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Effect of Foliar Application of Manganese on Plant Growth, Nodulation and Biochemical Attributes of Mungbean (*Vigna radiata* L.) under Salinity Stress

Swati Shahi, Malvika Srivastava

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ABSTRACT

Background: Salinity is one of the considerable factors which wanes crop productivity especially in arid and semi-arid realms of the world. The stress created by high soil salinity can cause osmotic stress, specific ion toxicity, nutritional imbalance, hormonal dysfunction and oxidative damage. A study was carried out to determine the effect of manganese (Mn) on growth rate index (GRI), Nodulation status, total nitrogen content, total amino acid content and total protein content of mungbean plants under salinity.

Methods: Three indices were used to evaluate the effect of salt stress on development of mungbean plants (100, 200 and 300 mM NaCl). Untreated plants served as control expect. Mn was supplied to the plants in form of manganese chloride ($MnCl_2$). The plant samples were analyzed for 65 days at every 10-day interval.

Result: The results revealed that low level of salinity (100 mM NaCl) showed a significant increment in all the above observed parameters, while higher concentrations (200 mM and 300 mM) decreased the mentioned attributes. Foliar spraying with Mn (0.15%) mitigated the deleterious impacts of salinity and enhanced growth, nodulation and biochemical parameters. Thus, foliar treatment with Mn can be used in increasing the tolerance capacity of the plant and to enhance the nitrogen fixing ability of the plant under salinity.

Key words: Amino acid, Manganese, Nitrogen, Nodulation, Plant growth, Protein, Salinity stress.

INTRODUCTION

India is the largest producer, consumer and processor of the pulses in the world (Srivastava *et al.,* 2010) and accounts for about 65% of the world acreage and 54% of the world production of mungbean crop (Sehrawat *et al.,* 2013). It is the third most important pulse crop in India, occupying nearly 3.72 million ha area with 1.56 million tons production (Ali and Gupta, 2012). Besides being rich in dietary protein content,the symbiotic association of mungbean roots and Rhizobia reduces the cost for nitrogen fertilizers (Limpens and Bisseling, 2003).

Plants often experience abiotic stress like salinity, drought, high or low temperature, flooding, metal toxicity, ozone, UV-radiations, herbicides, *etc.*, which pose serious threat to the crop production (Ahmad and Prasad, 2012). Of the various abiotic stresses, soil salinity is a global issue that limits the growth and productivity of plants that causes considerable crop losses (Ashraf *et al*., 2008). Although soil salinity occurs predominantly in arid and semiarid regions, it has been found in all the climatic zones (Munns and Tester, 2008). Abiotic stresses severely reduce the productivity of almost all pulses, including mungbean (Hasanuzzaman *et al*. 2013).

Soil salinity may disrupt symbiotic N_2-fixation systems in several ways. Salts can limit nodule formation by reducing the population of *Rhizobium* in the soil or by impairing their ability to infect root hairs (Zahran, 1999). The direct effects of salinity on the host plant can limit N fixation, independent of the effects of salinity on the Rhizobium bacteria and the nodulation process (Keck *et al*.,1984; Fageria, 1992). Saline conditions may affect the legume-*Rhizobium* symbiosis by reducing the survival of rhizobacteria, inhibiting the infection process, affecting nodule development and function and reducing plant growth (Singleton and Bohlool, 1984).

Salinity stress disturbs the normal uptake and distribution of essential nutrients in plants. Salt stress causes an induction or inhibition of some polypeptides in the leaves and the changes in protein synthesis can be determined as new synthesis, complete loss, increase or decrease.

Status of mineral nutrient in plants play an important role in improving the confrontation to any environmental perturbation like water stress, salt stress and heavy metal stress *etc.* Application of some plant micronutrients has

Plant Physiology and Biochemistry Laboratory, Department of Botany, Deen Dayal Upadhyay Gorakhpur University, Goarkhpur-273 009, Uttar Pradesh, India.

Corresponding Author: Swati Shahi, Plant Physiology and Biochemistry Laboratory, Department of Botany, Deen Dayal Upadhyay Gorakhpur University, Goarkhpur-273 009, Uttar Pradesh, India. Email: swatishahi06@gmail.com

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increased the salt tolerance of many crop plants (Tawfik *et al*., 2013; El-Fouly *et al*., 2011).

Manganese (Mn) is an essential plants nutrient involved in vital metabolic processes including photosynthesis, respiration, amino acid biosynthesis, activation of phytohormones, *etc* (Brunell, 1988). It also participates in the biosynthetic pathway of isoprenoids (Lidon *et al*., 2004) and assimilation of nitrate (Ducic and Polle, 2005).

