Influence of Manganese on Nutrient Uptake in Rice Plants under Saline Conditions

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT
Salinity-induced nutritional disorders adversely affect the performance of crops. The consequence of salinity disorders may result on nutrient availability, competitive uptake, transport or partitioning within the plants. Present study was aimed to investigate the effects of Mn application on nutrient uptake contributing to the salinity tolerance of the rice. A hydroponic experiment was carried out in National Agriculture Research Centre, Islamabad, Pakistan, on two rice varieties. Three NaCl salt concentrations, namely 0, 25, 50 mmol were used. Manganese sulphate was used for foliar and root application with four concentrations (0, 2, 4, 8 mg Mn L⁻¹). Salinity decreased the uptake of P, K, Ca, Mg, Mn and Zn in both applications methods while application of Mn increased the uptake of almost all the investigated macro and micronutrients. Root applications of Mn and foliar application methods were compared where root application method was found better for most of the nutrient uptake. Interaction effect i.e salinity × Mn treatment × methods of application was significant. The results showed that Mn application can improve the nutrient uptake capability contributing to the salinity tolerance in rice.

Keywords: Manganese; salinity; nutrient uptake; rice.

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1. INTRODUCTION

During growth and development, plants may face many stresses such as heat, drought, cold anaerobiosis and salt. Salinity is considered a major stress disturbing agricultural productivity damagingly especially in arid land, as many major crops show relatively less salt tolerant [1]. Salinity can be described as the occurrence of excessive number of soluble salts in the root zone or soil. The key contributory cations of salinity are Na+, Ca2+, Mg2+, K+ and anions are Cl−, SO42−, NO3−. Existence of trace ions such as B, Sr, Li, Rb, F, Mo, Ba, and Al also contribute toward salinity. This excessive amount of soluble salts in root zone weaken plant growth because salinity negatively affect the ability of plants to extract water [2].

Ions present in the soil solution needed to be transported into the root, crossing both cellular and organelar/organellar membranes and distributed all over in the plant parts, [3]. Crop performance may be adversely affected by salinity-induced nutritional disorders. These disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant. For example, soil salinity reduces phosphate uptake and accumulation in crops primarily by reducing phosphate availability but in solution cultures ion imbalances may primarily result from competitive interactions. Salinity dominated by Na+ salts not only reduce Ca2+ availability but reduces Ca2+ transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs. Salinity can directly affect nutrient uptake, such as Na+ reducing K+ uptake or by Cl− reducing NO3− uptake. Salinity can also cause a combination of complex interactions that affect plant metabolism, susceptibility to injury or internal nutrient requirement [4].

Micronutrients play many complex roles in plant nutrition and plant production. For example, Zinc and manganese function in many plant enzyme systems as bridges to connect the enzyme to the substrate upon which it is meant to act [3]. Manganese (Mn) is an important micronutrient for plant growth and development and sustains metabolic roles within different plant cell compartments. The metal is an essential cofactor for the oxygen-evolving complex (OEC) of the photosynthetic machinery, catalyzing the water-splitting reaction in photosystem II (PSII). Despite the importance of Mn for photosynthesis and other processes, the physiological relevance of Mn uptake and compartmentation in plants has been underrated [5]. The most-well-studied function in plant metabolism that depends on Mn is the water-splitting reaction in PSII, which is the first step of photosynthesis. This process requires the tetra-Mn cluster Mn4O2Ca to split two water molecules into four electrons, four protons, and molecular O2 [6]. The quantity of the Mn in the plant tissues is related to the growth rate [7].

In saline and sodic soils, the availability of micronutrients is particularly low, and the plants grown in the soils often experience deficiencies in these elements [8]. Salinity reduces the uptake of manganese (Mn) and induces a Mn deficiency in shoots of plants, which reduces the growth [9]. Mn deficiency can be a serious plant nutritional disorder in soils with high pH and high partial pressure of O2 (pO2) where the bio-availability of Mn can decrease far below the level that is required for normal plant growth [10]. Several studies also revealed that supplemental Mn plays an important role in the adaptive responses of plants under various environmental stresses [11].

