INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–1225/2019/22–6–1407–1414
DOI: 10.17957/IJAB/15.1215
http://www.fspublishers.org

***Full Length Article***

# Effect of Multi-Strain Bacterial Inoculation with Different Carriers on Growth and Yield of Maize under Saline Conditions

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## Abstract

Soil salinity being a significant character of the arid and semi-arid climate, causes enormous reduction in the crop production. It does so by disturbing the hormonal and nutritional balances which are important for plant growth. Using plant growth promoting rhizobacteria to mitigate salinity stress is an emerging potential technology. By employing various mechanisms of actions, they have the ability to improve the crop production under saline conditions. However, when present in the natural environments, their survivability may reduce drastically due to various environmental stresses. But using carrier materials during inoculation can enhance bacterial survivability because carriers generally provide the bacteria with better suited micro climate, nutrition and help to withstand the stresses. This study was carried out to evaluate five different carrier materials for the improvement of maize crop growth and production. Bacterial consortium was applied along with the carriers including peat, pressmud, biogas slurry, biochar and compost to improve the crop production at salinity levels normal (1.53 dS $m^{-1}$), 4 and 8 dS $m^{-1}$. An un inoculated treatment as a control and a liquid inoculation application directly to the soil as a treatment were also maintained. Results revealed that with the increase in salinity, there was a significant reduction in maize plant growth and production. However, where consortium of PGPR strains (S5, S14 and S20) was applied, the growth and production of the maize plant was significantly improved as compared to control at all salinity levels. The grain yield was increased up to 12.87, 13.36 and 13.59% more compared with un-inoculated control in case of pressmud based inoculation at 1.53, 4 and 8 dS $m^{-1}$, respectively. The results help to make a conclusion that pressmud was the carrier with best potential among all five carriers for significantly improving maize growth and yield. © 2019 Friends Science Publishers

**Keywords:** Consortium; Carriers; PGPR; Inoculation; Maize

## Introduction

Salinization of the soils is the key reason among all others that are responsible for soil degradation and decreasing crop yields worldwide with even more drastic effects in arid to semi-arid climates (Batool *et al*., 2014). In the recent years, the figure of saline soils has been increased to about 800 million ha of agricultural land worldwide (Munns, 2005). Soil salinity may develop by various factors including extreme temperature, minimal rainfall per annum, high transpiration rate and bad quality irrigation water with high salts content (Plaut *et al.*, 2013). Plants growing in saline environments show reduced growth and yield due to various stress factors including osmotic stress, mineral and nutritional imbalances, specific ion toxicity and hormonal imbalances (Munns and Tester, 2008). Various strategies can be applied to counter these drastic consequences of salinity on plant growth and production which include developing salt tolerant and resistant varieties of crops, discharging and leaching salts deep down to lower aquifers and salt accumulation in the aerial plant parts (Ramadoss *et al*., 2013). Salinity stress enhances ethylene levels beyond optimum concentration which retards the root from growing and enlarging inside the soil (Schaller, 2012). During any stress especially salinity and drought condition, ACC-synthase enzymes are triggered which result in the production of 1-aminocyclopropane 1 carboxylate (ACC) and ultimately ethylene because ACC is the precursor of ethylene (Rajput *et al*., 2013). Plant growth promoting bacteria (PGPR), having the ability of producing ACC-deaminase enzyme also possess the ability to regulate ethylene level within the plants through ACC-deaminase enzyme which catalyzes ACC and produce ammonia and α-ketobutyrate as by-products. Ammonia, being a nitrogen source for the microbes, is taken up by the microbes (Chernin and Glick, 2012; Nadeem *et al*., 2013b). PGPR that

**To cite this paper:** Irfan, M., Z.A. Zahir, H.N. Asghar, M.Y. Khan, H.T. Ahmad and Q. Ali, 2019. Effect of multi-strain bacterial inoculation with different carriers on growth and yield of maize under saline conditions. *Intl. J. Agric. Biol*., 22: 1407–1414

reside close to or within the root cells, have the potential for plant growth promotion by various mechanism of actions (Bhattacharyya and jha, 2012). Among these mechanisms are the release of regulatory biochemical substances, enhancing nutritarians solubility and availability to roots and/or reducing deleterious effects of various compounds or control of various pathogens (Ahemad and Kibret, 2013). Applying microbes in consortium increased plant growth more than the single microbial strain inoculation due to various synergistic mechanisms (Raja *et al*., 2006). However, it is an established fact that when present in natural environmental conditions, bacteria face many stress factors that may reduce their survivability and growth. So, using carrier materials during the inoculum preparation can effectively enhance the bacterial growth and survival by not only providing nutrient to bacteria but also creating a suitable microclimate to the bacteria. Carriers may have organic or inorganic origin. Selective carriers made from specific molecules and materials are also available (Pandey and Maheshwari, 2007). Carriers have a specific function of transferring viable microbes in optimum population from the laboratory to the field (Brahmaprakash and Sahu, 2012). They also share a major portion of the biofertilizer inoculant (Khavazi *et al*., 2007). A good quality carrier must be featured with higher moisture retaining capacity, easily processing and sterilization, cheap and abundantly available. Carriers with good buffering capacity and better structural stability are preferred. Carriers must be sticky which helps it to adhere with seed and it should not be toxic to PGPR (El-Fattah *et al*., 2013a).

