31 l | 21.75 kl | 38.82 kl | 22.03 jk | 21.69 kl | 37.99 jk | 26.60 D |
| 2 | 22.54 jk | 23.07 kl | 49.15 j | 369.21 e | 240.61 h | 237.88 h | 157.08 C |
| 4 | 24.87 kl | 25.97 kl | 52.32 j | 570.25 c | 323.55 f | 303.59 g | 216.74 B |
| 8 | 18.42 l | 29.16 kl | 132.73 i | 719.09 a | 457.32 d | 662.71 b | 336.57 A |
| Mean | 38.00 | | | 330.49 | | | |
| *LSD (≤ 0.05 ) = Treatment., 7.88; Application, 5.57; NaCl level, 6.82; Treatment * Application* NaCl level, 19.29 | | | | | | | |
| *SE = Treatment., 3.91; Application, 2.77; NaCl level, 3.39; Treatment * Application* NaCl level, 9.58 | | | | | | | |

For foliar application, increasing NaCl and Mn levels increased Mn concentration in the root tissues especially at 50 mM salinity, Mn concentration was 132.73 µg $g^{-1}$ for 8 µg Mn $mL^{-1}$ level.

**Effects on Chlorophyll Content**

Chlorophyll concentration was measured to characterize the isolated thylakoid membranes. Increasing NaCl decreased the chlorophyll contents in all the Mn treatments but significant increase over control was observed with increased Mn application in both varieties. Maximum chlorophyll contents were observed for 2 µg Mn $mL^{-1}$ treatment in both varieties (Fig. 3a). The chlorophyll contents in relation to methods of Mn application and Mn treatment showed significant difference between methods of Mn application and Mn treatments in Pakhal and non-significant difference in KS-282. Foliar application with increased chlorophyll contents was found better in both varieties (Fig. 3b).

Chlorophyll contents in relation to methods of application and NaCl significantly decreased in Pakhal and were non-significant in KS-282. More decrease was seen for root application as compared to foliar application in both varieties, which indicate that foliar Mn application is for the chlorophyll contents in the shoot (Fig. 3c).

Interactive effect showed that with increase in NaCl, decrease in chlorophyll contents was observed but increasing Mn application increased the chlorophyll contents. At 0 mM NaCl salinity, the maximum chlorophyll contents i.e., 2.55 mg $g^{-1}$ was observed for foliar applied and 2.48 mg $g^{-1}$ for root application at 2 µg Mn $mL^{-1}$ treatment and then gradual decrease due to toxic effect up to 8 µg Mn $mL^{-1}$ treatment observed but the Mn contents were better than 0 µg Mn $mL^{-1}$ treatment. In KS-282, interaction was non-significant.

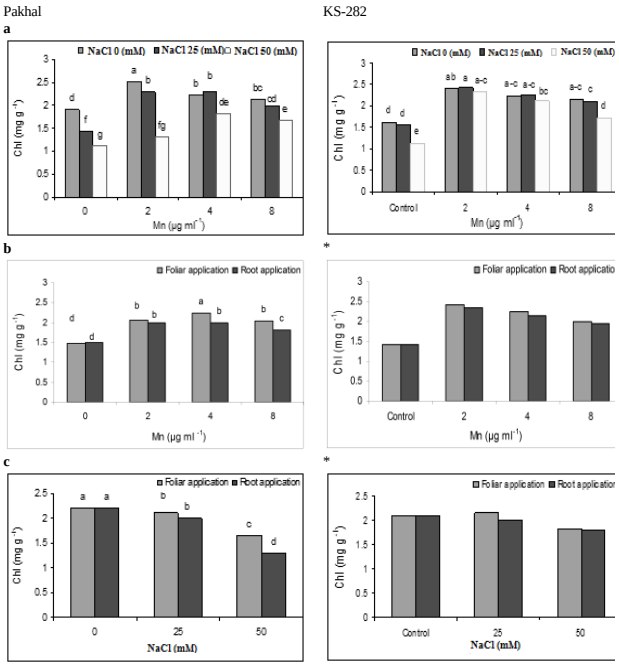


**Fig. 3:** Chlorophyll (Chl) contents in relation to (a) NaCl and Mn treatment (b) method of application and Mn treatment (c) method of Mn application and NaCl in rice varieties Pakhal and KS-282. Means with different letters differs significantly ($p \leq 0.05$)

