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COMPARATIVE EFFECTIVENESS OF DIFFERENT CARRIERS TO IMPROVE THE EFFICACY OF BACTERIAL CONSORTIUM FOR ENHANCING WHEAT PRODUCTION UNDER SALT AFFECTED FIELD CONDITIONS

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Abstract

Salinity is one of the most crucial problems for sustainable agriculture which is severely affecting crop growth and decreasing the food production. On another hand, burgeoning population in the world demands to produce more food. So, there is a need of hours to increase agricultural production particularly cereals from salt affected soils by adopting cost effective and environment friendly approaches. Use of bio-inoculants with salt tolerant plant growth promoting rhizobacteria (PGPR) could be a promising option to enhance the production of cereals in salt affected soils. Therefore, a field experiment was conducted to evaluate different carriers compost, peat, biogas slurry and press mud along with PGPR to enhance wheat production under salinity stress. Consortium containing equal proportion of three PGPR strains (*Bacillus cereus* strain Y5, *Bacillus* sp. Y14 and *Bacillus subtilis* strain Y16) was used with different carriers for seed coating. Finely ground and sterilized carriers were mixed in broth and coated on the surface of wheat seeds with different carriers. Coated seeds were sown in saline field with salinity range of 10−13 dS m^{-1}. Results revealed that multi−strain bacterial inoculation improved the gas exchange, ionic, biochemical, growth and yield attributes of wheat crop under salinity stress. However, use of different carriers further improved the efficacy of multi−strain inoculation and significantly increased growth, yield and physiological parameters of wheat. The results of compost, peat and biogas slurry as carrier for bio−inoculants were statistically similar.

Key words: Biofertilizer, PGPR, Inoculation, Saline conditions, *Triticum aestivum* L.

Introduction

Salinity stress is amongst the most severe environmental factors constraining plant growth and crop productivity by altering the ionic and osmotic potential and composition of ions (Nandwal *et al*., 2000; Arora *et al*., 2012). High salt concentrations in saline soils not only degrade soil structure but also change physiology of plant (Arora *et al*., 2012). These salts adversely affect photochemical reactions of photosynthesis, nutrient balance, relative water contents, membrane stability index, protein and carbohydrate synthesis (Mane *et al*., 2011; Talaat & Shawky, 2014). In Pakistan, salinity causes 65% average yield reduction annually (Shafi *et al*., 2010).

Stress tolerant microbial strains colonize roots of agronomic crops and enhance the plant growth by increasing the accessibility of primary nutrients and improving the soil conditions (Adesemoye & Egamberdieva, 2013). The mechanism of actions of PGPR could be direct i.e. biological fixation of atmospheric nitrogen, solubilization of mineral nutrients, production of plant growth promoting hormones, secretion of metabolites and enzymes which make the deficient element available to the plant (Compant *et al*., 2010). Reduction of high ethylene levels and oxidative damage and induction of biochemical changes (accumulation of proline, betains, anti−oxidants) and/or indirect i.e. favoring colonization by other beneficial soil microorganisms, mycorrhizal fungi and reducing the growth of plant pathogens are also important functions of PGPR (Upadhyay *et al*., 2012; Ahemad & Kibret, 2014). Nitrogen fixing bacteria e.g. *Azotobacter* sp., *Azospirillum* sp., plant growth promoting rhizobacteria (PGPR) e.g. *Rhizobium* sp., *Pseudomonas* sp. and arbuscular mycorrhizal fungi (AMF) e.g. *mycorrhizae* are all different types of bio fertilizers (Alizadeh *et al*., 2012).