It is obvious through the literature that no single carrier has all the characteristics which a good carrier should have. However, a carrier having most of the required characteristics is considered as best (Brahmaprakash and Sahu, 2012). As the carrier plays an important role in improving the efficacy of bacterial strains, therefore, this study is planned to hunt for the best carrier where different carrier materials including compost, biogas slurry, biochar, pressmud and peat along with the bacterial consortium were evaluated for the maize crop growth and yield promotion in a pot trial under saline soil conditions.

## Materials and Methods

In this study, efficacy of multi strain bacterial consortium along with the carrier materials *i.e.*, biochar, compost, peat, pressmud and biogas-slurry (Table 1) were utilized to increase maize performance under saline soil conditions.

Salt tolerant PGPR strains S5, S14 and S20 were collected from the Soil Microbiology and Biochemistry Lab., Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, which were pre-isolated and characterized against salinity stress and had already been proved worthy to enhance the growth of plant as single strain inoculants under saline conditions (Nadeem *et al*., 2013a). Strains were identified as *Pseudomonas syringae* (S5), *Enterobacter aerogenes* (S14) and *P. fluorescens* (S20).

These bacterial strains were checked for their compatibility for each other using the cross-streak method followed by Raja *et al*. (2006). The different isolates were grown on the same agar LB plates by following the cross-streaking method. Plates were placed under the incubator for 48–72 h and after that the plates were observed for the inhibition zones around the colonies showing their non-compatibility for each other in case of the development of the inhibition zones.

Multi-strain consortium was prepared by using the three broth cultures of PGPR in equal proportion with similar bacterial population ($\approx 10^{-8}$ CFU). After drying of carrier materials, they were ground to fine texture, sieved and sterilized thrice for 20 min at 121°C and 15 psi in autoclave. The carrier materials were inoculated using the selected PGPR strains @ 100 mL $kg^{-1}$ of the carrier material and incubated overnight. Maize seeds were surface sterilized by dipping in 70% ethanol for 1 min and then rinsed with sterilized distilled water. Seeds were then dipped in 5% solution of sodium hypochlorite for 4–5 min followed by 4–6 washings with sterilized distilled water. For seed coating, seeds were sprayed with 10% sugar solution and then the inoculated carriers along with the clay were mixed with the seeds until the uniform coating of the carriers on the seeds was achieved. For un-inoculated control, seeds were dipped in sterilized (autoclaved) broth for 15 min before sowing. Another inoculation treatment without any carrier material was maintained in which PGPR consortium was applied to soil at the rate of 100 mL/pot. Seeds after coating with carrier-based inoculum were sown in the pots containing soil at various salinity levels including 1.53, 4 and 8 dS $m^{-1}$. Soil used in the pots had the properties given in Table 2.

Calculated amount of salt (NaCl) was used to develop different salinity levels in pots. Three levels of salinity were used for pot trial which include original (1.53), 4 and 8 dS $m^{-1}$. The actual EC that developed in the pots was 4.12 and 8.06 dS $m^{-1}$ in 4 and 8 dS $m^{-1}$ pots respectively. Good quality water was used for irrigation. Recommended NPK fertilizer doses (160, 80, 60 kg $ha^{-1}$) were applied (P.A.R.C., 2019). Half of the nitrogen fertilizer was applied at sowing stage and half at second irrigation. All the agronomic and plant protection measures were ensured.

Soil texture class was assessed using the bouyoucos hydrometer method developed by Moodie *et al*. (1959). While saturation percentage of soil was determined following the method 27a described by U.S. Salinity Laboratory Staff, (1954). Soil $EC_e$ was determined from saturated paste extract using the EC meter (Rhoades, 1982) and CEC was figured by flame photometer (410 Sherwood) following the method 19 of US Salinity Laboratory Staff (1954). Organic matter contents in soil were estimated by the method of Moodie *et al*. (1959). Nitrogen was determined using Kjeldhal apparatus (VELP Scientifica, UDK 126 D) (Jackson, 1962). Available phosphorus was measured at the wavelength of 880 nm with Spectrophotometer (Thermo Electron Corporation, microlet evolution 300) (Watanabe