Rice, the most important cereal crop in many parts of the world, is considered to be salt sensitive. Sensitivity of rice to salinity stress varies with the growth stage. In general, rice plants are very sensitive to salinity stress at young seedling stages and less at reproduction [12]. In contrast, rice is more salt tolerant at germination stage [13]. As Mn plays very important role in photosynthetic activity and other enzyme systems, the foliar application of this nutrient may reduce the adverse effect of stress conditions, influencing root growth and nutrient uptake capacity. Hence the objective of the study was, to explore the effect of Mn application through root as well as foliar on nutrient uptake contributing to the salinity tolerance in rice.

2. MATERIALS AND METHODS

A Hydroponic experiment was conducted at Land Resources Research Institute, NARC, Islamabad, to explore the effect of Mn application on nutrient uptake. Seeds of rice varieties Pakhal and KS-282 were surface-sterilized with 0.1% NaOCl for five minutes, washed thoroughly with several changes of distilled water. The seeds were soaked for 24 hours in a beaker of distilled water and then spread on trays containing sand thoroughly washed with distilled water. For growth, two weeks old, four seedlings per pot
were transplanted to 2 cm plugged holes in black painted pots containing nutrient solution without any sodium and manganese contents. Complete randomized design with three replicates was applied. All the pots and solution culture studies were conducted in a glass house having exhaust fans and no any other environmental control. Inside the glass house, maximum temperature ranges from 35-45°C, minimum temperature 15-20 °C and bright sunlight, with active photoperiod of 7-9 hours. Yoshida nutrient solution [14] at pH 5.0 was used. Applied manganese levels were 0, 2, 4, 8 mg Mn L\(^{-1}\) solution as MnSO\(_4\) for root application as well as foliar spray. The pH was adjusted every second day with 1 N KOH or 1 N HCl and nutrient solution was changed once a week. After two weeks salt stress was applied at the rate of 0, 25, 50 mmol NaCl with three increments. Plants were harvested after 36 days of transplantation and washed first with running tape water then with distilled water, blotted and air dried. Nutrients in the plant samples were analyzed by using wet digestion method. Digested samples were determined for Zn, Fe, Cu, Mn, Ca and Mg by using Atomic Absorption Spectrophotometer and Na and K by flame photometer. Phosphorus in the digested samples was determined according to the ammonium-vanadomoly bdate method [15]. The data was subjected to analysis of variance (ANOVA), and the mean differences were compared by Fisher’s LSD using Statistix 8.1 software (2015). Difference at \(P \leq 0.05\) was considered significant.

3. RESULTS AND DISCUSSION

A higher amount of Na\(^+\) and a higher Na\(^+\)/K\(^+\) ratio disrupt ion homeostasis and decrease the uptake of other nutrients. Salt stress increase Na\(^+\) content and decrease K\(^+\) content in plants with the increasing duration of stress because of salt-induced Na\(^+\) influx and K\(^+\) efflux. Mn supplementation decreased Na\(^+\) accumulation and increased K\(^+\) and Mn accumulation as well as improved ion homeostasis in both the roots and shoots under the salt-stress condition [11].

Salinity-mineral nutrient interaction in the soil solution, particularly P, K and the micronutrients, are controlled by the solid phase and concentrations are much lower than those in nutrient culture where concentrations of salts and nutrients are easily controlled over the course of an experiment. [13].

3.1 Phosphorus Uptake

Increasing salinity significantly decreased the uptake of P in both the methods of application in both varieties of rice but increase in Mn application showed significant increase in P uptake with maximum uptake in 2 mg Mn L\(^{-1}\) treatment in Pakal while in 4 mg Mn L\(^{-1}\) for KS-282. Two methods of application also showed significant difference in both varieties where Root application was found better. Interaction of salinity x Mn treatment x methods of application also presented a significant effect on P uptake with maximum uptake was recorded in 2 mg Mn L\(^{-1}\) at 0 mmol salinity level of root application for Pakal while in KS-282, it was 8 mg Mn L\(^{-1}\) at 0 mmol salinity level of root application (Table 1).

