Research Article

Application of PGPR Enhances Development and Nodulation of *Vigna Radiata* L. Grown under Salt Stress

Muhammad Shafique, Nosheen Noor Elahi*, Muhammad Rashid, Amjad Farooq and Kausar Hussain Shah

Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, 60800, Multan, Pakistan.

Abstract | Salinity is a major soil problem limiting plant growth and development. Application of plant growth promoting rhizobacteria (PGPR) is effective against this stress. Mung bean is an important crop that is used as food. Present experiment was conducted under natural environment to evaluate the effect of PGPR on biomass production, nitrogen and proteins percentage of four mung bean varieties under different NaCI levels in sand culture after 5,7 and 9 weeks of sowing. Both inoculated and uninoculated plants were grown on mineral medium that were N-free either without NaCI or with a range of NaCI (20, 50,100, 200 and 300mM). Dry weight of plants was increased at 0-50mM NaCl and decreased at 100-300mM NaCI concentration. Inoculation effectively increased the dry weights of plants at salinity levels of 0-50 mM NaCI as compared to uninoculated plants but was not effective at 200-300mM NaCI. Number and fresh weight of pods were not affected at 0-100 mM NaCI levels of salinity but strongly decreased at 200-300 mM NaCl. Number and fresh weights of nodules increased slightly at lower levels (20- I00 mM) of salinity but strongly decreased at 200 mM NaCI. Nodulation and pods were completely inhibited at 300mM NaCl. The nitrogen percentage was decreased in salinity stress. The total protein contents increased with increasing levels of NaCI concentration. The plants in 50 mM NaCI maintained maximum amount of protein contents as compared to those at 0mM NaCI level. Varieties MI and NM92 had higher protein contents than M6 and NM98. From these findings it is concluded that PGPR can play an effective role in increasing crop production in saline soil.

Received | March 20, 2019; **Accepted** | May 22, 2019; **Published** | July 15, 2019
***Correspondence** | Nosheen Noor Elahi, Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, 60800, Multan, Pakistan; **Email:** nosheenilahi@yahoo.com
Citation | Shafique, M., N.N. Elahi, M. Rashid, A. Farooq and K.H. Shah. 2019. Application of PGPR enhances development and nodulation of *Vigna Radiata* L. Grown under salt stress. *Sarhad Journal of Agriculture,* 35(3): 763-769.
DOI | http://dx.doi.org/10.17582/journal.sja/2019/35.3.763.769
Keywords | Abiotic stress, Mung bean, Nitrogen, PGPR, Vigna radiata

Introduction

Abiotic stresses include salinity, water deficiency, heavy metal, extreme temperature and flooding. All these abiotic stresses disturb the plant growth, development and yield. Among these stresses, salinity is the basis of nourishing restraints and leads to the osmotic stress as it lowers the intake of calcium, potassium, phosphorus and nitrate (Wang et al., 2013; Nadeem et al., 2019). In saline soil the chlorophyll degradation occurs, and plant biomass production is decreased. Leaf area and photosynthetic pigments are also affected. As a result of this stress, the plants change their morphology, physiology and anatomical structure (Alam et al., 2015; Munns, 2006; Wang et al., 2013). So, Soil salinity is a major deleterious abiotic stress factor for food production (Kotagiri and Kolluru, 2017).

In response to salinity, plants adopt different tolerance mechanisms including repair and detoxification, osmotic adjustment and growth modulation. Under salinity stress, the production of ROS (reactive oxygen species) increased that results in increasing the degradation of proteins and important enzymes (Läuchli and Grattan, 2007). Plant growth promoting rhizobacteria (PGPR) are effective against stress conditions as they provide mineral nutrients and increased resistance of plants to abiotic stresses (Figueiredo et al., 2008). Among various minerals, nitrogen is an important mineral that is required to plant for its growth and development. The microorganisms add the nitrogen to plant by symbiotic or non-symbiotic association. It is considered that 175 million tons nitrogen is fixed in soil every year by these microorganisms, these are cheap source of nitrogen fixation (Orhan et al., 2006).