The present study aimed to investigate the effect of manganese in improving the salinity tolerance of mungbean plants with respect to plant growth, nodulation and certain biochemical attributes.

MATERIALS AND METHODS

This study was carried out in the Department of Botany, DDU Gorakhpur University, Gorakhpur during the year 2017-18. Intact seeds, which were homogeneous and identical in size and colour and free from wrinkles, were chosen and sterilized with ethyl alcohol. Sterilized seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibition, seeds were inoculated with 96 h grown Rhizobacteria, placed in Petri plates and were kept in Growth chamber under controlled conditions (Temp. 25±2°C). After 3 days of germination, seedlings were transferred to earthenware pots containing 5 kg of acid washed sand. The whole experiment was conducted in completely randomized block design (CRBD) with 3 replications per treatment. Three salinity levels of 100 mM NaCl, 200 mM NaCl and 300 mM NaCl were prepared by dissolving sodium chloride in distilled water and used as treatment to impose salt stress. The control treatment was without sodium chloride. All the plants were watered every day and Hoagland's nutrient solution was applied to the plants at every 5-day interval. Foliar application of microelement (Mn) was done at 15, 30 and 45 days of plant growth in form of $MnCl_2$ (0.15%) prepared by dissolving $MnCl_2$ in double deionized water.

Plant growth in terms of GRI (growth rate index), nodulation (number, fresh weight, color and activity) and biochemical attributes (nitrogen, amino acid and protein contents) were measured at 25 days to 65 days after transplanting. GRI was measured by the amount of plant growth in terms of dry biomass in a specified time period and was calculated by difference of initial and final biomass. Nitrogen fixation by nodules was done following the method of Akkermans *et al*., (1978). Section of nodule was placed in 0.1% T.T.C (Triphenyltetrazolium chloride) solution for 4 hours and production of red coloured Formazon crystal was noted under the microscope.

Plant samples were oven dried at 60±2°C and were used in estimation of biochemical components. Total nitrogen content was estimated by the method of Doneen (1932). For total nitrogen 50 mg dried sample was powdered and kept in boiling test tube. To each fraction, (insoluble and total) 1.0 ml concentrated sulphuric acid (containing 5% salicylic acid, w/v) was added in digestion tube. It was then heated gently over a hot plate until fumes appeared. A small pinch of sodium thiosulphate ($Na_2S_2O_3$) was added to above heated mixture. The digestion tube was cooled to room temperature and then 1.0 ml of perchloric acid (containing 0.1% $CuSO_4.5H_2O$ w/v) was added. The tubes were again heated on the hot plate placed behind an exhaust fan for a period till the content become clear. Each digested sample which contained nitrogen as ammonium sulphate was cooled and diluted to 100 ml with distilled water. To 1.0 ml of this solution 1.0 ml Nessler's reagent was added. The absorbance of pale yellow colour so developed was measured at 440 nm.

The total free amino acids were estimated by the method of Yemm and Cocking (1955). 50 mg of dried leaves were crushed in 10 ml of 80% ethanol. The extract was centrifuged at 10000 rpm for 10 minutes. and for each 2.0 ml of supernatant, 2.0 ml of Ninhydrin (1% w/v prepared in isopropanol) was added. The mixture was heated on water bath for 20 minutes followed by cooling. The volume of violet colour solution so developed was diluted with 5 ml aqueous isopropanol. The intensity of violet colour was measured at 570 nm.

For measurement of protein content in dried leaves the amount of insoluble nitrogen fraction, as obtained by microkjeldahl digestion method was multiplied by a factor of 6.25.

Means of three replicates as well as their standard deviation (SD) from mean were determined. The test of significance between the treatments was done using atwo-way analysis of variance (ANOVA) and Least significance difference (LSD) has been calculated for the data where F-test was found significant.

RESULTS AND DISCUSSION

Results presented in Fig 1 showed that higher levels of salinity decrease Growth Rate Index throughout the experiment. It was found that the general trend of the treatment reflects a gradual decrease in the GRI with the increase of salt concentration, compared with the plants of the control experiment, except for the 100 mM treatment, which did not lead to the decrease in the GRI of the plants. Gupta and Huang (2014) reported that one of the initial effects of salinity on plants was the reduction of growth rate. Our results also matched with those of Neto *et al*., (2004). Crop growth rate decreases in abiotic stress condition because of increase in respiration and decrease of photosynthesis (Goldani and Rezvani, 2007).