Interaction between salinity and phosphorus (P) nutrition of plants is complex. Salt concentration adversely affected the uptake of phosphorus by plants [9], shoot P reduced at elevating salinity level [16]. In most cases, salinity decreases the concentration of P in plant tissue but the results of some studies indicate salinity either increased or had no effect on P uptake. Plant-growing conditions, plant type and even cultivar play a large role in P accumulation. Most studies demonstrated that salinity increased tissue-P concentration in sand or solution cultures [13], not in soils. Phosphate concentration in solution cultures are often orders of magnitude higher than that in soil solutions (e.g.2 mM vs. 2 mM). Phosphate availability is reduced in saline soils not only because of ionic strength effects that reduce the activity of phosphate but also because phosphate concentrations in soil solution are tightly controlled by sorption processes and by the low-solubility of Ca-P minerals. Therefore, it is understandable that phosphate concentrations in field-grown agronomic crops decreased as salinity (NaCl or CaCl\(_2\)) increased [17]. Robert et al. [18] reported that in maize salinity stimulated P uptake; its translocation from the root to shoot, and accumulation in root tip cytoplasm. In rice plants, Mn application either through root or shoot enhanced the uptake of phosphorus which is an essential macronutrient and had positive effects on growth parameters.

3.2 Potassium Uptake

Data regarding K uptake of both rice varieties (Table 2) revealed a significant decrease with salinity increase in foliar as well as root application. Minimum K uptake was recorded at
50 mmol salinity level. Manganese application by root as well as foliar significantly increased the K uptake in both rice varieties with maximum uptake at 2 µg ml⁻¹ in Pakhal and at 8 µg ml⁻¹ Mn level in KS-282. Statistically significant difference in K uptake was observed between foliar and root application in Pakhal where maximum value was presented in foliar application but for KS-282 it was non-significant. Interactive effect (salinity × Mn treatment × method of application) was statistically significant where maximum K uptake was recorded at 2 µg ml⁻¹ in root application of Pakhal and at 8 µg ml⁻¹ Mn level in foliar application of KS-282 at 0 mmol salinity level. Maintenance of adequate levels of K is essential for plant survival in saline habitats. K nutrition is known to be disturbed under salt stress. The transcript level of several K transporter genes is changed, the deposition rate into growing cells is reduced, the concentration of K in the xylem, shoot and expanding tissue of the leaf reduced and K efflux from the root increased [16].

Potassium concentration in plant tissue, expressed on a dry mass basis, declines as the Na-salinity or as the Na/Ca in the root media is increased. Sodium-induced K deficiency has been implicated in growth and yield reductions of various crops [13]. A significant decrease in K uptake was observed with increasing salinity level [19]; shoot K decreased with elevating salinity level [15]. Subbarao et al. [20], Sultana et al. [21], Salama [22] and Othman et al. [23] also reported that K concentration in the plant tissue was reduced as the Na salinity in the root media was increased.

### 3.3 Sodium Uptake

Increase in salinity levels significantly elevated the sodium uptake in both varieties of rice through Mn foliar as well as root application. Although the sodium uptake was higher compared to control in Mn treatments but it deceased while increasing Mn concentration in Pakhal however in KS-282, the trend although was significant compared to control but it was non-significant among Mn treatments. Two rice varieties showed non-significant difference while interactive effect between salinity, Mn treatment and method of application was found significant in both rice varieties (Table 3). In plants, concentration of Na increases with the increase of salinity levels [3,24]. Under saline conditions, Na in the growth medium might compete with other cations such as K, Ca and Mg, among others, resulting in the low absorption of the latter by the roots, and significant increases in the Na concentration in the leaves [25]. Salinization induced increases in Na ion contents in shoot and root of rice seedling [26]. Haq et al. [27] reported that Na concentration increased significantly with an increase in salinity from 1.2 to 15 dSm⁻¹ and this increase was 13.3-fold as compared to Na in plants grown under non-saline conditions.