**Table 1:** Physico-chemical characteristics of carriers used for maize pot trial

| Property | Unit | Carrier materials | | | | | |
|---|---|---|---|---|---|---|---|
| | | Peat | Pressmud | Sawdust | Biochar | Compost | Biogas slurry |
| pH | - | 6.1 ± 0.1 | 6.7 ± 0.3 | 6.8 ± 0.3 | 8.0 ± 0.2 | 6.8 ± 0.3 | 7.8 ± 0.3 |
| EC | dS $m^{-1}$ | 2.9 ± 0.05 | 4.7 ± 0.09 | 5.2 ± 0.07 | 5.9 ± 0.02 | 2.1 ± 0.09 | 7.1 ± 0.07 |
| WHC | % | 48.34 ± 2.3 | 89.67 ± 1.1 | 66.4 ± 1.5 | 71.24 ± 0.5 | 60.01 ± 1.1 | 52.6 ± 1.5 |
| Inherent Moisture Capacity | % | 2.56 ± 0.2 | 8.05 ± 0.15 | 5.13 ± 0.2 | 7.06 ± 0.05 | 9.72 ± 0.15 | 8.86 ± 0.2 |
| Total C | % | 51.01 ± 0.2 | 37.51 ± 0.18 | 66.33 ± 0.16 | 89.54 ± 0.12 | 14.93 ± 0.18 | 50.12 ± 0.16 |
| Total N | % | 0.80 ± 0.2 | 0.42 ± 0.2 | 0.22 ± 0.4 | 0.10 ± 0.5 | 0.94 ± 0.2 | 5.81 ± 0.4 |
| Total P | % | 4.0 ± 0.7 | 8.2 ± 0.6 | 1.2 ± 0.5 | 2.34 ± 0.5 | 1.2 ± 0.6 | 1.05 ± 0.5 |
| Total K | % | 0.26 ± 0.7 | 0.63 ± 0.9 | 0.62 ± 1.1 | 0.17 ± 0.8 | 1.4 ± 0.9 | 1.44 ± 1.1 |
| C:N | - | 63.75 | 89.30 | 301.5 | 890 | 15.87 | 8.63 |

**Table 2:** Physico-chemical characteristics of soil used for maize pot trial

| Characteristics | Unit | Value |
|---|---|---|
| Textural class | | Sandy clay loam |
| Saturation percentage | % | 31 |
| pHs | | 7.5 |
| ECe | dS $m^{-1}$ | 1.53, 4 and 8 |
| CEC | $cmol_c\ kg^{-1}$ | 7.2 |
| Organic matter | % | 0.62 |
| Total nitrogen | % | 0.043 |
| Available phosphorus | mg $kg^{-1}$ | 9.5 |
| Extractable potassium | mg $kg^{-1}$ | 182 |

and Olsen, 1965). Flame photometer was used for the determination of extractable potassium by using the method 11a of U.S. Salinity Laboratory Staff (1954).

At the tasseling stage, gas exchange parameters of the maize plant were recorded. These include photosynthetic rate (A), substomatal conductance (Ci), stomatal conductance (gs), transpiration rate (E), water use efficiency (WUE) and vapor pressure deficit (VPD) by using CIRAS-3, a Portable Photosynthesis System (PLC3, USA). Relative water contents were determined using the formula of Mayak *et al*. (2004).

$$\text{RWC}\ (\%) = \frac{(\text{FW} - \text{DW})}{(\text{FTW} - \text{DW})} \times 100$$

Chlorophyll pigments were determined by Chlorophyll meter SPAD 502 Plus, Spectrum technologies Inc., USA and proline was measured following the method of Bates *et al.* (1973). The plants samples were digested using the method of Wolf (1982) and nitrogen, phosphorus and potassium were determined with Kjeldhal apparatus, Spectrophotometer and Flame Photometer, respectively (Richards, 1954; Olsen and Sommers, 1982). All the agronomic parameters were recorded by following the standard methods.

Treatments were arranged in three-time replications and completely randomized design in factorial settings was applied to the experimental units (Steel *et al*., 1997). Treatment means were then compared using Duncan's multiple Range test (Duncan, 1955). Statistix 8.1 software was used for analyzing the data statistically.

## Results

Results revealed significant consequences of salinity on maize crop. A significant decrease in the maize growth and yield with increase in salinity was observed as compared to original level (1.53 dS $m^{-1}$). This significant decrease was observed in many plant growth parameters. Salinity decreased grain yield up to 2 and 4.4% (Fig. 2), cob weight up to 1.6 and 11%, cob length up to 2.5 and 22% (Fig. 1), plant height up to 5.46 and 8% and root length up to 6 and 17% at 4 and 8 dS $m^{-1}$, respectively (Table 3). The 100 grain weight**,** relative water contents (Fig. 1), $K^+$ concentration in straw, $K^+/Na^+$ (Table 3) and chlorophyll contents (Fig. 2), were significantly reduced with the increase in salinity. However, proline contents (Table 3) were increased up to 11 and 45% at salinity levels 4 and 8 dS $m^{-1}$, respectively comparing with the normal soil conditions. Salinity also significantly decreased crude protein up to 20.38 and 47% (Fig. 2), nitrogen in straw up to 30 and 47%, nitrogen in grain up to 9.5 and 26% and $K^+$ in grain up to 27 and 40% at 4 and 8 dS $m^{-1}$, respectively (Table 4). It also decreased phosphorus contents in straw and also in grain when salinity stress was increased. Gas exchange parameters (Fig. 3) of leaf were also significantly reduced with increase in salinity. At 4 and 8 dS $m^{-1}$ salinity levels, decrease in sub-stomatal $CO_2$ level (Ci) was 6.5 and 19%, respectively, as compared to normal soil. While, reduction in photosynthetic rate (Fig. 2) was 17 and 34.5%, respectively, as compared to normal soil. Salinity levels 4 and 8 dS $m^{-1}$ caused 10 and 38% decrease in transpiration rate, 6 and 15% decrease in stomatal conductance, 11 and 24% reduction in photosynthetic water use efficiency and 10 and 31% decrease in vapor pressure deficit, respectively, as compared to respective control (Fig. 3).

