Effect of exogenous nitric oxide (NO) supply on germination and seedling growth of mungbean (cv. Nm-54) under salinity stress

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ABSTRACT

The present study evaluated the effects of exogenous NO supply, using sodium nitroprusside (SNP) as a source of NO, on germination and seedling growth of mungbean (Vigna radiata) under salt stress conditions. The results showed that the seeds treated with NO solution (0.2 mM SNP) exhibited 80% and 109% higher germination percentage and germination stress tolerance index (GSI) than untreated seeds (control) under salt stress conditions. Similarly, the seedlings fertigated with NO maintained the highest values of 77.8%, 84.3%, 77.2%, 60.5% and 100.3% for plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), shoot fresh weight stress tolerance index, root fresh weight stress tolerance index (RFSI) and dry matter stress tolerance index (DMSI), respectively. Moreover, the NO fertigated seedlings maintained 57% higher chlorophyll contents than control seedlings. It is concluded that exogenous NO supply is an effective approach to ensure uniform stand establishment in saline regions of the world.

Key words: Mungbean, NO, Salinity stress, Seed germination, Seedling growth.

INTRODUCTION

Salinity stress is one of the major environmental stresses that results in the accumulation of Na⁺ in plant growth medium which eventually leads to plant death as a consequence of growth arrest and metabolic damage (Hasanuzzaman et al. 2012; Sehrawat et al. 2013a). Accumulation of salts also decreases the uptake of many essential nutrients for plants due to competitive effect of Na⁺ with NH₄⁺, Mg²⁺ and K⁺ on uptake sites (Shahala and Munns, 2012) hence the improvement of seed germination and seedling growth under salt stress conditions has remained the research priorities of plant scientists.

The lack of suitable techniques for introgression of desirable agronomic traits or resistant genes makes it difficult to develop salt tolerant varieties (Singh et al. 2011) hence attempts have been made to develop shotgun approaches such as seed treatment, foliar or soil application of nutrients (Hussain et al. 2016), hormones (Jisha et al. 2013), osmoprotectants (Ashraf and Foolad, 2007) and stress signaling molecules (Fan et al. 2013) to enhance salinity tolerance in plants however, the fertigation and foliar spray are considered the most effective techniques due to simplicity and practicability (Nawaz et al. 2014).

Nitric oxide (NO) has been recognized as an important stress-signaling molecule with multifaceted physiological roles in biological systems. It may either act as a pro-oxidant or an antioxidant depending on its concentration in cells (Corpas et al. 2011). Recently, the importance of NO in seed germination has gained particular importance due to its association with the regulation of various nitrogenous compounds in germinating seeds (Sírová et al. 2011). The nitrate availability in soils and presence of reductants and prevalence of acidic conditions in seed apoplast increases the non-enzymatic production of NO in seeds (Bethke et al. 2007). Moreover, the activities of nitrate reductase (NR) and NO synthetase (NOS) further contribute to increased NO production in germinating seeds (Sírová et al. 2011). Exogenous NO donors such as sodium nitroprusside (SNP) constitute a powerful way to supplement plants with NO (Corpas et al. 2011).

The present study was carried out to evaluate the effects of exogenous NO supply on germination and seedling growth of mungbean under salinity stress which would help to develop a promising and effective approach to induce salinity tolerance in mungbean at early growth stages. The study was carried out with the hypothesis that NO fertigation induces salinity tolerance in mungbean at early growth stages.
MATERIALS AND METHODS

Seed collection and experimental conditions: The seeds of indigenous mungbean variety (NM-54), reported as salt tolerant by Wahid et al. (2004), were obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan to be used as plant material for this study. The experiment was laid out in a completely randomized design with four replicates for each treatment. The treatments scheme was as follows: normal water supply (0 mM NaCl) + 0 mM SNP, normal water supply + 0.2 mM SNP, 100mM NaCl + 0 mM SNP and 100 mM NaCl+ 0.2 mM SNP. The randomly selected healthy, physically pure seeds of mungbean were sterilized with 5% sodium hypochlorite solution for 5 minutes, washed three times with distilled water and air dried before sowing. Ten seeds were sown in plastic pots (15cm dia x 11 cm length) containing fine, sterilized river sand (600 g in each pot) as a growth medium. Data regarding promptness index (PI), emergence index (EI) and germination stress tolerance index (GSI) were recorded daily for eight days and the seeds with an approximate 2 mm radicle length were considered germinated. The seedlings were thinned to five after emergence and the nutrient requirements of the seedlings were met by supplying Hoagland solution in each pot (Hoagland and Arnon, 1950). The leaf chlorophyll contents of the seedlings were measured two days before harvesting using a SPAD chlorophyll meter (Hansatech CL-01).

