



Improving salt tolerance and yield by Mn supplementation in *Vigna radiata*

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Received: 06 October 2018; Accepted: 19 July 2019

ABSTRACT

The present study was carried out in the year 2016–17 to understand the effects of salinity stress on relative water content (RWC), superoxide dismutase (SOD) activity, proline content, reducing sugar content and yield attributes in *Vigna radiata* and role of foliar application of manganese in improving salinity tolerance. Plants were subjected to varying levels of NaCl induced salinity (0, 100, 200 and 300 mM). The plant samples were analyzed from 25 days to 65 days of plant growth at every 10-day interval. Yield attributes were recorded at 85 days. Results revealed that there was a slight increase in RWC, SOD activity and yield attributes at 100 mM NaCl concentration as compared to control plants, whereas, an abrupt decrease was recorded at higher salt regimes (i.e. 200 and 300 mM NaCl). However, increase in salinity resulted in increased proline and total reducing sugar contents. The foliar application with Mn (0.15%) ameliorated the negative effects of high salinity and increased the yield. Hence, it was concluded that foliar application of Mn may alleviate the adverse effects of excess salinity on *Vigna radiata* plants.

Key words: Proline, Salinity, SOD, Yield

Mungbean [*Vigna radiata* (L.) R. Wilczek] is a fast-growing annual legume crop grown mostly in rotation with the cereals. It is high in protein content (24.5%) and has better digestibility than any other pulse crop (Tabasum *et al.* 2010) and is also used as an animal feed. It has the unique ability to fix the atmospheric nitrogen (58–109 kg/ha) in symbiotic association with *Rhizobium* bacteria, enabling it to meet its own nitrogen requirement while also benefiting the succeeding crops (Ali 1992).

Salinity is one of the major abiotic stresses negatively affecting soil productivity, growth and productivity of a variety of crops all over the world. Saline soils have excess amounts of soluble salts consisting of the chlorides and sulphates of Na⁺, Ca²⁺ and Mg²⁺, giving rise to soil saturation extract electrical conductivity (EC_e) values ≥ 4 dS/m. Plant growth in saline soils is hampered initially by the osmotic stress (i.e. physiological drought) and subsequently by the specific ion toxicities caused by excess Na⁺ and Cl⁻. Na⁺ and Cl⁻ ions often accumulate to the toxic levels causing a variety of harmful effects in plants including osmotic and oxidative stresses, nutrient toxicities and imbalances, and ultimately cell damage and death (Munns 2002, Singh and Sharma 2018). Salinity stress creates

oxidative stress in plants by producing reactive oxygen species (ROS) such as superoxide radical (O²⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH⁻) (Joseph *et al.* 2010). Below a threshold level, ROS are not detrimental to plant cells. Furthermore, these can also act as messenger molecules to initiate signal transduction cascades involving mitogen-activated protein kinase (MAPK) under various environmental cues (Opdenakker *et al.* 2012). Vighi *et al.* (2017) reported that there is differential expression of genes encoding protein of each antioxidant enzyme isoform whose transcription imparts salt tolerance. The presence of salt in the growth media often results in the accumulation of low molecular mass compounds, termed as compatible solutes, including proline, glycine betaine and sugars (Parida and Das 2005), which do not interfere with normal biochemical reactions (Zhifang and Loescher 2003). Their higher accumulation often protect the plants from salt injury, indicating that their higher tissue levels may positively be correlated with plant salt tolerance (Singh and Sharma 2018).

Micronutrients are required in relatively small amounts typically below 500 ppm (0.05%) of the plant dry weight (Chesworth 2008) for normal plant development. Mn plays an overriding role in the activation and as cofactor of various enzymes in plants (Burnell 1988), such as: Mn-superoxide dismutase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase. As a cofactor of superoxide dismutase (SOD), manganese participates in the plant's defense against oxidative stress, produced by elevated levels of activated forms of oxygen and free radicals (reactive oxygen species, ROS). It has been proposed that Mn

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can act as a scavenger of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Ducic and Polle 2005). Manganese facilitates the production of carbohydrates and is required for optimum utilization of macro nutrients in plants thus its deficiencies decrease photosynthetic efficiency thereby reducing crop yield and quality (Diedrick 2010). This study was done to understand the influence of foliar application of manganese as micronutrient in improving the tolerance of *Vigna radiata* plants against the injurious levels of NaCl by modulating various morphological, enzymatic and biochemical parameters.