Results showed that inoculation with PGPR helped ameliorate salinity stress in all the growth parameters. PGPR significantly enhanced maize growth parameters like plant height, root length and weight (Table 3), cob length and weight (Fig. 1) and also, the grain yield of maize (Fig. 2) when compared with the un-inoculated control.

Application of PGPR also significantly improved the 100 grain weight by 10, 10.3 and 12.9% (Fig. 1), $K^+$ contents up to 2.7, 2.2 and 2.4%, $K^+/Na^+$ up to 2.7, 2.8 and 3.5% (Table 3), relative water contents up to 7.9, 8.5 and 9% (Fig. 1) and chlorophyll contents up to 13.5, 16 and 16.5% (Fig. 2) at salinity stress levels of 1.53, 4 and 8 dS $m^{-1}$, respectively. Inoculation showed significant decrease in proline contents which were up to 5, 4 and 10% at 1.53, 4 and 8 dS $m^{-1}$, respectively compared to respective controls (Table 3).

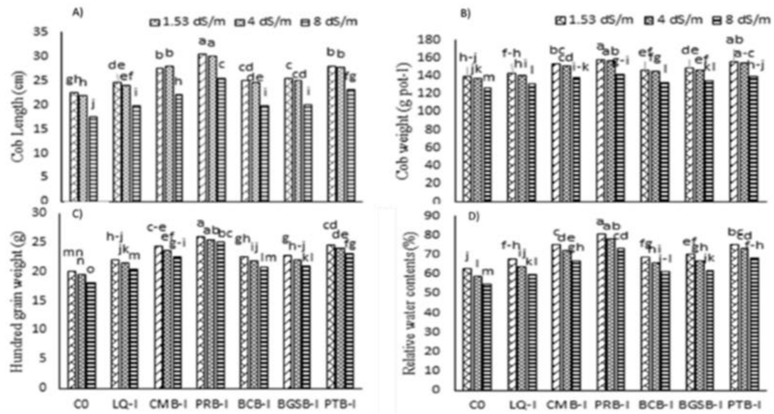


**Fig. 1:** Effect of multi-strain inoculation with different carrier materials on cob length, cob weight, 100 grain weight and relative water contents of maize under salinity stress

$C_0$= control, LQ-I= liquid inoculum, CMB-I= compost based inoculation, PRB-I= pressmud based inoculation, BCB-I= biochar based inoculation, BGSB-I= biogas slurry based inoculation, PTB-I=peat based inoculation

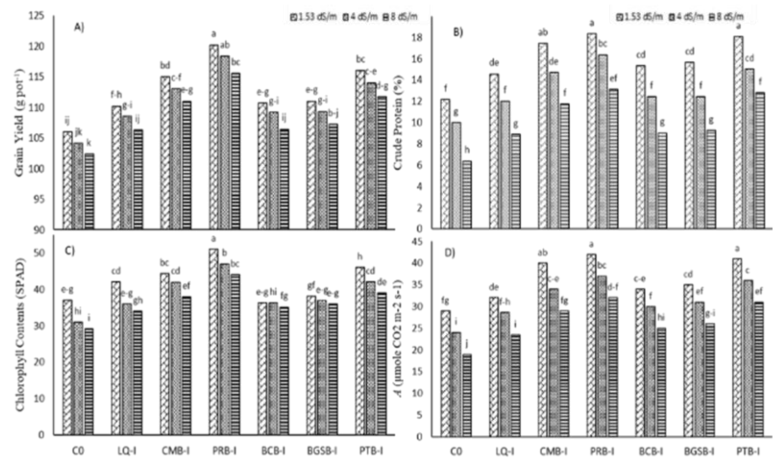


**Fig. 2:** Effect of multi-strain inoculation with different carrier materials on grain yield, chlorophyll contents, crude protein and photosynthetic rate of maize under salinity stress

$C_0$= control, LQ-I= liquid inoculum, CMB-I=compost based inoculation, PRB-I= pressmud based inoculation, BCB-I= biochar based inoculation, BGSB-I= biogas slurry based inoculation, PTB-I=peat based inoculation