Salinity stress and NO treatments: The seedlings were exposed to salt stress (0, 100 mM NaCl) in first week of seedling emergence. Salt stress solutions were prepared by dissolving Analar grade sodium chloride (NaCl) in de-ionized water and kept at room temperature (25°C) before application to the pots. Reports of Maswada and El-Kader (2016) showed that exogenous SNP supply (0.2 mM) significantly enhanced salinity tolerance in bread wheat so we used the same SNP treatment, developed by dissolving 0.06 g sodium nitroprusside (SNP), obtained from Sigma-Aldrich (USA), in 1000 mL of de-ionized water, to evaluate the role of improving salt stress tolerance in mungbean. The seedlings were fertigated with NO solution after emergence by seeds, which reduces the hydrolytic enzyme activity resulting in poor germination (Khodarahmpour, 2011). The leaf chlorophyll contents of the seedlings were measured two days before harvesting using a SPAD chlorophyll meter (Hansatech CL-01).

Statistical analysis: The results were statistically analyzed using analysis of variance (ANOVA) technique and significantly different means between treatments were compared by Tukey test at the 0.05 significance level of probability using STATISTIX software (version 8.1).

RESULTS AND DISCUSSION

Seed germination: Salinity stress had a marked effect (p<0.05) on germination attributes of mungbean seedlings (Table 1) as accumulation of salts inhibits the water uptake by the seeds, which reduces the hydrolytic enzyme activity resulting in poor germination (Khodarahmpour, 2011). The seeds applied with NO solution (0.2 mM SNP) resulted in the highest GSI (57.7%), whereas the 0 Mm SNP application gave the lowest value (27.6%) for this index (Fig. 2a). Similar results were noted for GP as mungbean seeds applied with NO in combination with NaCl induced salt stress (100 mM) exhibited higher GP (67.5%) than no NO supply (37.5%), however a non-significant effect of NO supply on GP (Table 1) was noted under normal conditions (Fig. 1a). Likewise, exogenous NO supply did not significantly (p>0.05) affect the PI (Fig. 1b) and EI (Fig. 1c) of mungbean seeds though a marked difference (p<0.05) was observed between seeds grown under normal and salt stress conditions (Table 1). NO mediated increase in germination, EI and PI might be attributed to stimulatory effects of NO on the availability of nitrates and inorganic nitrates in germinating seeds (Bethke et al. 2007) which is further promoted by hydroxylamine and azide by inhibition of catalase activity (Sirová et al. 2011). NO has been proposed to be involved in the light-mediated events in the plants including seed germination (Batak et al. 2002).
Table 1: Summary of the ANOVA for germination percentage (GP), promptness index (PI), emergence index (EI) and leaf chlorophyll contents (CC) of mung bean. Significant differences are indicated by an asterisk (*); * p≤0.05; ** p≤0.01; *** p≤ 0.001; NS, non-significant; SOV, source of variation; CV, coefficient of variation.

<table>
<thead>
<tr>
<th>SOV</th>
<th>GP</th>
<th>PI</th>
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<th>CC</th>
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<tr>
<td>Salinity Stress (S)</td>
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<td>MS</td>
<td>DF</td>
<td>MS</td>
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<tr>
<td>NO Treatments (T)</td>
<td>1</td>
<td>4900***</td>
<td>1</td>
<td>1.13*</td>
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<tr>
<td>S x N</td>
<td>1</td>
<td>1600***</td>
<td>1</td>
<td>0.66*</td>
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<td>CV</td>
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<td>25.92</td>
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<td>18.81</td>
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Table 2: Summary of the ANOVA for germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), shoot fresh weight stress tolerance index (SFSI), root fresh weight stress tolerance index (RFSI), dry matter stress tolerance index (DMSI) and leaf area stress tolerance index (LASI) of mung bean. Significant differences are indicated by an asterisk (*); ** p≤0.01; *** p≤ 0.001; NS, non-significant; SOV, source of variation; CV, coefficient of variation.

