Research Article

# Improving the Growth and Yield of Maize Through Multi-Strain Inoculation (PGPR) under Saline Conditions

**Hafiz Imran Iqbal[1*], Zahir Ahmad Zahir[2], Obaidur Rehman[1], Rizwan Khalid[1*], Abdul Waheed[1], Raja Abad Raza[1], Shahid Saleem[1], Muhammad Rashid[1], Sarosh Tariq Alvi[1] and Asia Munir[1]**

*[1]Soil and Water Testing Laboratory for Research, Data Ganj Bukhsh Road, Rawalpindi, Pakistan; [2]University of Agriculture, Faisalabad, Pakistan.*

**Abstract** | Growth of maize plant affect by saline stress conditions. Salt stress can be reduced through beneficial rhizobacteria that may increase the growth of plants. An experiment in pot was conducted to assess the effect of combined use of rhizobia and plant growth promoting rhizobacteria (PGPR) (containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase) on the growth and yield of maize under salt affected conditions. In pots three levels of salinity were developed i.e. 1.2 (original), 6 and 12dSm$^{1}$ by sodium chloride (NaCl) salt. The inoculants being used for maize seeds included PGPR (S1, S2) and *Rhizobium* (Mn2) strains. Recommended doses of NPK were applied @ 180:140:90 kgha$^{-1}$. Growth and yield of maize plants improved significantly by inoculation. Root length was increased by 32% with S1+S2+Mn2 strains at original EC. The rhizobacterial strains (either alone or in combination) significantly increased the1000-grain weight, fresh and dry plant biomass up to 35%, 36% and 48% at 6 dSm$^{-1}$ respectively. Dry matter of the crop (40%) was recorded with *Rhizobium* strain Mn2 (alone) compared to un-inoculated control at 12 dSm$^{-1}$. Results showed that the strains consortia (S2+Mn2) might be helpful in elevating the productivity of maize under saline conditions.

**Received** | November 20, 2018; **Accepted** | March 08, 2021; **Published** | May 14, 2021
***Correspondence** | Hafiz Imran Iqbal and Rizwan Khalid, Soil and Water Testing Laboratory for Research, Data Ganj Bukhsh Road, Rawalpindi, Pakistan; **Email:** imran.agro@yahoo.com, agrichemist.rwp@gmail.com
**Citation** | Iqbal, H.I., Z.A. Zahir, O. Rehman, R. Khalid, A. Waheed, R.A. Raza, S. Saleem, M. Rashid, S.T. Alvi and A. Munir. 2021. Improving the growth and yield of maize through multi-strain inoculation (PGPR) under saline conditions. *Pakistan Journal of Agricultural Research,* 34(2): 400-406.
**DOI** | https://dx.doi.org/10.17582/journal.pjar/2021/34.2.400.406
**Keywords** | Maize, Growth, Salinity, PGPR, Inoculation, Ethylene

## Introduction

Soil salinity is a major problem in arid and semi-arid areas of world including Pakistan (Parida and Das, 2005; Abuelgasim and Ammad, 2019). The estimated area affected by soil salinity/sodicity is over 7 m ha in Pakistan (FAO, 2017). The extent of useful soil conversion to saline soils extends up to 40,000 ha year$^{-1}$ (Khan, 1998). These losses from salinity are of great concern in countries like Pakistan, whose economy is highly dependent on agriculture (GOP, 2010). Crop production is badly affected by abiotic stress like soil salinity which is the main cause of food insecurity (Kotagiri and Kolluru, 2017).

Salinity stress shows various negative impacts on growing plants and their yield (Wu *et al.*, 2015). It not only inhibits the germination of seed and activities of enzymes (Seckin *et al.*, 2009) but also causes the decrease in photosynthetic ability, membrane destabilization and high ethylene concentration in the plant body (Parida and Das, 2005). Soil salinity


Links Researchers

also significantly decreases the process of mitosis and deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and synthesis of protein (Tabur and Demir, 2010; Javid *et al*., 2011). Plants absorb and build up larger quantity of toxic ions like sodium and chloride instead of absorbing useful nutrient elements in such soils (Mari *et al*., 2018). The phosphorus (P) uptake reduces as a result of phosphate precipitation with calcium ions under saline conditions (Bano and Fatima, 2009).