Mung bean is one of the major caloric (347K Cal.) and protein source in many countries of Asia. Like other pulse spp. its protein is deficient in methionine, cysteine and tryptophan and rich in tyrosine (Yi-Shen et al., 2018). Mung bean is used in the preparation of mung food items. The popular food is dhal which is consumed in almost every Asian country. The mung bean sprout is utilized as high-quality vegetables all over the world (Ahmad et al., 1988). Mung bean like other pulses acts as soil building crop because of most effective system of biological nitrogen fixation through its symbiotic relationship with rhizobium. It has potential to fix from 30-60 Kg /ha of nitrogen depending on soil and environmental conditions (Verma, 2016).

Keeping in view the above information, the present study was designed to assess the effect of PGPR inoculation on four varieties of mung bean grown in a growth medium that were N-free either without NaCI or with a range of NaCI (20, 50,100, 200 and 300mM).

Materials and Methods

The experiment was carried out in Botanical Garden of Bahauddin Zakariya University Multan, Pakistan. The germplasm of four varieties of *Vigna radiata* L. (MI, M6, NM92 and NM98) were taken from Nuclear Institute of Agriculture Biology (NIAB), Faisalabad. The Rhizobium inoculum for Mung bean (Bio power -Bio fertilizer for legumes) was obtained from National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad. The experiments were set in a completely randomized design with six replicates per treatment for each variety. Plastic pots of 20 cm diameter were used for plant cultivation. The pots were labeled as U/0, I/0, I/20, I/50, I/100, I/200, I/300, I/400 indicating the range of NaCI concentrations (mM) used whereas U and I stand for un-inoculated and inoculated, respectively.

Before sowing seeds, nitrogen free nutrient media was applied to each pot. The nitrogen free media was used as given by Elahi and Akhtar (2004). Half strength of the nutrient media (one liter) was given to each pot uniformly. The seeds were treated in 5ml inoculum and sown in sand (pure sand which at first was treated with 0.1 M HCl solution and then thoroughly washed with running tap water). Inoculation was repeated twice in week and while inoculated pots were not given any media. The pots were supplied with half strength of the nitrogen free media twice week when plants were young. After two weeks when the plants were fully grown then full-strength nitrogen free media was applied to each pot once a week. The salinity treatment (0, 20, 50, 100, 200 and 300 mM) was started two weeks after sowing. The salinity treatment along with nutrient solution (1L) were applied once a week to each pot. Before each application the pots were thoroughly leached with tap water to remove the unabsorbed molecules or ions. Leaching was done to maintain the required concentration of nutrients and NaCl.

First harvest was taken after 5 weeks of sowing, i.e., at flowering stage and second harvest was taken after 7 weeks of sowing i.e., at the beginning of pod growth stag. While third harvest was taken after 9 weeks of sowing i.e., at the middle of pod growth stage. The total number of flowers, pods and nodules of each plant were counted. Fresh weight of root, shoot, pods and nodules was measured by using digital balance. Then the shoot and root were kept into oven for one week for drying. After one week, the dry weight of root and shoot was measured. The total nitrogen contents were measured by using the method of Guebel et al. (1991).

Statistical analysis of the data including analysis of variance (ANOVA) and Duncon's Multiple Range Test (DMRT) at 5% probability level was done by using COSTAT.

Results and Discussion

Fresh weight of shoot and root of all the four varieties of *Vigna radiata* L. M 1, M6, NM92 and NM98 decreased gradually with the increasing level of salt concentration as compared to inoculated control (Figure 1 and 2). While at 200mM NaCI and above fresh weight of shoot and root per plant decreased drastically.