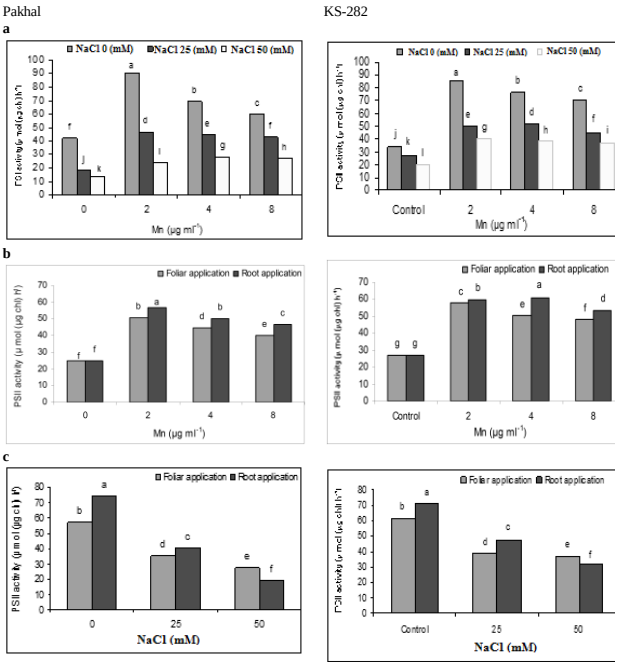


**Fig. 4:** PS-II activity in relation to (a) NaCl and Mn treatment (b) method of application and Mn treatment (c) method of Mn application and NaCl in rice varieties Pakhal and KS-282. Means with different letters differs significantly ($p \leq 0.05$)

### Effect on PS-II Activity

The PS-II activity was used as an indicator of total plant photosynthetic performance. Inhibition and recovery of PS-II activity under saline conditions and supplemental Mn showed significant difference with increased Mn concentration over control. On increasing NaCl level, the PS-II activity was inhibited significantly but increasing Mn application increased PS-II activity over control. The trend was same in both varieties and the maximum PS-II recovery was seen at 2 µg Mn $mL^{-1}$ treatment at all the NaCl levels except 50 mM NaCl in Pakhal.

PS-II activity in relation to methods of Mn treatment showed significant difference between two methods in both varieties (Fig. 4b). The maximum PS-II activity in relation to Mn treatment was measured with root application as compared to foliar but their interaction (Fig. 4c) was different and increasing NaCl inhibited PS-II activity. At 0 and 25 mM NaCl level root application showed enhanced PS-II activity but at high NaCl level i.e., 50 mM foliar applied method performed better with similar trend is same in both varieties.

Significant effect of NaCl and Mn application was seen in Pakhal and KS-282 (Table 4). In both methods of Mn application, increased NaCl inhibited the PS-II activity but increasing Mn application recovered the PS-II activity. At 0 mM NaCl, maximum PS-II activity in foliar i.e., 79.69 {µmol (µg chl) $h^{-1}$} and root application i.e., 102.1 {µmol (µg chl) $h^{-1}$} was observed at 2 µg Mn $mL^{-1}$ treatment. KS-282 showed same behavior, with maximum PSII activity recovery with foliar i.e., 80.88 {µmol (µg chl) $h^{-1}$} and root application i.e., 102.1 {µmol (µg chl) $h^{-1}$} observed at 0 mM NaCl and 2 µg Mn $mL^{-1}$ treatment.

## Discussion

The relationship between salinity and trace element nutrition is complex and salinity may increase, decrease, or have no effect on the micronutrient concentration in plant shoots. In saline and sodic soils, the solubility/availability of micronutrients (e.g. Cu, Fe, Mn, Mo and Zn) is particularly low, and plants grown in these soils often experience deficiencies of these elements (Page *et al*., 1990).