### 3.4 Calcium Uptake

Calcium plays an essential role in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behavior as well as cell wall enzyme activities [28,29]. Increase in salinity levels significantly decreased the Ca uptake by Mn foliar application as well root application in both rice varieties. Manganese treatments responded positively by significantly increasing the Ca uptake against control with maximum uptake at 2 µg ml⁻¹ in Pakhal and at 8 µg ml⁻¹ Mn level in KS-282. Both methods of Mn application behaved significantly different and root application was better in Pakahl and foliar application in KS-282. Interactive effect (salinity × Mn treatment × methods of application) was statistically significant where maximum Ca uptake was recorded at 2 µg ml⁻¹ in Pakhal in root application and at 8 µg ml⁻¹ Mn level in KS-282 in foliar application at 0 mmol salinity level (Table 4). Salinity dominated by Na salts not only reduces Ca availability but reduces its transport and mobility to growing regions of plant, affecting the quality of both vegetative and reproductive organs. Sodium chloride induce Ca-deficiency symptoms in several plant species, reduce Ca contents in plant tissues including leaves, leaf primordia and growing tissues of the leaf [18]. Under high levels of NaCl-salinity, calcium uptake and transport to all organs was significantly reduced [30,31]. Salinity reduced Ca uptake and concentration in barley [7], rice [21]. The hazard to crops, which are susceptible to Ca-related disorders even in the absence of salinity, becomes greater under saline conditions. As the salt concentration in the root zone increases, plant requirement for Ca also increases. At the same time, the uptake of Ca from the substrate may be depressed because of ion interactions, precipitation, and increases in ionic strength. These factors reduce the activity of Ca in solution thereby decreasing Ca availability to the plant. Severity of the calcium disorder depends on the kinds of ions that contribute to salinity and environmental conditions [4].
3.5 Magnesium Uptake

A significant decrease in Mg uptake with increase in salinity levels by both methods of Mn application in both varieties of rice is evident in Table 5. Increasing Mn concentration significantly increased the Mg uptake with maximum value at 2 μg ml⁻¹ level in both rice varieties. Methods of Mn application were significantly different from each other where in rice variety Pakhal, root application was found better and in KS-282 foliar application methods presented higher Mg uptake. Interactive effect between salinity, Mn treatment and methods of application was significant in Pakhal while non-significant in KS-282. Calcium is strongly competitive with Mg and the binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg than for Ca [29]. Salinity declined Mg concentration in barley [7] maize and barley [22]. According to Ruiz et al. [32] NaCl salinity reduced leaf Mg concentrations in citrus. However, increases in salinity are not always associated with decreases in leaf Mg. Neves-Piestun and Bernstein [18] found that increases in salinity (NaCl+CaCl₂) only reduced leaf Mg concentration in beet and had little or no effect in leaves from five other vegetable crops that they examined.

3.6 Micronutrients Status under Saline Conditions

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 of micronutrients (e.g. Cu, Fe, Mn, Mo and Zn) is particularly low, and plants grown in these soils often experience deficiencies in these elements [8].

3.7 Manganese Uptake

Main objective in this hydroponic study was to investigate the effect of Mn by different method of application under saline conditions. Manganese is involved in the oxidation reduction process in the photosynthetic transport system. Biochemical research shows that this element plays a structural role in the chloroplast membrane system, and also activates numerous enzymes. As availability of micronutrient is low under saline conditions, Mn uptake significantly decreased with increasing salinity levels, however increasing Mn application significantly elevated the Mn uptake and maximum value was recorded at 8 mg Mn L⁻¹ in application methods in both rice varieties (Table 6). Significant difference was observed between two methods of application where root application presented better Mn uptake compared to foliar application. Significant interaction between salinity, Mn treatment and methods of application was observed where maximum Mn uptake was observed in root application method at 0 mmol salinity in both rice varieties. It was observed that salinity reduced the Mn uptake in shoots [9] as well as in roots of plants. Examples of decrease in Mn concentration under saline conditions include rice [21] barley [7] maize [22], bean [33], corn [34,35] pea [36], squash, Cucurbita pepo L. [37], wheat [19] cucumber, Cucumis sativus L. [38] and tomato [39]. According to Cramer and Nowak [40] supplemental Mn improves the growth and Mn concentration of salt stress plant.