Single strain inoculation often fails to compete with soil microflora and has poor survival efficiency and root colonization percentage under varying soil and environmental field conditions (pH, moisture and, temperature) (Bashan, 1998; Elkoca *et al*., 2010). Multi−strain inoculation (consortium) improves plant growth through the cumulative effect of various mechanisms adopted by different microbial strains (Khan & Zaidi, 2007). Consortia of multiple bacterial strains in natural environment form biofilms that protect them against desiccation, grazing and anti−microbial agents (Bashan, 1998). Moreover, microbes communicate synergistically with each other in consortium to promote plant growth by decreasing inhibitory products, enhancing absorption of nutrients and more balanced plant nutrition (Wani *et al*., 2007). Moreover, application of liquid inoculum in natural conditions may not be successful due to different environmental constraints i.e. salinity, aridity, drought and erosion (Malusa *et al*., 2012). Besides, survival of bacteria may be poor on seed surface due to desiccation, high temperature and release of acid exudates from seed coat which are toxic to bacterial inoculum (Bashan, 1998; Pandey & Maheshwari, 2007).

To improve the efficiency of bio fertilizers under field conditions formulation of bacterial inoculants in suitable carriers is necessary for their easy handling, long term storage, transmission to the target, protection from

harmful environmental factors, and for enhancing the activity of the organisms (Bashan & Gonzalez, 1999; El−Fattah *et al*., 2013). Carriers are such type of materials required for the application of inoculants, having the potential to support the microbial growth and delivery to the rhizosphere, and they also preserve the microbes till they are applied (Brahmaprakash & Sahu, 2012). The nature of carriers may be organic (e.g. compost, peat, biogas slurry, crushed corn cob) or inorganic (e.g. zeolite, talc, perlite) (Gunjal *et al*., 2012). Keeping in view the above information, present study was planned to evaluate suitability and efficiency of different locally available carrier materials for enhancing the efficacy of rhizobacterial consortium (*Bacillus cereus* strain Y5, *Bacillus* sp. Y14 and *Bacillus subtilis* strain Y16) to improve growth and yield of wheat under salt affected field conditions.

Materials and Methods

Location and soil properties: A field study was carried out to assess the comparative effectiveness of various carriers i.e. compost, peat, biogas slurry and press mud to improve the efficacy of bacterial consortium for enhancing wheat production under saline soil conditions. The experiment was conducted on the research area of the Post Graduate Research Station (PARS), University of Agriculture Faisalabad (UAF). Prior to sowing soil samples were collected for various physico−chemical properties. The textural class of the soil was sandy clay loam having saturation percentage of 34.35%. The chemical characteristics were pH 8.2, CEC 7.15 $cmol_c$ kg^{-1}. Organic matter content of the soil was 0.62%, total nitrogen 0.04%, available phosphorus 7.3 mg kg^{-1} and extractable potassium was 182 mg kg^{-1}. The salinity range of the selected field was 12 dS m^{-1}.

Preparation of inoculums: Three pre−isolated strains i.e. *Bacillus cereus* strain Y5 (KM652420), *Bacillus* sp. Y14 (KM652421) and *Bacillus subtilis* strain Y16 (KM652422) were collected from the Soil Microbiology and Biochemistry Laboratory, Institute of Soil & Environmental Science, University of Agriculture Faisalabad were used for inoculum formulation. Fresh inocula of these selected pre−isolated strains were prepared by taking 100 mL of sterilized Luria Bertani (LB) media broth in four conical flasks having 250 mL volume and sterilized at 15 psi pressure and 121 ºC temperature for 20 minutes. Each sterilized conical flask was inoculated with a strain (along with an un−inoculated control) and incubated at 28 ± 1ºC in a shaking incubator at 100 rpm. After gaining proper population (10^7−10^8 CFU mL^{-1}) of each strain in the flasks, all possible combinations were prepared by mixing respective inoculum in equal proportion (10 mL each) in separate sterilized conical flasks.

Inoculation of carriers and seed treatment: All the selected carrier materials were processed (dried, ground, sieved and sterilized). Each of the selected carrier material was inoculated with a mixture of all bacterial inoculum (100 mL kg^{-1}) and was incubated for overnight and each carrier material was also inoculated with sterilized broth to segregate the effect of carrier from inoculation. For seed coating, seed dressing was carried out with different inoculated carriers mixed with clay and 10% sugar solution. For un−inoculated control, seeds were coated by following same procedure but autoclaved inoculum suspension was used for inoculation of different carriers. One another treatment of multi−strain inoculation with no carrier material was also maintained, where wheat seeds were dipped in liquid inoculum for 15 minutes.