MATERIALS AND METHODS

This study was carried out in the Department of Botany, DDU Gorakhpur University, Gorakhpur (26.7606°N, 83.3732°E) during the year 2016–17. After surface sterilization with ethyl alcohol for 5 min, well matured and vigorous seeds of *Vigna radiata* were inoculated with 96 h grown culture of *Rhizobium* for 12 h at 25–30°C. Seeds were then allowed to germinate in growth chamber under controlled conditions (25±2°C). Three days old germinated seedlings were then transferred to sand filled earthen pots. Hoagland's nutrient solution was applied to pots periodically at 5-day interval. Plants were treated with salt solution of different concentrations of NaCl (100, 200 and 300 mM) weekly. Control plants were supplied with same amount of distilled water. All the plants were watered daily to keep the sand moist and to maintain the salinity of the medium. Foliar sprays of manganese were applied as solution of $MnCl_2$ (0.15%) in double de-ionized water at 15, 30 and 45 days after planting. Plants were observed for 85 days. The treatments were: Control, T1: Untreated+Mn, T2: 100mM NaCl, T3: 100mM NaCl + Mn, T4: 200mM NaCl, T5: 200mM NaCl + Mn, T6: 300mM NaCl, T7: 300mM NaCl + Mn.

The experiment was laid in a randomized complete block design with three replicates for each treatment. Collection of plant samples was done at every 10 days and parameters like relative water content (RWC) and antioxidant enzyme superoxide dismutase activity (SOD) were analyzed from 25 days up to 65 days of plant growth. Fresh leaves were harvested on same days on which growth and enzyme activity were measured. Samples were dried in oven at 60 ± 2°C and were used to estimate biochemical compounds such as proline and reducing sugar.

Relative water content was calculated as described by Schonfeld *et al.* (1988). The superoxide dismutase [E.C.1.15.1.1] activity was measured in the primary leaves by the modified method of Giannopolites and Ries (1977). Proline was estimated by the method of Bates *et al.* (1973). Total reducing sugar was estimated by Somogyi's method (1952).

Yield parameters were studied at 70 DAS in terms of number of seeds per pod, number of pods per plant, average pod length, number of seeds per plant, weight of 10 seeds and harvest index. Harvest index (HI) was calculated by the method of Gardner *et al.* (1985).

A two-way analysis of variance (ANOVA) of data for all attributes was calculated using SPSS software (version 23). LSD (least significant difference) was used to compare the mean values of all treatments where F-test was found significant. In yield parameters standard deviation (SD) was calculated.

RESULTS AND DISCUSSION

Relative water content is one of the important indices showing the water status of plants. RWC significantly decreased at higher salinity levels (T4 and T6) but mild (T2) salt stress did not have any significant effect on RWC as compared to control (Fig 1a). Kumar *et al.* (2018) showed that leaf RWC consistently declined with increase in salinity in *Cicer arietinum* cultivars. Abbasi *et al.* (2014) found that while higher salinity significantly decreased RWC, mild salt stress did not cause significant decrease in RWC in *Zea mays*. Decrease in the RWC indicates decrease in turgor pressure in the plant cells and this eventually impairs the plant growth (Takkar and Wolker 1993). However, when Mn was applied, RWC increased and increase was the maximum in T5. Khan *et al.* (2016) reported an increase in RWC in drought stressed *Brassica juncea* plants sprayed with Mn. Higher RWC in Mn-treated plants might be due to improved fatty acid production; there is a possibility that Mn could be involved in the production of certain wax compounds reducing the transpirational water loss and thus increased water-use-efficiency (Buchanan *et al.* 2000).

In the present study, imposition of salt stress caused a significant increase in superoxide dismutase activity (SOD) in *Vigna radiata* (Fig 1b). The results are in conformity with Srivastava and Shahi (2018) in *Triticum aestivum* and Vighi *et al.* (2017) in *Oryza*. However, SOD accumulation did not increase linearly at T6, reflecting that SOD accumulation may not keep pace with excess salinity beyond a threshold and ROS produced due to salt stress cannot be scavenged efficiently, leading to the reduction in the growth and yield. The highest activity of the enzyme was obtained at 45 days. However, when Mn was sprayed, SOD activity even increased in T7 implying that Mn could be involved in the activation of SOD (Santandrea *et al.* 2000), especially Mn dependent SODs.