Nadeem *et al*. (2007) reported that the toxic effects induced by ethylene accumulation in plant decreased to some level by inoculation with rhizobacteria having ACC-deaminase. This enzyme can reduce ACC (1-aminocyclopropane 1-carboxilic acid), which is an instant precursor of ethylene, into ammonia and α-ketobutyric acid. There could also be different benefits of PGPR inoculation that increase plant growth and development through variety of mechanisms; increasing disease resistance, improving plant nutrient uptake and release of plant hormones (Khalid *et al*., 2009). The strain *Rhizobium*, having ability to increase growth and yield of non-nodulating plants have also been reported as PGPRs (Noel *et al*., 1996). They are capable of growth promotion in non-nodulating plants through growth promoting mechanisms while inhabiting in rhizosphere/rhizoplane (Mehboob *et al*., 2011). However, pronounced results in plant growth promotion may be achieved if the consortia of different rhizobium strains are used (Shaharoona *et al*., 2006a).

Maize (*Zea mays* L.) is one of the most important crop in Pakistan cultivated on 1.23 million hectares area with 5.7 million tones production of grain per annum having 4640 kg ha$^{-1}$ average yield (GOP, 2018). However, net return in terms of maize yield in our country is very less as compared to the developed countries of the world. High salinity level decreases the maize seedlings growth and cause low yield (Nadeem *et al*., 2007).

Keeping in view the decreased yield due to salt stress, expanding saline areas in the county and incremental impact of PGPRs and rhizobium strains on growth and yield of crops even in salt stress, an experiment on maize crop was planned to evaluate the impact of pre-isolated strains of *Rhizobium* and PGPRs on crop growth and development under different saline stress conditions.

## Materials and Methods

Sandy clay loam, sieved, well mixed and air-dried soil @12 kg pot$^{-1}$ was used in the experiment. The nutrients were applied from Urea, DAP and SOP with doses N P K @180:140:90 kg ha$^{-1}$. Phosphorus and potassium were applied at the initial stage of sowing while urea was used in two split doses. Three salinity levels were developed (1.2 (original), 6 and 12 dS m$^{-1}$) by using calculated amount of sodium chloride salt. Pre-isolated strains of PGPR (S1-*Pseudomonas syringae*), (S2-*Pseudomonas fluorescens*) and Rhizobium Mn$_2$ (*Rhizobium phaseoli*) containing ACC-deaminase activity, which were identified and characterized by Nadeem *et al*. (2007) were cultured and inoculants for each isolate was prepared following standard procedures.

Treatments were as; T1: Un-inoculated control; T2: S1 (PGPR); T3: S2 (PGPR); T4: Mn2 (*Rhizobium*); T5: S1 + S2; T6: S1 + Mn2; T7: S2 + Mn2; T8: S1 + S2 + Mn2.

Sterilized peat-clay mixture (3:1) and (10%) solution of sugar in 4:5:1 ratio was used as coating material for seeds inoculation with *Rhizobium* and PGPR alone and in consortia. While in un-inoculated control, coating of seeds was done with the same mixture but autoclaved. Five seeds of maize were sown in each pot that was prefilled with soil while after germination, only one plant per pot was maintained for further study. Pots were arranged according to completely randomized designs (CRD) in factorial arrangement in wire house. Irrigation was done by high quality water. The data related to growth and yield of maize was recorded at maturity of the crop. Various soil characteristics i.e particle size, saturation percentage (SP), electrical conductivity (EC$_e$) and pH were determined before sowing (Table 1); using the procedures illustrated by US Salinity Laboratory Staff (1954).

Concentration of sodium (Na$^+$) and potassium (K$^+$) was determined from the supernatant of leaf sap, by flame photometer as depicted by Ryan *et al*. (2001). Recorded data was statistically analyzed using CRD factorial design through the software MSTATC (Steel *et al*., 1997) while means were separated through Tukey's HSD test.