3.8 Zinc Uptake

Different behavior was observed in both rice varieties related to Zn uptake where increasing salinity increase Zn uptake in foliar application as well as root application in Pakhal while in KS-282 increasing salinity decreased Zn uptake (Table 7). Increasing Mn levels increased Zn uptake in both rice varieties with maximum uptake at 2 mg Mn L⁻¹ in Pakhal and 4 mg Mn L⁻¹ in KS-282. Methods of Mn application showed significant difference in Pakhal while it was non-significant in KS-282. Interactive effect between salinity, Mn treatment and methods of applications was significant in both varieties of rice where maximum uptake was observed at 2 mg Mn L⁻¹ in root application of Pakhal and at 4 mg Mn L⁻¹ in root application method of KS-282 at 0 mmol salinity. The majority of studies in the literature have shown salinity increased Zn concentration in shoot tissue such as in bean [33] citrus [32], maize [35] and tomato [37,41,42], but in other studies it has not affected [34] or actually decreased Zn concentration as in cucumber leaves [43]. Mn application enhanced the uptake of Zn which affected the growth parameters positively, because according to Fox and Guerinot [44] Zn is an essential catalytic component of over 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, Cu-Zn superoxide dismutase, and carbonic anhydrase. Zn also plays a critical structural role in many proteins.
Table 1. Response of Mn application through different methods on P (mg pot⁻¹) uptake in different rice varieties

| Treatment Mn (µg m⁻²) | Foliar application | Root application | Mean | Foliar application | Root application | Mean |
|-----------------------|--------------------|------------------|------|--------------------|------------------|------|
|                       | Pakhal             | KS-282           |      |                     |                  |      |
| Salinity level (mmol) |                    |                  |      |                    |                  |      |
| 0                     | 25                 | 50               | 0    | 25                 | 50               | 0    |
| Control               | 0.08                | 0.07             | 0.09 | 0.06               | 0.06             | 0.25 |
| 2                     | 13.98g              | 17.05d           | 12.79| 12.45e             | 12.18            | 16.95A|
| 4                     | 12.50i              | 15.25e           | 14.08e| 19.51c             | 16.77            | 15.32B|
| 8                     | 0.09j              | 12.60hi          | 14.22e| 22.54b             | 14.49ef          | 14.47C|
| Mean                  | 12.04              | 15.30            |      | 19.85A             | 14.07            | 12.25D|
| Foliar application    | 11.14E              | 12.91 C          | 12.06| 19.58A             | 12.06            | 12.25D|

LSD (0.05) Method = 0.58; Trt = 0.82; Method * Sal = 1.00; Method * Sal * Trt = 2.00

Table 2. Response of Mn application through different methods on K (mg pot⁻¹) uptake in different rice varieties

| Treatment Mn (µg m⁻²) | Foliar application | Root application | Mean | Foliar application | Root application | Mean |
|-----------------------|--------------------|------------------|------|--------------------|------------------|------|
|                       | Pakhal             | KS-282           |      |                     |                  |      |
| Salinity level (mmol) |                    |                  |      |                    |                  |      |
| 0                     | 25                 | 50               | 0    | 25                 | 50               | 0    |
| Control               | 0.86                | 0.67             | 0.714| 0.90               | 0.66             | 0.25 |
| 2                     | 13.98g              | 17.05d           | 12.79| 12.45e             | 12.18            | 16.95A|
| 4                     | 12.50i              | 15.25e           | 14.08e| 19.51c             | 16.77            | 15.32B|
| 8                     | 0.09j              | 12.60hi          | 14.22e| 22.54b             | 14.49ef          | 14.47C|
| Mean                  | 12.04              | 15.30            |      | 19.85A             | 14.07            | 12.25D|