However, when Mn was applied to the plants GRI increased and maximum increment was found in 200 mM NaCl treated plants. This was due to increase in biomass of mungbean plants under the influence of manganese under salinity (Shahi and Srivastava, 2016).

Nodulation status was observed in terms of number of nodules (Table 1), fresh weight of nodules (Table 2), colour of nodules (Table 3) and nitrogen fixing ability (Table 4). 200 mM and 300 mM NaCl treatment caused a significant decline in all the nodulation parameters. But there was a slight increment recorded in 100 mM NaCl treated plants as compared to the control sets. Our results are in conformity with those obtained by Younesi and Moradi (2015) in alfalfa

plants and Song *et al*. (2017) in soyabean. However, the parameters significantly increased with application of foliar spray of manganese under salinity and maximum increment was observed in 200 mM NaCl treated plants when sprayed with Mn. This may be due to the fact that optimal levels of manganese increase the uptake of copper (Malvi, 2011) and also due to its role in respiratory proteins that are required for N_2 fixation in *Rhizobia* (Delgado *et al*., 1998).

Salt stress treatment showed a marked decline in total nitrogen content in mungbean plants. The extent of retardation enhanced drastically with the progressive increase in salt concentrations (Fig 2). Raptan *et al*., (2001) reported that salinity decreased total nitrogen in mungbean plants. Elevated salinity has shown a decrease in leaf nitrogen concentration in *Gazania* (García-Caparrós *et al*., 2016). However, total nitrogen content enhanced on foliar application with Mn and maximum increment was observed

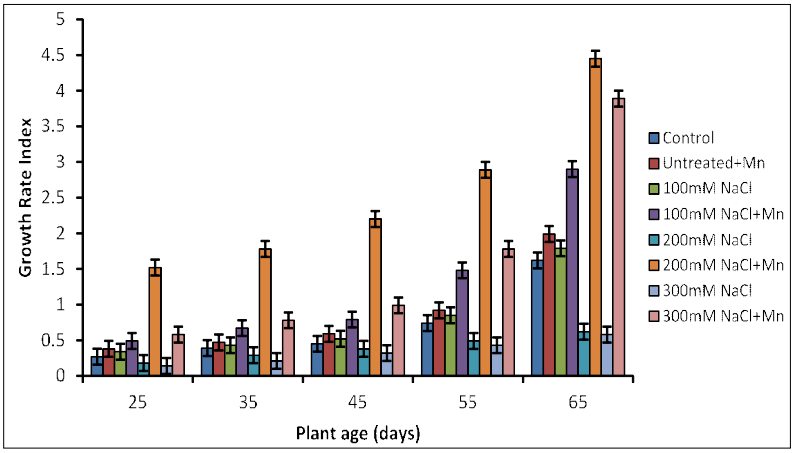

Fig 1: *Vigna radiata*: Growth rate index at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

Table 1: *Vigna radiata*: Number of nodules at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

Treatments	Number of nodules per plant				
	Plant age (days)				
	25	35	45	55	65
Control	33.67±3.78*	43.33±2.69*	48.33+4.52*	35.66±3.99*	28.67±1.98*
Untreated+ Mn	62.33±4.29	79.66±5.44	91.33±5.89	69.00±2.98	59.67±3.99
100 mM NaCl	38.67±4.76	47.33±4.99	50.67±5.82	41.67±4.43	32.66±3.29
100 mMNaCl +Mn	77.00±4.98	86.66±5.29	110.66±5.99	79.67±3.66	61.67±2.98
200 mM NaCl	21.33±2.99	27.33±3.68	33.66±2.83	25.67±3.88	18.33±3.66
200 mMNaCl +Mn	98.00±4.90	132.66±5.77	146.33±7.06	110.67±5.0	70.67±4.54
300 mM NaCl	10.00±1.51	17.33±2.98	22.67±2.67	14.00±1.66	8.55±1.22
300 mMNaCl + Mn	89.00±3.89	107.66±4.66	122.0±5.43	97.00±5.77	63.66±3.67

* Mean ± standard deviation (n=3).