**Table 1:** *Physical and chemical characteristics of soil used.*

| S. No. | Characteristics | **Values** |
|---|---|---|
| 1 | Saturation percentage | 27 % |
| 2 | Texture | Sandy clay loam |
| 3 | Electrical Conductivity (ECe) | 1.2 $dSm^{-1}$ |
| 4 | pH | 6.97 |
| 5 | Soil organic matter | 0.96% |
| 6 | Total N | 0.06 %, |
| 7 | Extractable P | 7.5 mg $kg^{-1}$ |
| 8 | Extractable K | 110 mg $kg^{-1}$ |

## Results and Discussion

### *Root length (cm)*

The data in Figure 1 revealed that at original EC (1.2 dS $m^{-1}$), the root length of plants increased by almost all the strains (either alone or in combination) by showing 32% increase by using S1+S2+Mn2 strains over uninoculated control. At 6 dS $m^{-1}$ the treatment (S1+Mn2) in consortium showed increments of 34% followed by S1+S2+Mn2 (31%) while maximum (30%) root length was recorded when seeds were co-inoculated with S1+Mn2 at 12 dS $m^{-1}$.

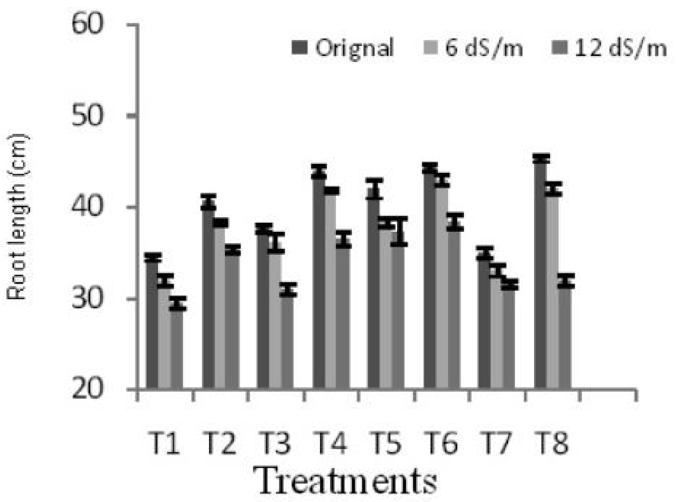


**Figure 1:** *Effect of Rhizobium and PGRR containing ACC-deaminase inoculation on root length (cm) under salinity in pots.*

### *Fresh plant biomass (g/pot)*

It was observed that the plant fresh biomass increased significantly over untreated control (Figure 2), by all the treatments but highest value (36%) was observed with inoculation treatment of S1+Mn2 strains at 6 dS $m^{-1}$ followed by (33%) at EC 1.2 dS $m^{-1}$. However, both S1+S2 and S1+Mn2 consortia were significantly indifferent by showing 30% increase over un-treated control at 12 dS $m^{-1}$ salinity level.

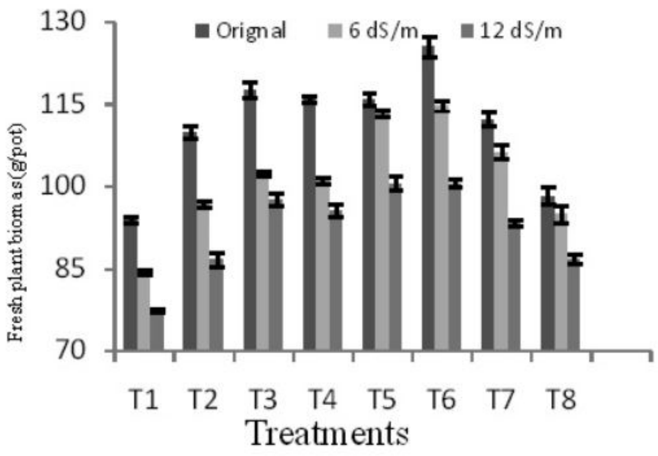


**Figure 2:** *Effect of Rhizobium and PGRR containing ACC-deaminase inoculation on fresh plant biomass (g/pot) under salinity in pots.*

### *Dry plant biomass (g/pot)*

The *Rhizobium* and PGPR (containing ACC-deaminase) inoculation either sole or in combination significantly enhanced dry plant biomass at all salinity levels (Figure 3). However, co-inoculation proved more effective in increasing the plant dry biomass as compared to sole inoculation. The inoculation of S1+Mn2 combination increased plant dry biomass 32% and 48 % at 1.2 and 6 dS $m^{-1}$ salinity levels, respectively. *Rhizobium* strain (Mn2) alone increased the plant dry biomass by 38 % at 12 dS $m^{-1}$ compared with control.