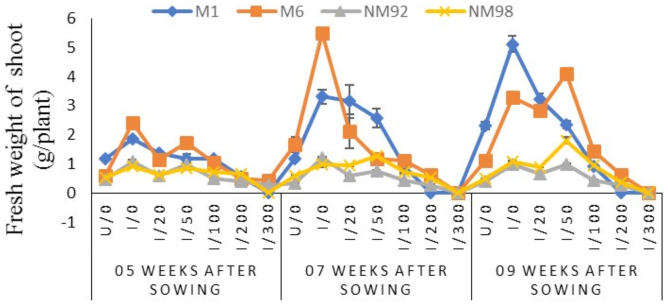

Figure 1: *Effect of NaCI concentration on shoot fresh weight of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

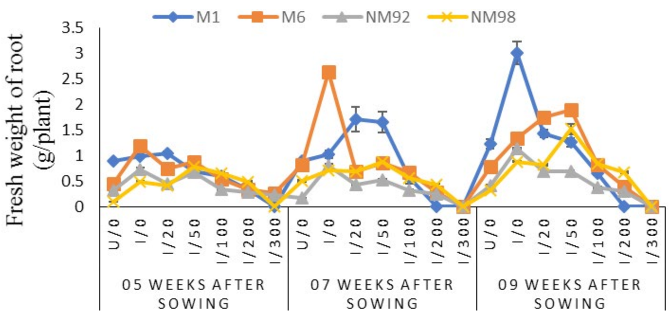

Figure 2: *Effect of NaCI concentration on root fresh weight of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

No growth was observed at 400mM NaCI level in first harvest in all the four varieties (Figure 1 and 2) at first, second and third harvest. A slight increase in root and shoot fresh weight was observed with slight increase in salinity. In variety M6 and NM92, fresh biomass of plants slightly increased at 50mM NaCI level as compared to 20mM NaCI level. At second harvest non-significant differences between all the treatments for fresh weight of shoot per plant were noted, which drastically decreased at 200mM NaCl. At third harvest non-significant differences were observed in all the treatments of salt concentration.

Dry weight of shoot and root per plant was decreased with increasing salinity level and the differences between them were significant for various treatments (Figure 3 and 4). Dry weight of whole plant biomass was slightly increased with increase in salt concentration in NM98 at all three harvests. At 50mM NaCl the plant growth of M6 and NM92 was slightly increased as compared to 20mM NaCl (Figure 3 and 4).

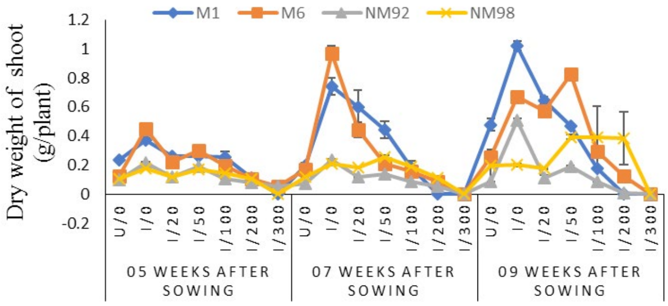

Figure 3: *Effect of NaCI concentration on dry weight of shoot of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

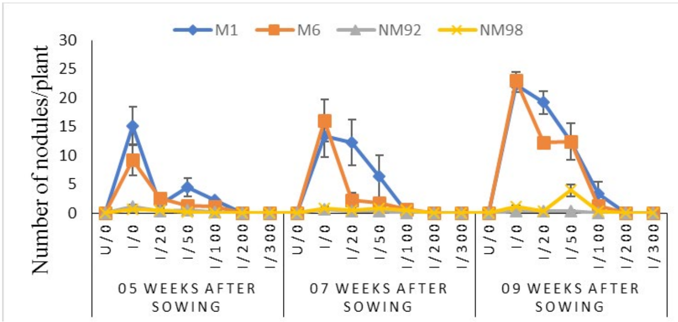

Figure 4: *Effect of NaCI concentration on dry weight of root of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

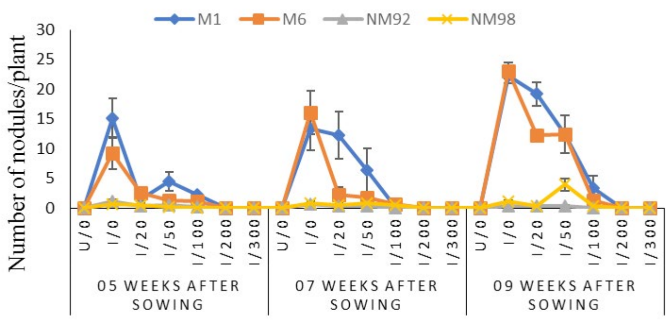

Figure 5: *Effect of NaCI concentration on number of nodules of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