It was observed that salinity reduced the Mn uptake in shoots (Pandya *et al.,* 2004) as well as in roots of plants. Examples of decrease in Mn concentration under saline conditions include rice (Sultana *et al.,* 2002), maize (Salama, 2001), bean (Doering *et al*., 1984), corn (Izzo *et al*., 1991; Rahman *et al*., 1993), pea (Dahiya and Singh, 1976), squash, *Cucurbita pepo* L. (Maas *et al*., 1972), wheat (Sangwan *et al*., 2003) cucumber, *Cucumis sativus* L. (Soyergin and Moltay, 2002) and tomato (Alam *et al*., 1989). Mn application enhances the Mn concentration at all the salinity levels. Significant difference was observed

**Table 3:** Interactive effect of application methods, NaCl levels and Mn treatment on the chlorophyll content (mg $g^{-1}$) of rice varieties

| Mn treatment (µg $mL^{-1}$) | Foliar application | | | Root application | | | Mean |
|---|---|---|---|---|---|---|---|
| | ---------NaCl level (mM)--------- | | | --------- NaCl level (mM)--------- | | | |
| | 0 | 25 | 50 | 0 | 25 | 50 | |
| Pakhal | | | | | | | |
| 0 | 1.83 f | 1.46 g | 1.12 h | 1.97 ef | 1.45 g | 1.12 h | 1.49 D |
| 2 | 2.55 a | 2.17 d | 1.46 g | 2.48 ab | 2.39 a-c | 1.14 h | 2.03 B |
| 4 | 2.27 cd | 2.30 b-d | 2.18 d | 2.21 cd | 2.30 b-d | 1.46 g | 2.12 A |
| 8 | 2.14 de | 2.11 c-f | 1.87 ef | 2.11 de | 1.87 f | 1.46 g | 1.93 C |
| | 1.96 | | | 1.83 | | | |
| *LSD (≤ 0.05) = Treatment., 0.08; Application, 0.06; NaCl level, 0.07; Treatment * Application* NaCl level, 0.19 | | | | | | | |
| *SE = Treatment., 0.04; Application, 0.03; NaCl level, 0.03; Treatment * Application* NaCl level, 0.10 | | | | | | | |
| KS-282 | | | | | | | |
| 0 | 1.62 | 1.56 | 1.11 | 1.63 | 1.56 | 1.11 | 1.43 D |
| 2 | 2.43 | 2.50 | 2.34 | 2.38 | 2.37 | 2.33 | 2.39 A |
| 4 | 2.24 | 2.34 | 1.20 | 2.22 | 2.18 | 2.05 | 2.20 B |
| 8 | 2.10 | 2.25 | 1.66 | 2.17 | 1.93 | 1.78 | 1.98 C |
| | 2.03 | | | 1.98 | | | |
| *LSD (≤ 0.05 )= Treatment., 0.12; Application, 0.08; NaCl level, 0.10; Treatment * Application* NaCl level, ns | | | | | | | |
| *SE = Treatment., 0.06; Application, 0.04; NaCl level, 0.05 | | | | | | | |

**Table 4:** Interactive effect of application methods, NaCl levels and Mn treatment on the PS-II activity of rice varieties