LSD (0.05) Application = 0.36; Trt = 0.51; Application * Trt = 0.72; Application * Salinity * Trt = 1.25

Table 3. Response of Mn application through different methods on Na (mg pot⁻¹) uptake in different rice varieties

| Treatment Mn (µg m⁻²) | Foliar application | Root application | Mean | Foliar application | Root application | Mean |
|-----------------------|--------------------|------------------|------|--------------------|------------------|------|
|                       | Pakhal             | KS-282           |      |                     |                  |      |
| Salinity level (mmol) |                    |                  |      |                    |                  |      |
| 0                     | 25                 | 50               | 0    | 25                 | 50               | 0    |
| Control               | 0.08                | 0.07             | 0.09 | 0.06               | 0.06             | 0.25 |
| 2                     | 13.98g              | 17.05d           | 12.79| 12.45e             | 12.18            | 16.95A|
| 4                     | 12.50i              | 15.25e           | 14.08e| 19.51c             | 16.77            | 15.32B|
| 8                     | 0.09j              | 12.60hi          | 14.22e| 22.54b             | 14.49ef          | 14.47C|
| Mean                  | 12.04              | 15.30            |      | 19.85A             | 14.07            | 12.25D|

LSD (0.05) Method = 0.58; Trt = 0.82; Method * Sal = 1.00; Method * Sal * Trt = 2.00

Method = 1.47; Trt = 2.07; Method * Sal = 2.54; Method * Sal * Trt = 5.08
Table 4. Response of Mn application through different methods on Ca (mg pot\(^{-1}\)) uptake in different rice varieties

| Treatment                      | Foliar application Mean | Root application Mean | Method =0.09; Trt=6.10: Method*sal=7.48; Method *Sal*trt=14.95 |
|--------------------------------|--------------------------|------------------------|---------------------------------------------------------------|
|                               |                          |                        |                                                                 |
| Control                       | 0.35f                    | 0.37g                  | 0.36f                                                          |
| 2                             | 1.30d                    | 1.39e                  | 1.38d                                                          |
| 4                             | 2.35c                    | 2.41d                  | 2.39c                                                          |
| 8                             | 3.35b                    | 3.40c                  | 3.37b                                                          |
| Mean                          | 0.26B                    | 0.28D                  | 0.26B                                                          |
| Foliar application through different methods on Ca (mg pot\(^{-1}\)) uptake in different rice varieties

Table 5. Response of Mn application through different methods on Mg (mg pot\(^{-1}\)) uptake in different rice varieties

| Treatment                      | Foliar application Mean | Root application Mean | Method =0.34; Trt=0.48; Method*sal=0.58; Method *Sal*trt=1.17 |
|--------------------------------|--------------------------|------------------------|---------------------------------------------------------------|
|                               |                          |                        |                                                                 |
| Control                       | 0.35f                    | 0.37g                  | 0.36f                                                          |
| 2                             | 1.30d                    | 1.39e                  | 1.38d                                                          |
| 4                             | 2.35c                    | 2.41d                  | 2.39c                                                          |
| 8                             | 3.35b                    | 3.40c                  | 3.37b                                                          |
| Mean                          | 0.26B                    | 0.28D                  | 0.26B                                                          |
| Foliar application through different methods on Mg (mg pot\(^{-1}\)) uptake in different rice varieties

Table 6. Response of Mn application through different methods on Zn (µg pot\(^{-1}\)) uptake in different rice varieties

| Treatment                      | Foliar application Mean | Root application Mean | Method =ns; Trt=6.10: Method*sal=7.48; Method *Sal*trt=14.95 |
|--------------------------------|--------------------------|------------------------|---------------------------------------------------------------|
|                               |                          |                        |                                                                 |
| Control                       | 0.35f                    | 0.37g                  | 0.36f                                                          |
| 2                             | 1.30d                    | 1.39e                  | 1.38d                                                          |
| 4                             | 2.35c                    | 2.41d                  | 2.39c                                                          |
| 8                             | 3.35b                    | 3.40c                  | 3.37b                                                          |
| Mean                          | 0.26B                    | 0.28D                  | 0.26B                                                          |
| Foliar application through different methods on Zn (µg pot\(^{-1}\)) uptake in different rice varieties