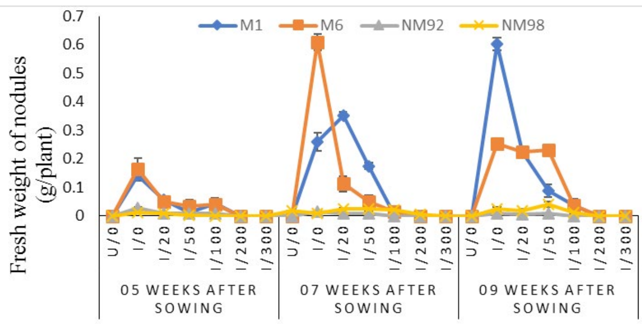

Figure 6: *Effect of NaCI concentration on fresh weight of nodules of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

Dry weight of all the four varieties was drastically decreased at 200-300mM NaCI (Figure 3 and 4). No plants survived at 400mM NaCl. A gradual decrease in dry biomass of all the four varieties was observed in first, second and third harvest in Ml (Figure 3 and 4). Figure 5 and 6 shows a gradual decrease in number of nodules and their fresh weight with increasing salinity levels. Number of nodules at higher level of NaCI was drastically decreased. No nodulation was observed at 200mM NaCI and above.

The Figure 6 shows that the fresh weight of nodules drastically decreased at different salinity levels from inoculated control to l00mM NaCl level in all the four varieties. At second harvest fresh weight of nodules decreased abruptly at each level of salinity from 0mM to 100mM.

Figure 5 shows that at 100mM NaCl level nodules were present at third harvest but their number, fresh and dry weight were drastically decreased (Figure 5 and 6). The flowers were found at I/0, I/20, I/50 and I/ 100 level of salt concentration but at I/200 level of salt fewer or no flowers were formed in all the four varieties of *Vigna radiata* (MI, M6, NM92 and NM98) at all the stages of harvest as shown in Figure 7.

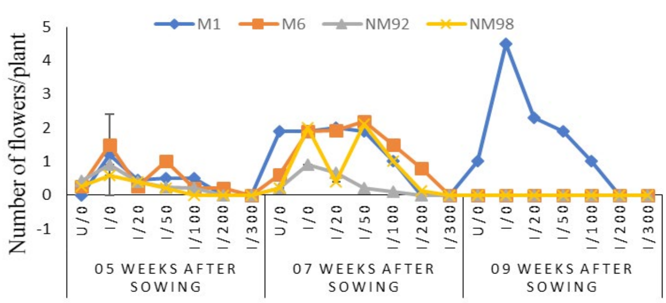

Figure 7: *Effect of NaCI concentration on number of flowers of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

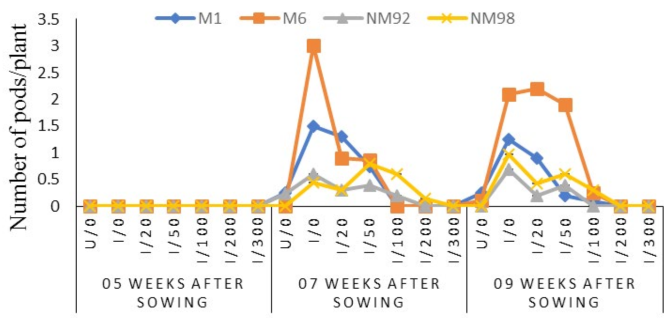

Figure 8: *Effect of NaCI concentration on number of pods of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

Number of pods and fresh weight decreased slightly with increasing salinity levels (Figure 8 and 9). Fresh weight of pods was gradually decreased in Ml at all the levels of salinity in all the three harvests (Figure 9). Figure 8 shows that number of pods decreased with increasing salt concentrations. At 2nd harvest pods were not formed at I/200-I/400 level of salinity in M6 (Figure 8). The pods were formed at I/200 level only in Ml, NM92 and NM98 at 2nd harvest. Figure 9 shows that Pod fresh weight was decreased gradually with increase in salt concentration in Ml, M6 and NM92. In M6 and NM98 fresh weight of pods decreased irregularly with increase in salt concentration (Figure 9).