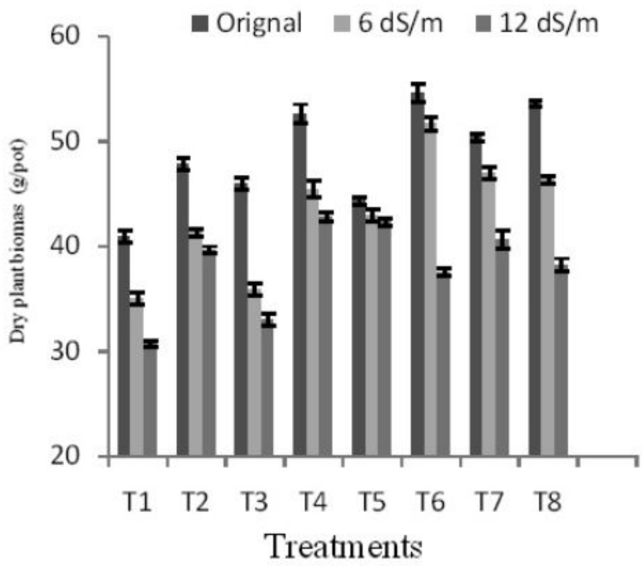


**Figure 3:** *Effect of Rhizobium and PGRR containing ACC-deaminase inoculation on dry plant biomass (g/pot) under salinity in pots.*

### *1000-grain weight*

*Rhizobium* and PGPR (containing ACC-deaminase activity) strains alone or in consortium significantly increased the grain weight of maize at all salinity


Links Researchers

levels over un-treated control (Figure 4). Maximum (35%) increase in grain weight was recorded with the strains (S2+Mn2) at 1.2 dS $m^{-1}$ followed by S1+Mn2 that showed 27% increase over control. Whereas the individual effect of strains S1 and S2 was non-significant at EC 1.2 dS $m^{-1}$. Highest increase (34%) at 6 dS $m^{-1}$ salinity level was recorded by inoculation with S2+Mn2 followed by S1+Mn2 (21%) increase over control. While inoculants S2+Mn2 and S1+S2 showed non-significant effect showing 29% increase over control at 12 dS $m^{-1}$ salinity level. However, the rest of isolates also showed increments in grain weight that ranged from 6 to 35% over un-inoculated control.

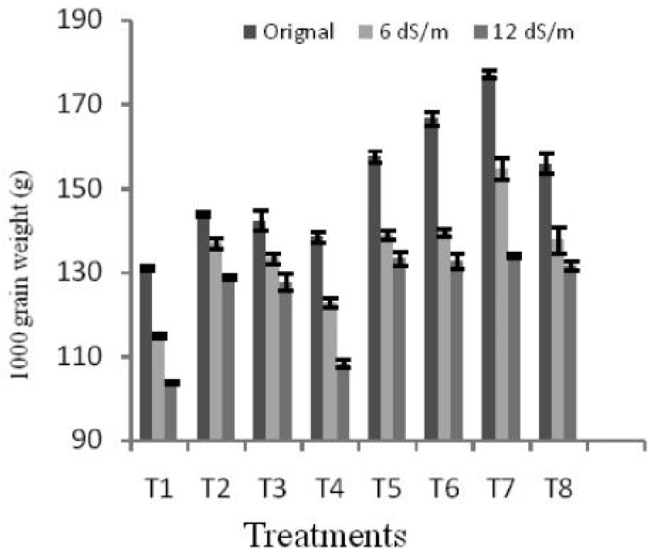


**Figure 4:** *Effect of Rhizobium and PGRR containing ACC-deaminase inoculation on 1000 grain weight of maize under salinity in pots.*

*Potassium to sodium ratio in leaves*

With increasing salinity level, $K^+/Na^+$ ratio in leaves decreased (Figure 5). Potassium to sodium ($K^+/Na^+$) ratio was affected by all the inoculants. But co-inoculation showed promising results at all levels of salinity. The inoculants in combination (S2+Mn2) showed the maximum (43%) increase in ratio at original salinity (1.2 $dSm^{-1}$) while the increments were 34% at 6 dS $m^{-1}$with the same inoculants. The treatment containing PGPR strains (S1+S2) remained the best among all treatments by showing 38% increase over control at salinity (12 dS $m^{-1}$).