Table 2: *Vigna radiata*: fresh weight of nodules at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

Treatments	Fresh weight of nodules (g)				
	Plant Age (days)				
	25	35	45	55	65
Control	0.174±0.10*	0.205±0.12*	0.257±0.17*	0.289±0.13*	0.316±0.16*
Untreated + Mn	0.257±0.10	0.399±0.15	0.522±0.22	0.579±0.24	0.629±0.28
100 mM NaCl	0.208±0.17	0.243±0.15	0.296±0.11	0.355±0.15	0.397±0.11
100 mMNaCl +Mn	0.377±0.14	0.470±0.18	0.633±0.25	0.690±0.30	0.732±0.33
200 mM NaCl	0.096±0.15	0.121±0.07	0.154±0.09	0.172±0.10	0.190±0.08
200 mMNaCl +Mn	0.759±0.37	0.884±0.40	1.09±0.42	1.15±0.41	1.19±0.37
300 mM NaCl	0.074±0.10	0.093±0.05	0.111±0.04	0.139±0.07	0.146±0.05
300 mMNaCl + Mn	0.475±0.21	0.594±0.34	0.725±0.29	0.845±0.32	0.916±0.30

* Mean ± Standard deviation (n=3).

with 200 mM NaCl treated plants. Plausible reasons for increment in nitrogen content may be attributed to the fact that Mn increases the nodulation status and nitrogen fixing ability (Orji *et al*., 2018).

Table 3: *Vigna radiata*: Colour of nodules at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

Treatments	Colour of nodules				
	Plant age (days)				
	25	35	45	55	65
Control	LP	LP	P	LP	LP
Untreated + Mn	LP	P	P	P	LP
100 mM NaCl	LP	P	P	P	LP
100 mMNaCl +Mn	LP	P	P	P	LP
200 mM NaCl	B	B	B	B	B
200 mMNaCl +Mn	LP	P	P	P	P
300 mM NaCl	B	B	B	B	B
300 mMNaCl + Mn	LP	P	P	P	P

W = white, LP = Light pink, P = pink.

Table 4: *Vigna radiata*: Triphenyltetrazolium chloride (TTC) test of nodules at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

Treatments	TTC test				
	Plant age (days)				
	25	35	45	55	65
Control	+	++	+	+	+
Untreated + Mn	++	++	+++	++	+
100 mM NaCl	+	++	++	++	+
100 mMNaCl +Mn	++	+++	+++	++	++
200 mM NaCl	+	+	+	+	+
200 mMNaCl +Mn	++	+++	+++	+++	++
300 mM NaCl	+	+	+	+	+
300 mMNaCl + Mn	++	+++	+++	++	++

(+) = low, (++) = moderate, (+++) = high.

Results presented in Fig 3 showed that increasing salinity decreased the total amino acid content of the plant at all observations. However, an increase was observed in amino acid content in 100 mM salt exposed plants. Decrease in amino acid content with salinity was also observed by Chakrabarti *et al*., (2003) and Dhingra *et al.,* (1993) in mungbean plants. This notable decrease in amino acid content, found in this study as a result of the treatment with increased concentrations of NaCl, could be explained by the negative effect of salt on amino acid synthesis (Angell *et al*., 2015). Foliar application of Mn increased total amino acid content at all levels of stress as compared to control. However, maximum increase was observed in plants with 200 mM NaCl stress. Foreseeably, this might be due to availability of nitrogen and other necessary elements in influence of Mn.

Compared with control, NaCl solution of 200 and 300 mM significantly reduced total protein content in mungbean plants. However, protein content increased in 100 mM NaCl treated plants (Fig 4). Jamil and Rha, (2013) reported that there was an increase in the concentration of total protein with the corresponding increase in NaCl level upto 100mM in mustard. Mohsan *et al*., (2013) studied the changes in protein metabolism induced by salinity in *Vicia faba* and reported that low salinity stimulated protein accumulation over control. Kumar *et al.,* (2018) stated that in chickpea cultivars, Protein content decreased with the increase in salinity stress. However, when $MnCl_2$ was applied, maximum protein content was obtained in 200 mM NaCl treated plants. Tawfic *et al*. (2013) recorded that moderate concentration of sea water increased the crude protein in *Leptochloa fusca* while foliar application of Mn positively affected the protein content. Jabeen and Ahmad (2011) reported a reduction in total protein with the increasing levels of salinity but foliar spray of Mn enhanced the total protein content in leaves. This is quite possible due to role of manganese in activation of RNA Polymerase enzyme which helps in synthesis of RNA transcript which ultimately forms proteins (Nagamine *et al*., 1978).