Proline accumulation has been suggested as an effective index for measuring abiotic stress tolerance in crop plants (Bates *et al.* 1973). In this experiment, proline content increased from 25 days up to 45 days and then decreased up to 65 days. The maximum proline content was noted in T5 when sprayed with Mn at 45 days (Fig 1c). Our findings are in agreement with previous studies in *Cicer arietinum* (Kumar *et al.* 2018) and *Vigna radiata* (Sehrawat *et al.* 2013) where salt induced increase in proline content was also observed. The most probable reason for increase in proline content under foliar treatments of Mn may be the role of Mn in nitrate assimilation (Ducic and Polle 2005), by which formation of glutamate occurs which acts as precursor of proline biosynthesis. The proposed functions of proline under stress conditions include osmotic adjustment,

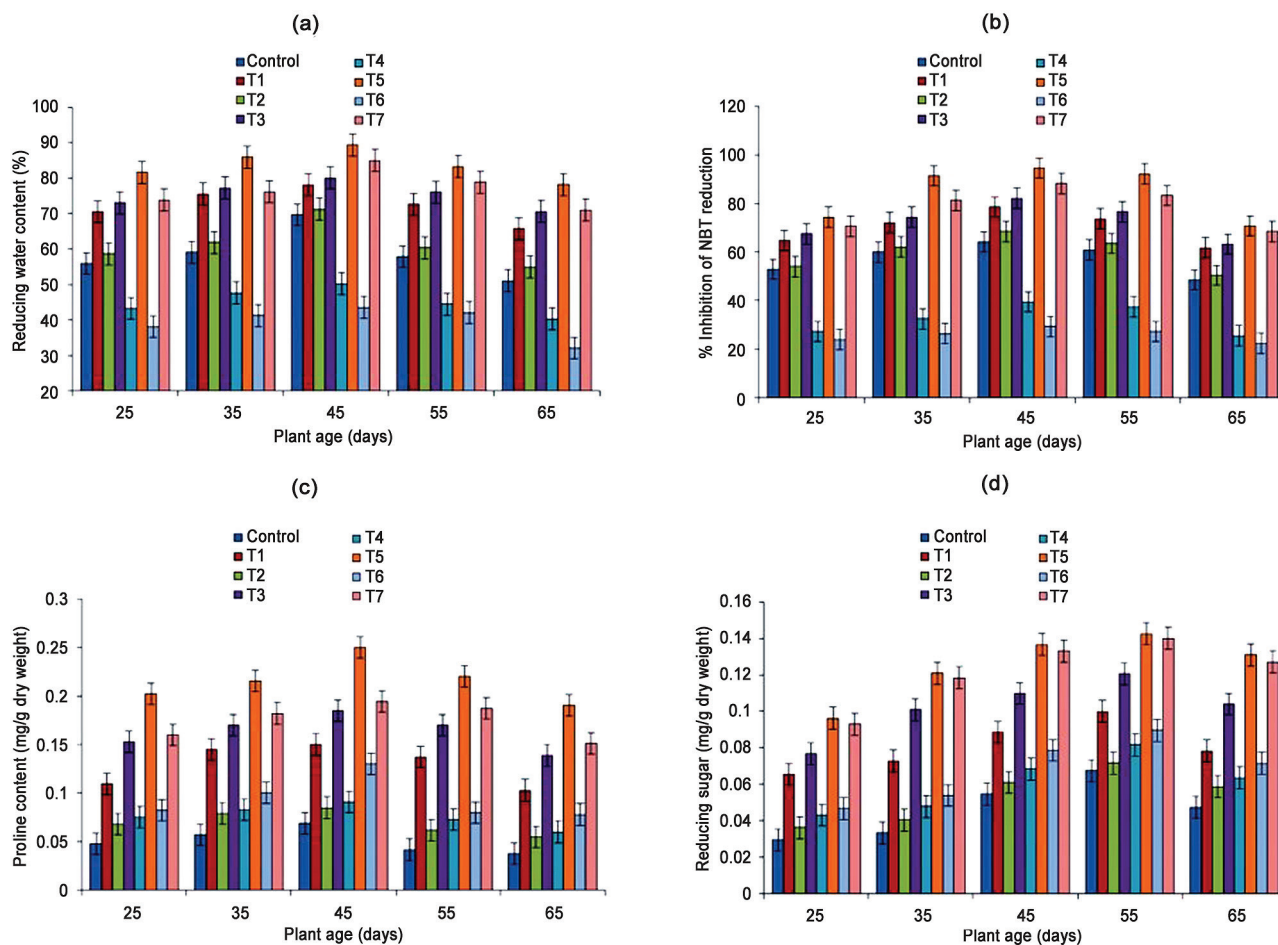


Fig 1 *Vigna radiata*: Relative water content, superoxide dismutase activity, proline content and total reducing sugar content at different days of plant growth under different salinity levels alone and in combination with MnCl₂.

protection of enzymes and membranes, as well as acting as a reservoir of energy and nitrogen for utilization during exposure to salinity (Perez-Alfocea *et al.* 1993).