The use of consortia as inoculants showed appreciable increments on growth and yield of maize crop all salinity levels. The increase in crop growth and development may be attributed to adaptation of these rhizobacterial strains producing hormones (Humphry *et al*., 2007), siderophores (Meyer, 2000), high uptake of nutrients (Hafeez *et al*., 1988) and certain other mechanisms such as biological control (Chandra *et al*., 2007) development of immunity in plants against various disease-causing agents (Mishra *et al*., 2006).

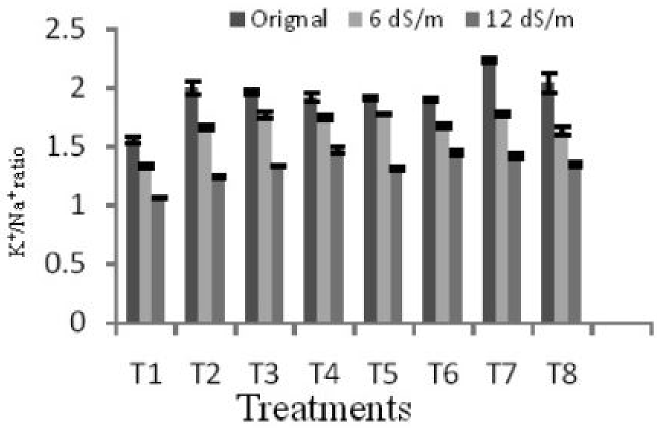


**Figure 5:** *Effect of Rhizobium and PGRR containing ACC-deaminase inoculation on K+/Na+ under salinity in pots.*

As far as the combine inoculation of PGPR and rhizobial strain (S2+Mn2) is concerned, the subsequent inoculants showed promising results over the rest of treatments at the 03 salinity levels, followed by S1+S2+Mn2 and S1+Mn2 which improved the growth and development at high salinity levels. It might be possible that their greater ability to colonize in roots and ACC-deaminase activity of PGPR made these amendments more vibrant under salt states. In this pot experiment, rhizobacterial strains alone as well as in combination, significantly increased the length of roots (up to 34 %), fresh and dry plant biomass (equal to 37 and 48%), 1000-grain weight (up to 35%), potassium to sodium ratio (43 %) of maize over un-inoculated control. Similar results were observed in some previous work (Naz *et al*., 2009) where inoculated species differed their capability to withstand at salty medium, thus variably promoting the plant growth and enhancing the crop yield. It has also been observed in another study that in rhizosphere, abilities of PGPR and rhizobia enhanced by the occurrence of each other (Tilak *et al*., 2006). The improved root architecture might have increased the nutrient uptake and enhance the growth of plants due to activity of rhizobacteria.

The increase in ionic concentration of sodium in foliage, as salinity levels increased, might have resulted the higher ionic concentration in rhizospheric soil, slow movement of cation exchange throughout affected membranes. Higher osmotic pressure might have buit up due to increased uptake of $Na^+$. The findings are also in conformity with Mansour *et al.* (2005). In an experiment, Mari *et al.* (2018) observed that increasing potassium to sodium ratio and production of osmo-protectants is highly useful to cope with salt inducing stress. These PGPR secrete some exopoloysaccahrides which can lower the $Na^+$ concentration by binding them (Geddie and Sutherland, 1993) hence the mechanism of less uptake of $Na^+$ by plant might have helped to lessen the salt stress in plants (Ashraf *et al.*, 2004).

## Conclusions and Recommendations

Rhizobium and PGPRs have incremental impact on maize crop growth and development that is due to AAC-deaminase activity, siderophore production and phytohormones release. Detrimental effect of salinity stress can be controlled, and salinity tolerance can be induced in plants being grown with the inoculants. It can be concluded from the results that the bio-augmentation of PGPR with *Rhizobium* might be used for maximum productivity of maize under saline conditions. However, further trails can be steered and planned under field conditions to elucidate the more benefits and use of such strains.