Planting and application of treatments: The treatments were press mud un−inoculated (PM_0), press mud inoculated (PM_1), compost un−inoculated (CM_0), compost inoculated (CM_1), biogas slurry un−inoculated (BS_0), biogas slurry inoculated (BS_1), peat un−inoculated (P_0), peat inoculated (P_1), control un−inoculated (C_0), control inoculated (C_1) arranged according to completely randomized design (RCBD) with three replications. Coated seeds were sown in field and recommended dose of N, P, K fertilizers (120, 60, 60 kg ha^{-1}) were applied into two splits i.e. one at the time of sowing and then along second irrigation water. Good quality water was used for irrigation and all agronomic as well as plant protection measures were adopted.

Assessments: Soil textural class was determined by bouyoucos hydrometer method (Moodie *et al*., 1959). Saturation percentage was figured by Method 27a, U.S. Salinity Lab. Staff (1954). Kent Eil 7015 pH meter was used to determined pH of saturated soil paste (Method 21a, U.S. Salinity Lab. Staff, 1954). Soil electrical conductivity (EC_e) was determined from saturated paste extracts (Rhoades, 1982). Cation exchange capacity (CEC) was estimated by using PFP−7 flame photometer (Method 19, U.S. Salinity Lab. Staff, 1954). Soil organic matter contents were measured according to the procedure explained by Moodie *et al*. (1959). Nitrogen was calculated by sulphuric acid digestion and distillation was done with macro Kjeldhal`s apparatus (Jackson, 1962). Phosphorus was determined by spectrophotometer using 880nm wavelength with the help of standard curve (Watanabe & Olsen, 1965). PFP−7 Flame photometer was used to measure extractable potassium (Method 1la, Salinity lab. staff, 1954).

When plants were at booting stage characteristics regarding gas exchange i.e. photosynthetic rate (*A*), transpiration rate (*E*), intrinsic CO_2 concentration (*Ci*), stomatal conductance (g_s) and water use efficiency (WUE) were measured by using CIRAS−3 (PP System, Amesbury, MA, USA) with PLC3 universal leaf cuvette, measuring both sides of the flag leaves. Cuvette was provided light via light emitting diodes (LED) and with a photon flux of 1000 µmol m^{-2} s^{-1}, ambient leaf temperature and 390 µmol mol^{-1} CO_2. Relative water content (RWC) and Membrane stability index (MSI) were determined by using the following formulas as described by Mayak *et al.* (2004) and Sairam (1994), respectively.

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

$$MSI\ (\%) = \left(1 - \frac{C1}{C2}\right) \times 100$$

Chlorophyll pigments were calculated by following the method and formulas given by Arnon (1949). Proline contents were calculated according to the procedure mentioned by Bates *et al*. (1973). The plant samples were digested to estimate N, P and K following the process of Wolf (1982). After that nitrogen was calculated by Kjeldhal method (Richards, 1954). Phosphorus was calculated by olsen method (Olsen & Sommers, 1982). Potassium was determined with Flame Photometer graded series of standards (ranging from 2−20 ppm) of K using KCl was prepared and standard curve was drawn. The values of soil K were determined from standard curve (Richards, 1954). Crude protein was determined by multiplying the grain N content with a factor of 6.25.

The growth characteristics (plant height, no. of grains per spike, spike length, grain yield, 1000 grain weight, straw yield, no. of tillers per m^2 and no. of spikelets per spike) were observed by following standard methods.

Statistical analysis: Collected data were subjected to analysis of variance (Steel *et al*., 1997) by using Statistix 8.1 software following completely randomized design (RCBD) and means were compared by Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

Gas exchange parameters: Data regarding gas exchange attributes (Table 1) revealed that inoculation in all cases (C_1, CM_1, P_1, BS_1 and, PM_1) significantly improved gas exchange attributes as compared to sole applied carrier materials (CM_0, P_0, BS_0 and, PM_0) and un−inoculated control (C_0) under salt affected field conditions. Maximum improvement in photosynthetic rate (23.95%), transpiration rate (16.87%), stomatal conductance (20.42%), sub stomatal conductance (10.81%), water use efficiency (6.30%), relative water contents (9.72%) and membrane stability index (15.24%) was observed with CM_1 followed by P_1, BS_1 and PM_1 over inoculated control (C_1). Results of alone use of carriers i.e. CM_0, P_0, BS_0 and, PM_0 were non−significant with control (C_0).