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<tr>
<th>SOV</th>
<th>GSI</th>
<th>PHSI</th>
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Seedling growth: The exposure to salt stress significantly (p<0.05) decreased the plant height and root length of mung bean seedlings (Table 2) however; NO fertigation markedly enhanced the physiological indices of plants. Salt induced reduction in seedling growth of mungbean might be due to reduced cell expansion and enlargement as a result of loss in turgor (Yamur and Kaydan, 2008). The seedlings fertigated with exogenous NO supply maintained higher PHSI (77.8%) with respect to seedlings grown (54.9%) without NO supply (Fig. 2b). The dehydration of protoplasm (Hussain et al. 2008) or the changes in cell wall extensibility due to hormonal imbalance (cytokinin, abscisic acid) might have attributed to decrease in PHSI under salinity stress. A significant increase in root length was observed in seedlings fertigated with NO and exhibited higher RLSI (84.3%) than control seedlings (39.89%) supplemented with 0mM SNP (Fig. 2c). A marked increase (p<0.05) in shoot and root fresh weight was also noted in seedlings fertigated with exogenous NO and gave significantly higher SFSI (77.2%) and RFSI (60.5%) than control seedlings with values of 59.5% (Fig. 2d) and 37.4% (Fig. 2e), respectively. Likewise, the fertigation of mungbean seedlings with 0.2 mM SNP resulted in higher DMSI (100.6%) than no NO supply (55.3%) (Fig. 2f). The positive effects of NO on SFSI, RFSI, DMSI and RLSI indicates that it is involved in seed germination just like in the pollen tube growth and determination of flowering time and flower development (Yu et al. 2014). Hebelstrup et al. (2013) reported that low doses of NO promotes biomass accumulation by increasing fresh weight and hypocotyl elongation however it may become toxic at higher doses during the vegetative growth phase of plant species. They were of the view that NO effects on plant growth are concentration-dependent so care must be taken to optimize NO doses before exogenous application to plant species. A significant increase in RLSI in present study might be due to increased activity of nitrate reductase (NR) and hence further supports the notion that this classic enzyme of nitrogen metabolism acts as a candidate for NO source in the root tissues (Peto et al. 2013). Our results are well in agreement with the reports of Yu et al. (2014) who suggested that NO participates in key processes responsible for primary root elongation, root hair differentiation and formation of adventitious and lateral roots.

Salinity stress also significantly (p<0.05) reduced the leaf area of mungbean seedlings (Table 2), which might be the result of loss of turgor due to closure of stomata (Yordanov et al. 2003) that disturbs the partitioning and translocation of photosynthates and consequently reduces plant growth and development (Miao et al. 2006). The seedlings fertigated with NO (0.2 mM SNP) maintained higher LASI (61.5%) than without NO supply (40.5%) (Fig. 2g) which suggests the contribution of optimal NO level in improving the normal growth of plants under abiotic stresses like salinity (Kolbert, 2016). Similar results were earlier reported by Frungillo et al. (2014) who observed that the Arabidopsis mutants containing low NO levels exhibited smaller leaf area and lower fresh weight than the wild type (WT) plants.
Fig. 1: Effect of NO fertigation on germination percentage (GP), promptness index (PI) and emergence index (EI) of mung bean exposed to normal (0 mM NaCl) and salt stress (100 mM NaCl) conditions. Values are mean±SE.
Fig. 2: Effect of NO fertigation on germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), shoot fresh weight stress tolerance index (SFSI), root fresh weight stress tolerance index (RFSI), dry matter stress tolerance index and leaf area stress tolerance index (LASI) of mungbean. Values are mean±SE
Chlorophyll contents: Mungbean seedlings grown under salt stress conditions exhibited a marked reduction (p<0.05) of 30% in chlorophyll contents with respect to normal conditions (Table 1). However, the fertigation of seedlings with NO (0.2 mM SNP) resulted in 57% higher chlorophyll contents (Fig. 3) than those without exogenous NO supply (control). The maximum value (7.85 SPAD value) was recorded in normal seedlings, whereas no NO supply resulted in the minimum (3.5 SPAD value) chlorophyll contents (Fig. 3). NO induced improvement in chlorophyll contents is in line with the early reports of Chen et al. (2014) who found a significant increase in chlorophyll content of salt stressed Aegiceras corniculatum leaves supplemented with 0.1 mM SNP. Similarly, Alavi et al. (2014) reported that NO positively counteracts the decay of chlorophyll in wheat leaves under oxidative stress.

CONCLUSION

The study revealed that exogenous NO supply is an effective and viable approach to ameliorate the negative effects of salt stress on germination and seedling growth of mung bean. NO plays a key role in improving the salt tolerance potential of mung bean and can be used as a potential stress ameliorant to improve early growth of crop plants in salt-affected soils of the world. However, further studies are suggested to explore the NO-mediated biochemical mechanisms responsible for inducing salt stress tolerance and would be interesting to investigate the interaction between NO and plant hormones particularly IAA actively involved in seed germination of plant species.

REFERENCES