## Novelty Statement

As Interactive use of different PGPR strains proved to be more effective and favourable for mitigating the salt stress in plants, in future, this eco-friendly biological approach could lead to developing an effective bio-formulation to get better crop productivity in salt affected soil.

## Author's Contribution

**Hafiz Imran Iqbal:** Data collection, conducted research and wrote the paper.
**Dr. Zahir Ahmad Zahir:** Overall guidance and supervision of research.
**Obaidur Rehman:** Incoporated reviewers comments and technical support.
**Rizwan Khalid:** Statistical analysis of data, helped in references and review of paper.
**Abdul Waheed:** Technical support in research work paper writing.
**Raja Abad Raza:** Support in analytical work.
**Shahid Saleem:** Support in analytical work.
**Muhammad Rashid:** Statistical and technical support.
**Serosh Tariq Alvi:** Support in analytical work.
**Asia Munir:** Supported in research work and collection of data.

*Conflict of interest*

The authors have declared no conflict of interest.

## References


Abuelgasim, A. and R. Ammad. 2019. Mapping soil salinity in arid and semi-arid regions using lands at 8 OLI satellite data. Remote Sens. Appl. Soc. Environ., 13: 415-425. https://doi.org/10.1016/j.rsase.2018.12.010

Ashraf, M., S.H. Berge and O.T. Mahmood. 2004. Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol. Fertil. Soil, 40: 157-162. https://doi.org/10.1007/s00374-004-0766-y

Bano, A. and M. Fatima. 2009. Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. Biol. Fertility Soils, 45: 405-413.

Chandra, S., K. Choure, R.C. Dubey and D.K. Maheshwari. 2007. Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotiniasclerotiorum* and enhances growth of Indian mustard (*Brassica compestris*). Braz. J. Microbiol. 38: 128-130. https://doi.org/10.1590/S1517-83822007000100026

FAO, 2017. National policy dialogue on salt-affected soils held by FAO on 13-03-2017 at Islamabad, Pakistan.

Geddie, J.L. and I.W. Sutherl. 1993. Uptake of materials by bacterial polysaccharides. J. Appl. Bacteriol., 74: 467-472. https://doi.org/10.1111/j.1365-2672.1993.tb05155.x

GoP, 2010. Agricultural statistics of Pakistan, Economic Wing. Islamabad, Pakistan.

GoP, 2018. Economic Survey of Pakistan. Ministry of Finance, Islamabad (Pakistan). pp. 18.

Hafeez, F.Y., Z. Aslam and K.A. Malik. 1988. Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vignaradiata* (L). Wilczek. Plant Soil. 106: 107-111. https://doi.org/10.1007/BF02371188

Humphry, D.R., M. Andrews, S.R. Santos, E.K. James, L.V. Vinogradova, L. Perin, V.M. Reis, S.P. Cummings. 2007. Phylogenetic assignment and mechanism of action of a crop growth promoting *Rhizobium* radiobacter strain used as a biofertiliser on graminaceous crops in Russia. Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol., 91: 105-113. https://doi.org/10.1007/s10482-006-9100-z

Javid, M.G., A. Sorooshzadeh, F. Moradi, S. A.M.M. Sanavy and I. Allahdadi. 2011. The role of phytohormones in alleviating salt stress in crop plants. Aust. J. Crop Sci., 5(6): 726-734.

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

Khalid, A., M. Arshad, B. Shaharoona and T. Mahmood. 2009. Plant growth promoting rhizobacteria (PGPR) and sustainable agriculture. In: M.S. Khan, A. Zaidi and J. Musarat (Eds). Microbial Strategies for Crop Improvement. Springer-Verleg, Berlin/ Heidelberg, Germany. (In press). pp.133-160. https://doi.org/10.1007/978-3-642-01979-1_7

Khan, G.S., 1998. Soil salinity/sodicity status in Pakistan. Soil Surv. Pak. Lahore. pp. 59

Mansour, M.M.F., K.H.A. Salama, F.Z.M. Ali and A.F.A. Hadid. 2005. Cell and plant response to NaCl in Zea mays L. cultivar differing in salt tolerance. Gen. Appl. Plant Physiol., 31: 29-41.