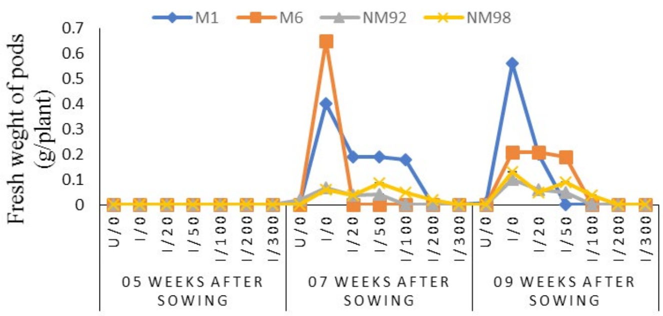

Figure 9: *Effect of NaCI concentration on fresh weight of pods of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

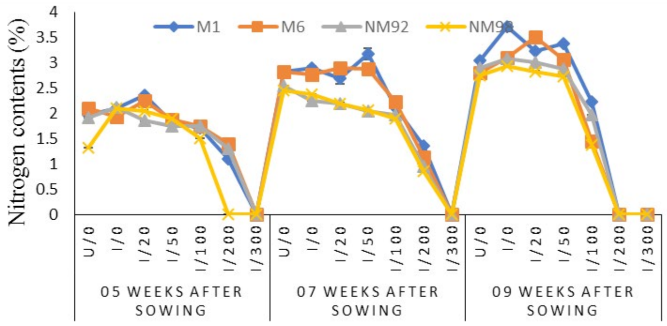

Figure 10: *Effect of NaCI concentration on nitrogen contents (%) of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

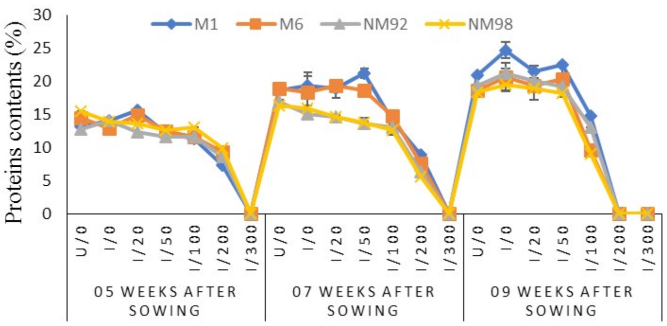

Figure 11: *Effect of NaCI concentration on proteins contents (%) of different varieties of Vigna radiata L. (MI, M6, NM92 and NM98) after 5,7 and 9 weeks of sowing.*

Salinity had a promontory/inhibitory effect on nitrogen contents of shoot dry weight at NaCl levels up to 50 mM but adversely affect at higher NaCI levels, at 1st harvest nitrogen contents were increased at 20mM NaCI levels of salinity in MI, M6, NM92 and NM98 as compared to inoculated control (Figure 10). Whereas, at 100mM NaCI the nitrogen content was 40% reduced as compared to inoculated control. A slight increase in nitrogen and total protein contents was observed at low salinity level, i.e., 20mM to 50mM NaCI in all the varieties of *Vigna radiate* L. (MI, M6, NM92 and NM98). At 100mM NaCl, 36-53% total protein contents were reduced in the four varieties at 3rd harvest (Figure 11).

The problem of salinity is worsening day by day in Pakistan as well as in the world and warrants the comprehensive study to understand the basis of growth and yield suppression due to salt stress especially in economically important crops. The magnitude of the problem can be minimized by appropriate drainage system, use of certain elements, leaching the salts from the root zone, growing salt tolerant varieties, etc. Construction of drainage, use of certain elements are very costly and out of reach of the farmer. While growing salt tolerant varieties seem to be more appropriate approach to minimize salinity problem (Qureshi et al., 2008; Qureshi et al., 2010). Therefore, the present study was designed to find the salt tolerant varieties from the locally available varieties of mung bean. Local germplasm with its unique character is an important gene pool for breeding varieties against different stresses. In the whole plant life cycle, the most critical phase is the seedling emergence from the soil as it effects the further growth and development of crops that ultimately influence the final yield (Maiti and Satya, 2014).