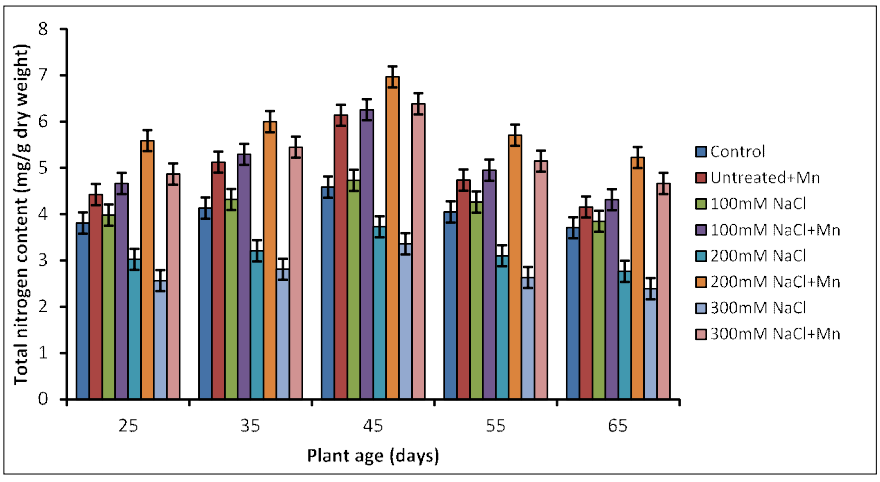

Fig 2: *Vigna radiata*: Total nitrogen content at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

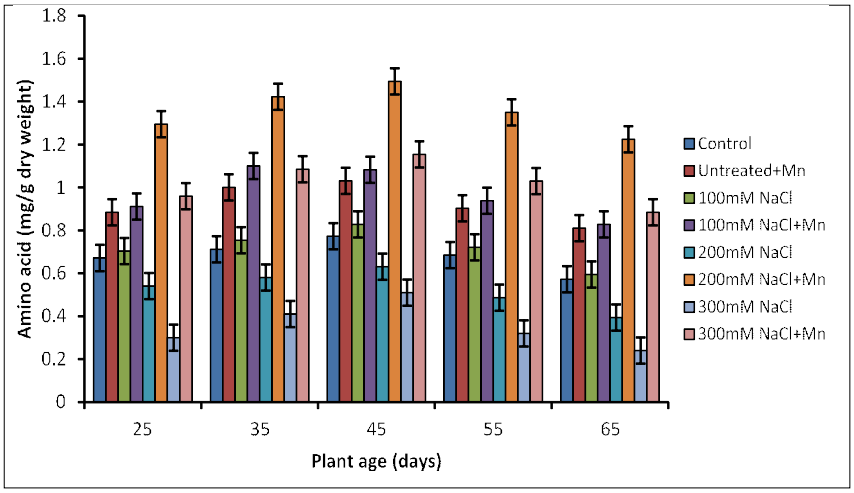

Fig 3: *Vigna radiata*: Total amino acid content at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

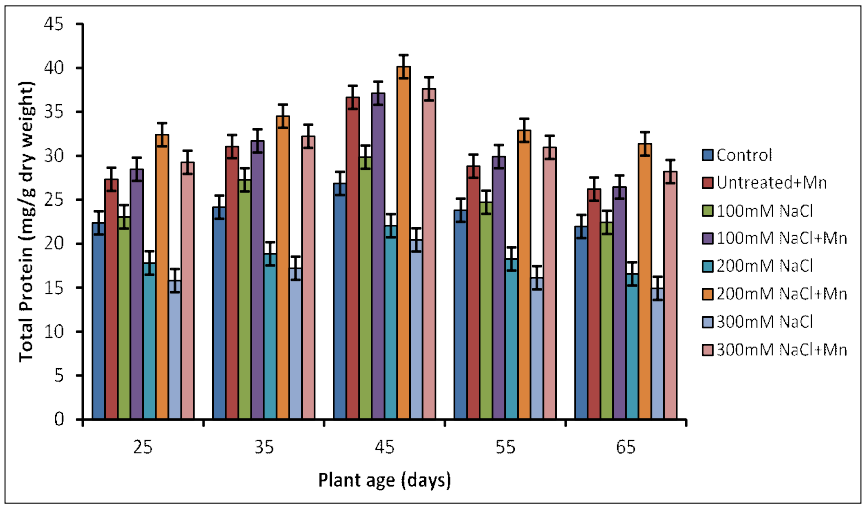

Fig 4: *Vigna radiata*: Total protein content at different days of plant growth under different salinity levels alone and in combination with $MnCl_2$.

CONCLUSION

From the present research, we present our current understanding about effects of soil salinity on mungbean crop and the approaches used to increase the tolerance of this crop towards salinity. Growth rate index, nodulation status and all the biochemical parameters decreased at high salt concentrations. Mn helped in better fixation of nitrogen which in turn improved the nitrogen status of plants. Better nitrogen availability enhanced the production of amino acids and protein. These proteins may be responsible for providing stress tolerance in mungbean plants.

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Conflict of interest: None.

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