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### Table 7. Response of Mn application through different methods on Cu (µg pot⁻¹) uptake in different rice varieties under saline conditions

| Treatment             | Pakhal                  | Mean | KS-282                  | Mean |
|-----------------------|-------------------------|------|-------------------------|------|
|                       | Foliar application      | Root application | Foliar application | Root application |
|                       | Salinity level (mmol)   |      | Salinity level (mmol)   |      |
|                       | 0          25   50   |      | 0          25   50   |      |
| Control 23.78±k       | 28.74±k     | 38.34±h     | 25.66±k     | 20.69±j     | 22.95±k      | 26.70±C     | 24.62±j     | 24.05j      | 25.87±j     | 25.72±j     | 26.85±g      | 26.37±g      | 26.21B |
| 2 41.73±g 64.02±f | 51.68±f   | 64.24±e   | 63.41±d   | 57.10±e   | 54.03±B     | 48.29±e     | 33.50±j   | 57.59±b   | 38.61±i   | 36.97±e   | 41.61±d-g   | 42.76A      |
| 8 33.95±i 49.60±f | 49.55±f   | 61.81±c   | 64.07±c   | 51.84±e   | 52.14±B     | 50.19±e     | 48.42±e   | 62.14±a   | 56.80±a-b | 38.85±i   | 27.36±h-j   | 47.29A      |
| Mean 42.94±B 56.40±A | 48.02B  | 56.94±A | 58.64±A   | 42.31C     | 25.87±j     | 35.87±j   | 37.35±A   | 49.70±B-C | 36.09±C   | 35.25C     |               |

LSD (0.05)= Method =8.63; Trt=2.46; Method*sal=2.84; Method *Sal*Trt=4.93

### Table 8. Response of Mn application through different methods on Mn (µg pot⁻¹) uptake in different rice varieties

| Treatment             | Pakhal                  | Mean | KS-282                  | Mean |
|-----------------------|-------------------------|------|-------------------------|------|
|                       | Foliar application      | Root application | Foliar application | Root application |
|                       | Salinity level (mmol)   |      | Salinity level (mmol)   |      |
|                       | 0          25   50   |      | 0          25   50   |      |
| Control 65±h 39±h     | 48±h       | 45±h     | 51±d       | 50±h       | 88±i   | 95±i     | 95±i      | 94±k      | 110±i     | 95±i      | 98D |
| 2 128±k 109±h 111±h  | 6067±b     | 3338±d  | 1245±g   | 1831C     | 182±k | 152±l | 159±l  | 285±f     | 233±g      | 235±g      | 1339C |
| 4 148±h 120±h 124±h  | 6016±b     | 3787±c  | 2359f   | 2092B     | 202±j  | 215±i | 171±l  | 417±b     | 314±d      | 301±e      | 182B |
| 8 133±h 127±h 138±h  | 6319±a     | 3853±c  | 2516±e  | 2163A     | 185±k  | 212±i | 177±l  | 746±a     | 396±e      | 194±h      | 2257A |
| Mean 107±B 9262±A     |               |      |        | 1613B     |       | 2596±A |          |          |           |          |
| Mean 118D 102D 98D   | 4615A 2730B | 1541C |       | 165±D | 167±D | 150D   | 3646±A   | 2287±B | 1855C |