In the present study, salinity caused a significant reduction in fresh and dry biomass of shoot and root at all three growth stages. However, the pattern of decrease was not consistent with increase in external NaCI regimes. The reduction in the fresh and dry biomass of shoot and root attributable to reduce cell division and cell enlargement (Shannon, 1997). In case of mung bean varieties Ml, M6 and NM98, at the initial growth stages inoculation with biological nitrogen fixers stimulate the growth of the plants at 20mM and 50mM NaCI while at later stages this effect was masked due to strong inhibitory effect of NaCI salt. While in mung bean variety NM92, even slight increase in salinity resulted in significant drop in growth at all the three stages of growth.

Due to the advancement in research of salt tolerance and adaptation mechanism of various crop species to salinity, it is concluded that there might be the existence of different mechanisms for mineral nutrition regulations in different genotypes (Munns et al., 2006). Similar findings for chickpea were reported by Elsheikh and Wood (1990). At latter phase of growth inoculation with rhizobium can stimulate the growth of non-salinized plants only. These results can be linked to the previous results of Singleton and Bohlool (1984), who found that high salt concentrations are not inhibitory to rhizobia survival nor colonization of root surface, but that high salt concentration of growth medium can weaken the contacts among rhizobium and host plant thus preventing the nodule development. In mung bean, results for root and shoot nitrogen clearly indicated that salt stress significantly reduces these attributes. Similar, reduction in shoot and root nitrogen was observed in wheat (Glick, 2012).

Similarly, crude protein contents of shoot and root were decreased as observed in various studies. This reduction in proteins are attributable to reduce rate of protein synthesis and or /protein degradation under salt stress (Volkmar et al., 1998; Hasegawa et al., 2000). Salinity inhibit the nitrogen fixation completely or partially by disturbing the signal exchanges and as result of this salinity disturb the formation of nodules (Miransari and Smith, 2007).

Conclusions and Recommendations

By summarizing the results, we may conclude that the use of PGPR may be helpful to mung bean in salt stress tolerance and improvement in development and nodulation. More compatible PGPR strains may be tested for mung bean crop in the presence and absence of salinity.

Novelty Statement

This research indicates that application of plant growth promoting rhizobacteria (PGPR) has potential to increase crop production in areas effected with salinity.

Authors's Contributions

Nosheen Noor Elahi designed the experiment; Muhammad Shafiq performed the experiment;

Nosheen Noor Elahi, Muhammad Rashid, Kausar Hussain Shah and Amjad Farooq prepared the manuscript.

References

Ahmad, S., Hajar and M.T. Muda. 1988. Effects of atmospheric carbon dioxide, oxygen and ethylene on the growth and quality of mung bean sprouts. Proceedings of Second International Mung bean Symposium. AUROC, Shanhua, Tainan, Taiwan pp.536-545.

Alam, M.A., A.S. Juraimi, M.Y. Rafii and A.A. Hamid. 2015. Effect of salinity on biomass yield and physiological and stem-root anatomical characteristics of purslane (*Portulaca oleracea* L.) accessions. BioMed. Res. Int. 2015105695-105695. https://doi.org/10.1155/2015/105695

Elahi, N. and W. Akhtar. 2004. Effects of nitrate on nodulation and growth of two mung bean (*Vigna radiata* [L.] Wilczek) cultivars. Biol. (Pak.).

Elsheikh, E.A.E. and M. Wood. 1990. Salt effects on survival and multiplication of chickpea and soybean rhizobia. Soil. Biol. Biochem. 22 (3): 343-347. https://doi.org/10.1016/0038-0717(90)90111-C

Figueiredo, M.V.B., C.R. Martinez, H.A. Burity and C.P. Chanway. 2008. Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). World. J. Microbiol. Biotech. 24 (7): 1187-1193. https://doi.org/10.1007/s11274-007-9591-4

Glick, B.R. 2012. Plant growth-promoting bacteria. Mechanisms and applications. Sci. 201215. https://doi.org/10.6064/2012/963401

Guebel, D., B. Nudel and A. Giulietti. 1991. A simple and rapid micro-Kjeldahl method for total nitrogen analysis. Biotech. Tech. 5: 427-430. https://doi.org/10.1007/BF00155487

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. Ann. Rev. Plant. Biol. 51: 463-499. https://doi.org/10.1146/annurev.arplant.51.1.463

Haytowitz, D. and R. Matthews. 1986. Legumes and legume products. Washington, DC: US Dep. Agric. 1-156.