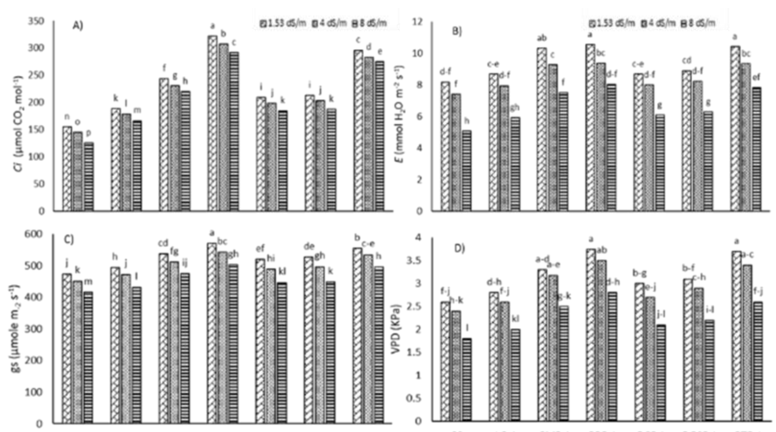


**Fig. 3:** Effect of multi-strain inoculation with different carrier materials on Sub-stomatal $CO_2$ (Ci), Transpiration rate (E), Stomatal conductance (gs) and Vapor pressure deficit (VPD) on maize under salinity stress

$C_0$= control, LQ-I= liquid inoculum, CMB-I=compost based inoculation, PRB-I= pressmud based inoculation, BCB-I= biochar based inoculation, BGSB-I= biogas slurry based inoculation, PTB-I=peat based inoculation

**Table 3:** Effect of multi-strain inoculation with different carrier materials on Plant height, Root length, Root weight, $K^+/Na^+$, Proline and $K^+$ in straw of maize under salinity stress

| Salinity | Treatments | Plant Height (cm) | Root Length (cm) | Root Weight (g) | $K^+/Na^+$ | Proline ($\mu$mol $g^{-1}$) | $K^+$ in Straw (%) |
|---|---|---|---|---|---|---|---|
| 1.53 dS $m^{-1}$ | C0 | 148.00 ij | 24.00 jk | 22.50 gh | 1.81 jk | 1.60 gh | 1.82 j-l |
| | LQ-I | 154.00 fg | 27.67 hi | 24.50 de | 1.86 g-i | 1.52 i | 1.87 e-g |
| | CMB-I | 166.00 b | 34.00 cd | 27.60 b | 1.93 b-d | 1.44 j | 1.92 bc |
| | PRB-I | 171.00 a | 39.67a | 30.50 a | 1.98a | 1.36 k | 1.98 a |
| | BCB-I | 158.67 cd | 30.50 fg | 25.00 cd | 1.88 e-g | 1.51 i | 1.88 d-g |
| | BGSB-I | 159.00 cd | 31.00 e-g | 25.50 c | 1.89 d-g | 1.51 i | 1.88 d-f |
| | PTB-I | 167.00 b | 35.30 b-d | 28.03 b | 1.94 bc | 1.53 j | 1.93 b |
| 4 dS $m^{-1}$ | C0 | 143.33 k | 23.00 k | 21.93 h | 1.78 k-m | 1.72 f | 1.79 l |
| | LQ-I | 148.33 ij | 27.00i | 24.00 ef | 1.83 h-j | 1.65 g | 1.83 h-j |
| | CMB-I | 157.00 d-f | 33.00 d-f | 27.87 b | 1.91 c-f | 1.55 hi | 1.89 c-e |
| | PRB-I | 165.00 b | 37.20 ab | 30.10 a | 1.96 ab | 1.45 j | 1.93 b |
| | BCB-I | 150.00 hi | 30.00 gh | 24.50 de | 1.86 g-i | 1.63 g | 1.85 g-j |
| | BGSB-I | 152.33 gh | 30.30 g | 25.00 cd | 1.87 f-h | 1.62 g | 1.85 f-i |
| | PTB-I | 158.00 de | 33.33 de | 27.77 b | 1.92 c-e | 1.53 i | 1.90 b-d |
| 8 dS $m^{-1}$ | C0 | 139.00 l | 20.27 l | 17.57 j | 1.70 n | 2.32 a | 1.69 n |
| | LQ-I | 144.00 k | 24.00 jk | 19.80 i | 1.76 m | 2.09 b | 1.73 m |
| | CMB-I | 155.00 e-g | 30.00 gh | 22.00 h | 1.81 j-l | 1.93 d | 1.80 kl |
| | PRB-I | 161.33 c | 36.00 bc | 25.53 c | 1.89 d-g | 1.82 e | 1.86 e-h |
| | BCB-I | 146.00 jk | 25.33 i-k | 19.87 i | 1.76 m | 2.01 c | 1.74m |
| | BGSB-I | 148.67 ij | 26.33 ij | 20.00 i | 1.77 lm | 1.99 c | 1.75 m |
| | PTB-I | 156.00 d-f | 31.33 e-g | 23.17 fg | 1.82 i-k | 1.92 d | 1.82 i-k |

Means sharing the same letter (s) are statistically non-significant at $p \leq 0.05$

$C_0$= control, LQ-I= liquid inoculum, CMB-I=compost based inoculation, PRB-I= pressmud based inoculation, BCB-I= biochar based inoculation, BGSB-I= biogas slurry based inoculation, PTB-I=peat based inoculation