LSD (0.05)= Method =26.97; Trt=47.40; Method*sal=54.74; Method *Sal*Trt=94.81

Method =26.81; Trt=37.92; Method*sal=46.44; Method *Sal*Trt=92.89

### Table 9. Response of Mn application through different methods on Fe (µg pot⁻¹) uptake in different rice varieties

| Treatment             | Pakhal                  | Mean | KS-282                  | Mean |
|-----------------------|-------------------------|------|-------------------------|------|
|                       | Foliar application      | Root application | Foliar application | Root application |
|                       | Salinity level (mmol)   |      | Salinity level (mmol)   |      |
|                       | 0          25   50   |      | 0          25   50   |      |
| Control 54±n 56±m 68±f | 53±m   | 51±m    | 62±k-m   | 54±C        | 71±h   | 76±j   | 90±g    | 65±l     | 89±k     | 9±2      | 81C  |
| 2 85±i 128±d 117±e-h | 198±a     | 158±c  | 78±j    | 127±A     | 127±i  | 113±l  | 154±d-f | 170±c    | 156±d-f  | 175±bc  | 149B |
| 4 86±i 122±e 115±h  | 171±b     | 187±d-f | 120±e   | 123±AB    | 155±df  | 129±h  | 165±c-e | 188±ab   | 140±h    | 176±bc  | 159A |
| 8 73±k 106±h 108±gh  | 180±b     | 126±f   | 135±d   | 121±B     | 151±-g  | 204±a  | 114±l   | 195±a    | 135±gh   | 123±h   | 154AB  |
| Mean 93±B          | 122±A     |       | 116±B   | 99±C      | 126±C  | 131±C  | 131±C   | 154±A    | 130±C    | 142B    |

LSD (0.05)= Method =3.72; Trt=5.26; Method*sal=6.43; Method *Sal*Trt=12.87

Method =5.17; Trt=7.31; Method*sal=8.96; Method *Sal*Trt=17.91
3.9 Copper Uptake

As evident in Table 8, increasing saline conditions did not impact uniformly in foliar application although the difference was significant but in root application, increase in salinity levels significantly decreased Cu uptake. Increasing Mn treatments presented significant positive response on Cu uptake with in Pakhal the maximum uptake was at 2 mg Mn L⁻¹ and 8 mg Mn L⁻¹ in KS-282. Root application method was found significantly better compared to foliar. Interactive effect i.e salinity x Mn treatment x methods of applications was also significant in both varieties of rice where maximum uptake was recorded at 2 mg Mn L⁻¹ of root application in Pakhal and 8 mg Mn L⁻¹ of foliar application in KS-282. Leaf and shoot Cu concentration decreased in salt-stressed maize grown in soil [11,45] and solution cultures [34] but NaCl-salinity substantially increased leaf Cu in hydroponically-grown tomatoes. As Cu is an essential redox component required for a wide variety of processes, including the electron transfer reactions of respiration (cytochrome c oxidase, alternate oxidase) and photosynthesis (plastocyanin), the detoxification of superoxide radicals (Cu-Zn superoxide dismutase) and lignification of plant cell walls (laccase) [46].

3.10 Iron Uptake

Table 9 presents the significant difference in the Fe uptake in the rice in relation to salinity and Mn treatment. Increasing salt level increased Fe uptake in foliar application but it decreased in root application. Increasing Mn application levels significantly enhanced Fe uptake with maximum value at 2 mg Mn L⁻¹ level in Pakhal and at 4 mg Mn L⁻¹ in KS-282. Root Mn application method was found significantly better compared to foliar application in both rice varieties. Interaction between salinity, Mn treatment and methods of applications was also significant in both varieties where maximum Fe uptake was recorded at 2mg Mn L⁻¹ level for Pakhal and 4 mg Mn L⁻¹ level in KS-282 at 0 mmol salinity level. Reports on the influence of salinity on the iron (Fe) concentration in plants are as inconsistent as those that concern Zn and Cu concentration. Salinity was demonstrated to increase, decrease or have no effect on leaf Fe contents under conditions which have reduced leaf growth [4]. Salinity increased the Fe concentration in the shoots of pea [36], tomato, soybean, Glycine max (L.) Merrill, squash [37], maize [18] and decreased its concentration in the shoots of barley and corn [47].

4. CONCLUSION

Rice is the most important but salt sensitive cereal crop in the world. Salinity reduces the uptake of essential nutrients for the growth. As Mn plays very important role in photosynthetic activity and other enzyme systems, its application reduces the adverse effect of stress conditions and enhance the nutrient uptake capacity contributing to the salinity tolerance in rice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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