Soil salinity decreased relative water contents and membrane stability index which might result in stomatal closure leading to least availability of CO_2 to plants and consequently might cause significant decrease in gas exchange parameters viz. photosynthetic rate, transpiration rate, water use efficiency and stomatal conductance, and increase in sub stomatal conductance of wheat leaves (Talaat & Shawky, 2014). Carrier based multi−strain inoculation noticeably increased photosynthetic rate, transpiration rate, water use efficiency and stomatal conductance, and decreased sub stomatal conductance of wheat leaves. This might be due to reduction of ethylene synthesis through ACC−deaminase activity and resultantly enhanced root growth to explore more soil for nutrient and water uptake. Thus, suppressed Na^+ absorption and increased K^+ and water uptake favored stomatal opening leading to more CO_2 availability (Mane *et al*., 2011). Moreover, this improvement in gas exchange parameters in carrier based multi−strain inoculation might be attributed to the fact that carriers contain organic matter, essential elements and low molecular mass bioactive substances i.e. hormones, humic acids and vitamins that maintain bacterial population (Gunjal *et al*., 2012). They protect microbes from adverse conditions and predatory protozoa, thus, enhance shelf life of inoculant and better survival on seed by its sticky nature (Malusa *et al*., 2012). They have neutral pH and high water retention capacity, and are also rich source of N, K and various trace elements (Mg, Fe, Cu, Co etc.). These elements are involved in carbohydrate metabolism, protein synthesis, enzyme activation and different growth processes of microbes (Pandey & Maheshwari, 2007).

Biochemical parameters: Biochemical parameters like chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, proline and crude protein are presented in (Table 2). Liquid inoculation of multi−strain (C_1) significantly improved biochemical attributes as compared to un−inoculated control (C_0). However, incorporation of multi−strain inoculum in different carrier materials further improved their performance. Carrier based multi−strain inoculations (CM_1, P_1, BS_1 and, PM_1) have a significant impact on biochemical parameters of wheat plant under salt affected field conditions (Table 2). The effect of inoculated carriers i.e. CM_1, P_1, BS_1 and, PM_1 was non−significant among themselves. Inoculated compost (CM_1) increased chlorophyll a by 3.47%, chlorophyll b by 4.20%, total chlorophyll by 4.16%, carotenoids by 9.51% and crude protein by 15.35%, and decreased proline by 12.76% as compared to inoculated control (C_1). Consequences of un−inoculated carriers (CM_0, P_0, BS_0 and, PM_0) were statistically non−significant with control (C_0).

In this study, the effect of carrier based multi−strain inoculation was observed on biochemical parameters i.e. proline, crude protein, chlorophyll a, b and carotenoids of wheat under salt affected field conditions. Results revealed that carrier based multi−strain inoculation with PGPR substantially increased chlorophyll a, b and carotenoids of wheat under saline soils. Prompted ethylene synthesis is responsible for causing senescence in plants (Arshad & Frankenberger, 2002). So, PGPR protected chlorophyll decay by suppressing ethylene synthesis. Plant growth promoting rhizobacteria might have raised the chlorophyll contents by enhancing the uptake of N and Mg, and suppressing Na uptake (Abbasi *et al*., 2011; Alizadeh *et al*., 2012). They might have also induced proline and glycinebetaine accumulation to protect thylakoid membrane from reactive oxygen species (ROS) (Mane *et al*., 2011; Talaat & Shawky, 2014). Moreover, increased total chlorophyll contents might be resulted in higher photosynthetic rate.

Table 1. Gas exchange characteristics of wheat.