**Table 4:** Effect of multi-strain inoculation with different carrier materials on Potassium in grain, Nitrogen in straw, Nitrogen in grain, Phosphorus in straw, Phosphorus in grain and Water use efficiency of maize under salinity stress

| Salinity | Treatments | Potassium in Grain (%) | Nitrogen in Straw (%) | Nitrogen in Grain (%) | Phosphorus in Straw (%) | Phosphorus in Grain (%) | WUE ($\mu$mole $CO_2$ $mmol^{-1}$ $H_2O$) |
|---|---|---|---|---|---|---|---|
| 1.53 dS $m^{-1}$ | C0 | 1.96 bc | 1.40 b-d | 1.7233 b-f | 0.33 de | 0.45 gh | 3.40 h-j |
| | LQ-I | 2.01 b | 1.57 a-c | 1.7900 a-e | 0.34 c-e | 0.65 d-f | 3.95 c-f |
| | CMB-I | 2.34 a | 1.63 ab | 1.8900 ab | 0.40 bc | 0.77 a-c | 4.30 a-c |
| | PRB-I | 2.54 a | 1.77 a | 1.9500 a | 0.46 a | 0.85 a | 4.48 a |
| | BCB-I | 2.02 b | 1.58 a-c | 1.8067 a-d | 0.36 c-e | 0.66 c-f | 3.97 c-e |
| | BGSB-I | 1.75 c-e | 1.61 ab | 1.8133 a-d | 0.36 c-e | 0.68 c-e | 3.99 cd |
| | PTB-I | 2.41 a | 1.69 a | 1.9233 a | 0.44 ab | 0.82 ab | 4.38 ab |
| 4 dS $m^{-1}$ | C0 | 1.58 e-g | 0.98 f-h | 1.55 gh | 0.23 g | 0.41 h | 3.00 k |
| | LQ-I | 1.64 dg | 1.12 eg | 1.63 e-h | 0.24 fg | 0.56 fg | 3.50 g-j |
| | CMB-I | 1.70 d-f | 1.28 de | 1.71 c-g | 0.30 ef | 0.62 d-f | 3.85 d-g |
| | PRB-I | 1.85 b-d | 1.36 c-e | 1.84 a-c | 0.36 cd | 0.73 b-d | 4.07 b-d |
| | BCB-I | 1.65 d-f | 1.17 d-f | 1.67 d-h | 0.25 fg | 0.56 fg | 3.60 f-h |
| | BGSB-I | 1.66 d-f | 1.21 d-e | 1.67 d-h | 0.26 fg | 0.60 ef | 3.61 e-h |
| | PTB-I | 1.81 b-d | 1.35 c-e | 1.83 a-d | 0.34 c-e | 0.72 b-d | 3.96 c-f |
| 8 dS $m^{-1}$ | C0 | 1.17 i | 0.81 h | 1.2400 j | 0.10 i | 0.20 j | 2.62 l |
| | LQ-I | 1.20 i | 0.83 h | 1.3200 j | 0.14 hi | 0.22 j | 2.99 k |
| | CMB-I | 1.43 g-h | 0.92 gh | 1.5200 h-i | 0.20 gh | 0.35 hi | 3.48 h-j |
| | PRB-I | 1.55 e-g | 0.99 f-h | 1.5600 f-h | 0.26 fg | 0.43 h | 3.60 f-h |
| | BCB-I | 1.22 hi | 0.87 h | 1.3433 j | 0.15 hi | 0.25 ij | 3.20 jk |
| | BGSB-I | 1.25 hi | 0.88 h | 1.3800 ij | 0.16 hi | 0.26 ij | 3.23 i-k |
| | PTB-I | 1.51 fg | 0.98 f-h | 1.5467 g-i | 0.24 fg | 0.42 h | 3.58 g-i |

Means sharing the same letter (s) are statistically non-significant at $p \leq 0.05$

$C_0$= control, LQ-I= liquid inoculum, CMB-I=compost based inoculation, PRB-I= pressmud based inoculation, BCB-I= biochar based inoculation, BGSB-I= biogas slurry based inoculation, PTB-I=peat based inoculation

Multi-strain bacterial inoculation improved the nutrients uptake and showed significant increment in nitrogen, potassium and phosphorus contents in both straw and grains at all salinity levels (Table 3–4). Bacterial inoculation also significantly increased gas exchange parameters *i.e.*, substomatal $CO_2$ level up to 21.5, 23 and 32.8%, stomatal conductance up to 4, 4.6 and 3.8%, transpiration rate up to 6, 7 and 17% (Fig. 3), water use efficiency up to 4, 16.6 and 14% (Table 4) and photosynthetic rate up to 10, 19 and 23.7% (Fig. 2) at 1.53, 4 and 8 dS $m^{-1}$, respectively.

Different carriers showed variable positive responses and significantly enhanced the potential of PGPR inoculation in consortium for increasing maize growth and yield. Pressmud and peat increased the growth significantly at all salinity levels when compared to un-inoculated control treatment, liquid inoculation and all others carrier material treatments. Compost, biochar and biogas slurry also improved significantly the effect of PGPR as compared to un-inoculated control treatment and liquid inoculum in many plant growth parameters.