Kotagiri, D. and V.C. Kolluru. 2017. Effect of salinity stress on the morphology and physiology of five different Coleus species. Biomed. Pharm. J. 10 (4): 1639-1649. https://doi.org/10.13005/bpj/1275

Läuchli, A. and S.R. Grattan. 2007. Plant growth and development under salinity stress p. 1-32. In: Jenks, M.A., P.M. Hasegawa and S.M. Jain (eds), Advances in molecular breeding toward drought and salt tolerant crops. Springer Netherlands, Dordrecht.https://doi.org/10.1007/978-1-4020-5578-2_1

Maiti, R.K. and P. Satya. 2014. Research advances in major cereal crops for adaptation to abiotic stresses. GM Crops Food. 5(4): 259-279. https://doi.org/10.4161/21645698.2014.947861

Miransari, M. and D.L. Smith. 2007. Overcoming the stressful effects of salinity and acidity on soybean nodulation and yields using signal molecule genistein under field conditions. J. Plant. Nutr. 30(12): 1967-1992. https://doi.org/10.1080/01904160701700384

Munns, R., R.A. James and A. Läuchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57(5): 1025-1043. https://doi.org/10.1093/jxb/erj100

Nadeem, M., J. Li, M. Yahya, M. Wang, A. Ali, A. Cheng, X. Wang and C. Ma. 2019. Grain legumes and fear of salt stress: Focus on mechanisms and management strategies. Int. J. Mol. Sci. 20(4): 799. https://doi.org/10.3390/ijms20040799

Orhan, E., A. Esitken, S. Ercisli, M. Turan and F. Sahin. 2006. Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Sci. Hort. 111: 38-43. https://doi.org/10.1016/j.scienta.2006.09.002

Qureshi, A.S., P.G. McCornick, M. Qadir and Z. Aslam. 2008. Managing salinity and waterlogging in the Indus Basin of Pakistan. Agric. Water Manage. 95: 1-10. https://doi.org/10.1016/j.agwat.2007.09.014

Qureshi, A.S., P.G. McCornick, A. Sarwar and B.R. Sharma. 2010. Challenges and prospects of sustainable groundwater management in the Indus Basin, Pakistan. Water Resour. Manage. 24(8): 1551-1569. https://doi.org/10.1007/s11269-009-9513-3

Shannon, M.C. 1997. Adaptation of plants to salinity. Adv. Agron. 60. https://doi.org/10.1016/S0065-2113(08)60601-X

Singleton, P.W. and B.B. Bohlool. 1984. Effect of salinity on nodule formation by soybean. Plant Physiol. 74: 72-76. https://doi.org/10.1104/pp.74.1.72

Verma, A.K. 2016. Weed management in mungbean (*Vigna radiata* L.) under custard apple (*Annona squamosa* L.) based agri-horticultural system. Inst. Agric. Sci., Banaras Hindu Univ. Varanasi.

Volkmar, K., Y. Hu and H. Steppuhn. 1998. Physiological responses of plants to salinity: a review. Can. J. Plant Sci. 78: 19-27. https://doi.org/10.4141/P97-020

Wang, M., Q. Zheng, Q. Shen and S. Guo. 2013. The critical role of potassium in plant stress response. Int. J. Mol. Sci. 14(4): 7370-7390. https://doi.org/10.3390/ijms14047370

Yi-Shen, Z., S. Shuai and R. Fitz Gerald. 2018. Mung bean proteins and peptides: nutritional, functional and bioactive properties. Food Nutr. Res. 6210.29219/fnr.v29262.21290. https://doi.org/10.29219/fnr.v62.1290