| Mn Treatment (µg $ml^{-1}$) | Foliar application | | | Root application | | | Mean |
|---|---|---|---|---|---|---|---|
| | ---------NaCl level (mM)--------- | | | --------- NaCl level (mM)--------- | | | |
| | 0 | 25 | 50 | 0 | 25 | 50 | |
| Pakhal | | | | | | | |
| 0 | 42.26 h | 18.41 n | 13.51 o | 42.65 h | 18.32 n | 13.55 o | 24.78 D |
| 2 | 79.69 b | 43.07 h | 29.61 l | 102.10 a | 49.82 e | 18.15 n | 53.74 A |
| 4 | 59.37 d | 40.55 i | 33.97 j | 78.89 b | 49.59 e | 22.58 m | 47.49 B |
| 8 | 47.40 f | 40.57 i | 31.68 k | 72.57 c | 45.18 g | 22.53 m | 43.32 C |
| Mean | 44.66 | | | 40.00 | | | |
| *LSD (≤ 0.05) = Treatment., 0.34; Application, 0.24; NaCl level, 0.29; Treatment * Application* NaCl level, 0.82 | | | | | | | |
| *SE = Treatment., 0.17; Application, 0.12; NaCl level, 0.14; Treatment * Application* NaCl level, 0.41 | | | | | | | |
| KS-282 | | | | | | | |
| 0 | 33.88 p | 27.04 q | 20.32 r | 33.77 p | 27.16 q | 20.31 r | 27.08 D |
| 2 | 80.88 c | 45.19 j | 47.33 i | 90.34 a | 54.21 h | 33.75 p | 58.62 A |
| 4 | 67.69 e | 42.35 l | 40.59 m | 85.71 b | 60.85 g | 36.14 o | 55.55 B |
| 8 | 63.24 f | 42.75 k | 38.46 n | 76.68 d | 47.32 i | 36.06 o | 59.75 C |
| | 45.81 | | | 50.19 | | | |
| *LSD (≤ 0.05 )= Treatment., 0.14; Application, 0.10; NaCl level, 0.12; Treatment * Application* NaCl level, 0.35 | | | | | | | |
| *SE = Treatment., 0.07; Application, 0.05; NaCl level, 0.06; Treatment * Application* NaCl level, 0.17 | | | | | | | |

between two methods of application, as significant shoot and root Mn accumulation. In root application, Mn was found high as compared to foliar application, which revealed that despite the presence of quite high Mn concentration with root application, it fails to alleviate injury to PS-II at high salinity level i.e., 50 mM and thus photosynthesis was reduced.

Salinity brought about a significant reduction in chlorophyll concentration (Vigo *et al*., 2002), NaCl decreased total chlorophyll concentration in leaves (Lutts *et al*., 1996; Salama, 2001; Lee *et al*., 2004) and photosynthetic pigments decreased significantly (Hassanein, 2000). Mn application either through root or shoot improved chlorophyll concentration in the leaves but when compared between methods, foliar application was better than root application. Sultana *et al*. (2001) also observed that foliar spray of Mn partially minimize the salt induced nutrient deficiency and photosynthesis. Abadia *et al*. (1986) concluded that examining the chlorophyll-protein and peptide composition of manganese deficient and control sugar beet thylakoids and found decrease in manganese decreased several thylakoid polypeptides, including chlorophyll *b* containing 30 kDa chlorophyll-protein complex. Supplementing Mn improved the chlorophyll contents; foliar Mn application rescued more chlorophyll contents as compared to root application.

The PS-II activities were used as an indicator of total photosynthetic activities of plants. It is reported earlier that one of the primary sites of damage to the photosynthetic apparatus by environmental stress is located in PSII (Lu and Vonshak, 1999). Salinity significantly reduced net photosynthetic rate and stomatal conductance in olive plants (Vigo *et al*., 2002); seawater salinity also decreased the net photosynthetic rate in rice (Sultana *et al*., 2002). Photosynthetic capacity of many plant species is reduced in the presence of salinity, which is associated with stomatal

closure. Photosynthetic membranes are stress sensitive sites but little information is available regarding the structural changes associated with salt stress and the exact mechanisms of membrane damage and protection are still unknown (Maslenkova *et al*., 1995). It has been reported by (Ozalp *et al.,* 2000) that PSII values are affected adversely by NaCl concentration and duration of time. Mn is necessary for photosynthetic reactions to proceed normally, being part of the water-splitting enzymes of photosystem II. Thus the decrease Mn concentration in the shoot may limit these enzymes activity.

## Conclusion

The application of Mn as $MnSO_4$ had positive effect on chlorophyll contents and PS-II activity of rice plants under saline conditions. Sodium chloride toxicity could be partially alleviated by foliar application of Mn. It is quite intriguing to note that despite presence of quite high Mn concentration in case of root application the injury to PS-II could not be alleviated at high salt concentration.

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**(Received 29 December 2015; Accepted 29 April 2016)**