Pressmud based inoculation showed the best results and increased plant height up to 15.5, root length up to 65, root weight up to 36 (Table 3), cob length up to 35.5 and cob weight up to 15.6% comparing with treatment without inoculation (control) at 1.53 dS $m^{-1}$ (Fig. 1)). Pressmud almost enhanced all parameters significantly than un-inoculated treatment and liquid inoculation at 4 and 8 dS $m^{-1}$. Other carrier-based formulations *i.e*., compost, biogas slurry and peat-based inoculations improved not only yield but also other parameters including the plant height, root length, root weight, cob length and cob weight of maize. All these parameters were statistically far better than un-inoculated treatment and also liquid inoculation at all three salinity levels. However, at 4 & 8 dS $m^{-1}$ pressmud based inoculation showed maximum gain in plant height which was up to 15.11 & 11.23% and 61.73 & 33.76% more than the un-inoculated and liquid inoculation treatments respectively (Table 3). Root length showed vigorous increase both at 4 and 8 dS $m^{-1}$ where pressmud along with the bacterial consortium was applied. Pressmud based inoculation also caused up to 13, 21 and 27% increase in grain yield (Fig. 2) when comparing to their respective controls having no inoculation at salinity stress levels 1.53, 4 and 8 dS $m^{-1}$, respectively. The 100 grain weight showed a significant increase where pressmud-based inoculation was applied. However, the treatments having biochar and biogas slurry-based inoculum, yielded statistically similar results with liquid inoculation treatment. Pressmud based inoculation also increased significantly the $K^+/Na^+$ as compared to un-inoculated control treatment and liquid inoculum (Table 3).

Similarly, proline contents were also significantly reduced at all salinity levels where bacterial consortium was applied along with different carrier materials. The maximum decrease of proline (15.69 and 12.21%) was found in the treatment where pressmud was applied at 4 dS $m^{-1}$ (Table 3). Similar improved results were also found in relative water contents (Table 4) and chlorophyll contents (Fig. 2) where pressmud based inoculum was applied compared to un inoculated control treatment and liquid inoculation.

Pressmud based inoculum showed best results in increasing nutrient elements concentration of maize crop (Table 3–4). Maximum increase in nitrogen in straw exhibited by pressmud at 1.53, 4 and 8 dS $m^{-1}$ was 26, 40 and 22% respectively more when compared to un-inoculated control treatment. Peat also showed significant increase than the un-inoculated and liquid inoculum control treatments and was at par with pressmud based inoculum. Similarly, maximum increase in $K^+/Na^+$, Nitrogen in grain and K in grain was also exhibited by pressmud and peat. However, peat-based carrier results were at par with pressmud based inoculant. Crude protein was also 26, 36 and 47.6% more as compared to liquid inoculation at 1.53, 4 and 8 dS $m^{-1}$ (Fig. 2).

Carrier-based inoculation also increased the gas exchange parameters of maize like transpiration rate, stomatal conductance and sub-stomatal $CO_2$ level, vapor pressure deficit, photosynthetic rate and water use efficiency. Pressmud and peat were found to be the best carriers for improving gas exchange parameters at all salinity levels (Fig. 3). Maximum increase in sub stomatal conductance exhibited by pressmud at 1.53, 4 and 8 dS $m^{-1}$ were 108, 72 and 75%, respectively, more comparing to un-inoculated control. Effect of peat was also significantly more than the control treatment where no inoculation was carried out and the treatment where liquid inoculum was applied but was statistically at par with pressmud-based inoculum.

## Discussion

Of all the stresses, the most devastating stress for the plant growth is salinity. It not only interferes with many physiological processes but also alters plant metabolic processes and hormonal balance (Perveen *et al*., 2013). It induces nutritional and water related imbalances that ultimately affect chlorophyll synthesis (Talaat and Shawky, 2014). Salinity stress causes to increase in the stress hormones concentration beyond the optimum levels in the plant tissues *i.e.*, roots and shoots. Stress hormones especially ethylene causes stunted root growth when present in higher concentration, ultimately, affecting overall plant growth at various growth stages. However, PGPR having ACC-deaminase activity bear the capability to break ACC into ammonia and α-ketobutyrate. This reduces the ACC (an ethylene precursor) concentration which ultimately causes ethylene reduction. This useful character of bacteria has the ability to improve plant growth during stress conditions (Cheng *et al*., 2007; Zahir *et al*., 2011).

Generally, use of PGPR for the plant growth is effective and yield positive results but there are several limitations of using these organisms also. One major concern is the survival of bacteria in the rhizosphere under natural environmental conditions (Abd-Alla *et al*., 2001). This limitation might be due to various factors *i.e.,* competition for nutrients and shelter (Bashan,1998). To cope with this situation, carrier-based inoculations are helpful with having higher shelf life than the one without carrier material.