Total reducing sugar content increased from 25 days up to 55 days and then decreased at 65 days. The maximum reducing sugar content was found in T6 at 55 days. Foliar spraying of MnCl₂ further increased the reducing sugar content in T5 (Fig 1d). Ruiz-Carrasco *et al.* (2011) found

that with increasing salinity, the accumulation of sugars and other compatible solutes such as proline, allow plants to maintain the cellular turgor pressure necessary for cell expansion under stressed conditions. Our results are consistent with the findings of Srivastava and Shahi (2017) in *Triticum aestivum* cultivars. Hakim *et al.* (2014) reported that reducing sugar increased up to 8 dS/m and thereafter decreased at 12 dS/m salinity level in *Oryza sativa*. The

Table 1 *Vigna radiata* yield attributes under different salinity levels alone and in combination with MnCl₂

Treatment	Number of seeds per pod	Number of pods per plant	Average length of pods (cm)	Number of seeds per plant	Weight of 10 seeds (g)	Harvest Index	Protein content in seeds (mg/g dry wt. of seed)
Control	9.0 ± 2*	13.33 ± 1.15*	6.7 ± 1.6*	119.97 ± 12.51*	0.37 ± 0.09*	35.48 ± 3.72*	23.31 ± 1.25*
T1	12.33 ± 1	18.33 ± 1	8.8 ± 1.5	226.0 ± 15.84	0.42 ± 0.07	44.38 ± 6.25	25.10 ± 1.50
T2	9.6 ± 0.57	15.33 ± 3.05	7.2 ± 1.5	147.168 ± 14.25	0.42 ± 0.05	37.48 ± 3.77	22.10 ± 1.07
T3	13.30 ± 1.15	19.33 ± 2.64	9.1 ± 1.2	257.08 ± 17.82	0.43 ± 0.08	45.31 ± 5.75	25.92 ± 1.66
T4	7.33 ± 2.30	7.33 ± 3.05	4.6 ± 1.8	53.72 ± 7.99	0.30 ± 0.08	31.62 ± 3.93	17.15 ± 0.98
T5	17.0 ± 1.52	28.33 ± 1.52	10.1 ± 1.5	481.61 ± 19.41	0.58 ± 0.13	55.97 ± 6.74	28.90 ± 3.33
T6	6.33 ± 1.15	5.0 ± 2	4.13 ± 2	31.65 ± 6.76	0.23 ± 0.09	30.23 ± 2.99	14.07 ± 0.89
T7	15.33 ± 0.57	23.66 ± 0.57	9.6 ± 2	362.70 ± 17.39	0.50 ± 0.11	50.98 ± 5.46	26.89 ± 2.69

* Mean ± Standard deviation (n=3)

increase in reducing sugar content after Mn application might be attributed to its role in improving photosynthesis and formation of photosynthates in the form of glucose and fructose.

Seed yield is a crucial consideration in any study relating to commercial cultivation as well as seed production of a crop. The yield parameters were severely affected at high salinity levels, i.e. T4 and T6; not withstanding slight increase in T2 (Table.1). The results are in conformity with the previous studies conducted in crops like *Triticum aestivum* (Srivastava and Shahi 2018) and *Sesamum indicum* (Singh et al. 2018). It was reported by Cruz et al. (2017) that the harvest index was significantly reduced at the highest salt concentration in *Manihot esculenta*. Moreover, it is seen that Mn application had a stimulatory effect on yield attributes of *Vigna radiata*. The results obtained were in conformity with Parmar et al. (2017) in potato. Nadergoli et al. (2011) observed that foliar spraying of Zn and Mn at shooting, flowering and podding stages enhanced the yield attributes in common bean.

Salinity decreases water potential and induces water scarcity for plant growth. Salt stress increases the free radicals in the cells hampering the normal plant physiology. Foliar spraying with Mn under these conditions can enhance the tolerance of *Vigna radiata* against salinity by increasing the ROS scavenging ability and by increasing the osmolytes, thus, increasing the yield. The tolerance mechanism of Mn treated plants includes morphological, enzymatic and biochemical changes. From above results it can be concluded that Mn treatment improves yield of *Vigna radiata* by improving the ROS scavenging ability of plants since it acts as cofactor in Mn SODs, and also by increasing osmolyte accumulation.

ACKNOWLEDGEMENTS

First author is thankful to Department of Science and Technology (DST), New Delhi for providing funds under DST INSPIRE fellowship program (Grant No. DST/INSPIRE/Fellowship/2014/109). Rajnish Kumar acknowledges University Grant Commission for providing funds under CSIR-UGC joint NET JRF scheme (Award no. 2121430382/Ref no. 21/12/2014 (ii) EU-V).

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