Mari, A.H., I. Rajpar, Zia-ul-Hassan, S. Tunio and S. Ahmad. 2018. Ions accumulation, proline content and juice quality of sugar beet genotypes as affected by water salinity. J. Anim. Plant Sci., 28(5): 1405-1412. ISSN: 1018-7081.

Mehboob, I., Z.A. Zahir, M. Arshad, A. Tanveer and Farooq-e-Azam. 2011. Growth promoting activities of different *Rhizobium* sp., in wheat. Pak. J. Bot., 43: 1643-1650.

Meyer, J.M., 2000. Pyoverdines: Pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas* sp. Arch. Microbiol., 174: 135-142. https://doi.org/10.1007/s002030000188

Mishra, R.P.N., R.K. Singh, H.K. Jaiswal, V. Kumar and S. Maurya. 2006. *Rhizobium*-mediated induction of phenolics and plant growth promotion in rice (*Oryza sativa* L.). Curr. Microbiol., 52: 383-389. https://doi.org/10.1007/s00284-005-0296-3

Nadeem, S.M., Z.A. Zahir, M. Naveed and M. Arshad. 2007. Preliminary investigation on inducing salt tolerance in maize through inoculation with Rhizobacteria containing ACC- deaminase activity. Can. J. Microbiol., 53: 1141-1149. https://doi.org/10.1139/W07-081

Naz, I., A. Bano and Tamoor-ul-Hassan. 2009. Morphological, biochemical and molecular characterization of rhizobia from halophytes of Khewra salt range and Attock. Pak. J. Bot., 41: 3159-3168.

Noel, T.C., C. Cheng, C.K. Yost, R.P. Pharis and M.F. Hynes. 1996. Rhizobium leguminosarum as a plant growth promoting rhizobacterium: Direct growth promotion of canola and lettuce. Can. J. Microbiol., 42: 279-283. https://doi.org/10.1139/m96-040

Parida, A.K. and A.B. Das. 2005. Salt tolerance and salinity effect on plant: A review. Ecotoxicol. Environ. Saf., 60: 324-349. https://doi.org/10.1016/j.ecoenv.2004.06.010

Ryan, J., G. Estefan and A. Rashid. 2001. Soil and plant analysis laboratory manual, jointly published by the international center agricultural research in dry areas (ICARDA), Aleppo, Syria and National Agric. Res. Centre (NARC), Islamabad.

Seckin, B., A.H. Sekmen and I. Turkan. 2009. An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in roots of wheat under salt stress. J. Plant Growth Regul., 28: 12-20. https://doi.org/10.1007/s00344-008-9068-1

Shaharoona, B., M. Arshad and Z.A. Zahir. 2006a. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axinic conditions and on nodulation in mung bean (*Vignaradiata* L.). Lett. Appl. Microbiol., 42: 155-159. https://doi.org/10.1111/j.1472-765X.2005.01827.x

Steel, R.G.D., J.H. Torrie and D.A. Dicky (eds.). 1997. Principles and Procedures of statistics-A Biometrical Approach. 3rd Ed. McGraw Hill Book International Co., Singapore. pp. 204-227.

Tabur, S. and K. Demir. 2010. Role of some growth regulators on cytogenetic activity of barley under salt stress. Plant Growth Regul., 60: 99-104. https://doi.org/10.1007/s10725-009-9424-6

Tilak, K.V.B.R., N. Rauganayaki and C. Manoharachari. 2006. Synergistic effects of plant-growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanuscajan*). Eur. J. Soil Sci., 57: 67-71. https://doi.org/10.1111/j.1365-2389.2006.00771.x

U. S. Salinity Lab. Staff. 1954. Diagnosis and improvement of saline and alkali soils. USDA Hand Book No. 60. Washington. DC, USA. 160p.

Wu, G.Q., Q.Z. Shui, C.M. Wang, J.L. Zhang, H.J. Yuan, S.J. Li and Z.J. Liu. 2015. Characteristics of Na+ uptake in sugar beet (Betavulgaris L) seedling under mild salt conditions. Acta Physiol. Plant, 37(4): 1-13. https://doi.org/10.1007/s11738-015-1816-9