This study revealed a positive impact of carrier-based inoculation upon the physiological and biochemical parameters of the maize crop. This positive increase in these parameters might be due to low concentration of ethylene, which acts as a stress hormone for plants at its high concentration. This low concentration of ethylene might be due to ACC-deaminase activity of the bacterial strains which splits ACC into ammonia and α-ketobutyric acid, hence improved the maize root length (Chernin and Glick, 2012). Besides ACC-deaminase, other characters may also be responsible for positive growth, e.g., nutrient solubilization, phytohormones production, exopolysaccharides and siderophores production (Ahmad *et al*., 2013).

This might also be due to the high survival rate of the bacteria due to better suited microclimate provided by the carrier materials (Kalra *et al*., 2010). Carrier materials with good physico-chemical properties, are also source of nutrients to the microbes which ensure their better survival (El-Fattah *et al*., 2013b). In this study, the better results obtained by the carrier based microbial inoculants might be due to this reason. Similar results were also found by Ramesh (*et al*. 2009) and Bashan (*et al*. 2013). In this study, peat showed potential for the crop improvement significantly under stress conditions. However, its performance was statistically at par with the pressmud based formulation. Similar findings were also reported by Janssen (1996).

Carbonbeing an integral part of the organic compounds, is found in abundant quantity in organic compounds *e.g*., biogas slurry and biochar where carbon was about 50 and 89% respectively. Rhizobacteria are also thought to be as carbon limited bacteria (Vance and Chapin, 2001). This abundant carbon content present in biogas slurry and biochar also assisted the bacterial survival in the unfavorable soil conditions. Many scientists suggested the carrier-based inoculant's use for the crop production instead of liquid culture inoculation (Sarma *et al.*, 2011; Gunjal *et al*., 2012).

Salinity is notorious for negatively effecting the plant photosynthetic, transpiration rates and stomatal conductance *etc*. (Talaat and Shawky, 2014). Results of this study showed that carrier-based inoculations significantly increased the gas exchange parameters *e.g*., photosynthetic rate, stomatal & sub stomatal conductance, water use efficiency and vapor pressure deficit *etc*. This might be because PGPR reduce chlorophyll degradation and improve water and nutrient use efficiency (Kanwal *et al*., 2011; Talaat and Shawky, 2014). Moreover, peat and pressmud both are good carriers having more nitrogen and potassium contents which might be favorable for the better growth and survival of the bacteria under the stressful environment. As potassium has a key role in enzymes activation, its availability might be increased with PGPR. Similarly, nitrogen is an integral part of chlorophyll and is essential for the chlorophyll synthesis. Moreover, high sugar contents present in the pressmud make it a promising carrier material. So, it helps the bacteria to survive and the plant to develop properly (Iqbal and Ashraf, 2013; Talaat and Shawky, 2014).

Relative water contents were reduced in maize crop when grown under the saline conditions as compared to control treatments and this could be because of alteration in water potential of the maize plant. However, when carrier-based inoculants were applied, a significant increase in relative water contents was observed at all salinity levels *i.e.*, 1.53, 4 and 8 dS $m^{-1}$. This improvement in relative water contents under salt stress conditions indicates that PGPR assisted the plant to withstand the stress and improved water uptake by plant by reducing the salinity stress (Ahmad *et al*., 2012). Reduction in salinity stress due to ACC-deaminase containing bacterial inoculation resulted in root elongation which covered large area as compared to control plant, ultimately taking up more water comparing with un-inoculated control and liquid inoculation treatment (Zahir *et al*., 2003).

High chlorophyll contents were also observed in pressmud-based inoculation and this might be due to the fact that pressmud has more water retention capacity which is a favorable trait for increasing the survival and shelf life of the inoculant during stressful conditions.

Proline which is produced due to stress was also reduced where carrier-based inoculums were applied. Least proline contents were observed where pressmud-based inoculum was applied. These results indicated that this decrease in proline contents might be because of stress reduction by multiple favorable mechanisms of actions of plant growth promoting rhizobacteria (Han and Lee, 2005).

Overall, multi strain bacterial inoculation along with the carrier materials, not only, were effective for stress reduction on the plant but also helped in significant increase in overall plant growth and yield. Crude protein concentration in the plant tissues was increased significantly comparing to control because its production is badly affected during salinity stress due to ethylene overproduction. So, this increase in protein concentration showed the reduction in salinity stress effect on the maize plant. Similar results were also observed when Hamdia *et al*. (2004) inoculated maize with *Azospirillum brasilense* under salt stress conditions.

## Conclusion

The study revealed that multi-strain bacterial inoculants have the potential to improve maize crop growth and yield growing under the saline conditions. However, their potential, efficacy and survival can be further improved when carrier-based inoculums are applied. Out of five carriers, pressmud and peat as carriers can be utilized in commercial biofertilizer formulations.

## Acknowledgements

We are highly obliged to the Higher Education Commission of Pakistan (HEC) for providing research funds. Special thanks to Mr. Usman Jamshed, Dr. Muhammad Naveed and Dr. Tasawar Abbas for their moral support and guidance.

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**[Received 31 Dec 2018; Accepted 08 Jul 2019; Published (online) 22 Dec 2019]**