Treatments	Photosynthetic rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration rate ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	Water use efficiency ($mmol\ CO_2\ mol^{-1}\ H_2O$)	Stomatal conductance ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	Sub−stomatal conductance ($\mu mol\ CO_2\ mol^{-1}$)	Relative water contents (%)	Membrane stability index (%)
PM_0	12.30 ± 0.36 c	2.16 ± 0.04 c	5.71 ± 0.25 c	311.33 ± 05.36 c	319.33 ± 3.76 a	66.50 ± 1.80 c	41.50 ± 0.76 c
PM_1	18.42 ± 0.65 a	2.74 ± 0.05 a	6.72 ± 0.30 a	460.33 ± 10.09 a	248.33 ± 3.18 c	79.84 ± 0.82 a	56.83 ± 1.17 a
CM_0	12.29 ± 0.45 c	2.16 ± 0.04 c	5.69 ± 0.31 c	311.67 ± 03.18 c	315.67 ± 6.94 a	67.83 ± 2.74 c	42.33 ± 0.44 c
CM_1	19.10 ± 0.96 a	2.84 ± 0.06 a	6.75 ± 0.44 a	475.67 ± 08.29 a	242.00 ± 5.51 c	80.83 ± 1.56 a	58.00 ± 2.08 a
BS_0	12.13 ± 0.32 c	2.12 ± 0.04 c	5.72 ± 0.05 c	311.00 ± 09.07 c	318.67 ± 8.41 a	66.67 ± 1.96 c	41.67 ± 1.30 c
BS_1	18.62 ± 0.68 a	2.81 ± 0.08 a	6.64 ± 0.43 a	470.67 ± 03.84 a	250.67 ± 6.89 c	80.00 ± 1.25 a	57.17 ± 1.88 a
P_0	12.23 ± 0.37 c	2.15 ± 0.04 c	5.69 ± 0.26 c	308.67 ± 09.02 c	319.00 ± 7.23 a	66.67 ± 2.03 c	41.83 ± 1.01 c
P_1	18.57 ± 0.81 a	2.76 ± 0.08 a	6.74 ± 0.21 a	464.67 ± 09.13 a	243.67 ± 4.33 c	80.13 ± 1.50 a	57.67 ± 1.20 a
C_0	12.22 ± 0.32 c	2.15 ± 0.06 c	5.71 ± 0.29 c	309.33 ± 07.62 c	320.33 ± 7.54 a	66.33 ± 1.96 c	41.33 ± 0.88 c
C_1	15.41 ± 0.49 b	2.43 ± 0.06 b	6.35 ± 0.04 b	395.00 ± 05.51 b	271.33 ± 3.76 b	73.67 ± 1.16 b	50.33 ± 1.20 b

Mean values followed by the different letter (s) in the same column are statistically different ($p \leq 0.05$)

PM_0 = press mud un−inoculated, PM_1 = press mud inoculated, CM_0 = compost un−inoculated, CM_1 = compost inoculated, BS_0 = biogas slurry un−inoculated, BS_1 = biogas slurry inoculated, P_0 = peat un−inoculated, P_1 = peat inoculated, C_0 = control un−inoculated, C_1 = control inoculated

Table 2. Biochemical characteristics of wheat.

Treatments	Chlorophyll a ($mg\ g^{-1}$)	Chlorophyll b ($mg\ g^{-1}$)	Total chlorophyll ($mg\ g^{-1}$)	Carotenoids ($mg\ g^{-1}$)	Proline ($\mu mol\ g^{-1}$)	Crude protein (%)
PM_0	2.75 ± 0.02 c	1.35 ± 0.01 c	4.420 ± 0.02 c	0.325 ± 0.01 c	1.04 ± 0.02 a	10.35 ± 0.03 c
PM_1	2.95 ± 0.01 a	1.47 ± 0.02 a	4.803 ± 0.01 a	0.386 ± 0.02 a	0.87 ± 0.03 c	14.00 ± 0.05 a
CM_0	2.76 ± 0.03 c	1.36 ± 0.02 c	4.447 ± 0.02 c	0.327 ± 0.02 c	1.05 ± 0.03 a	10.37 ± 0.02 c
CM_1	2.98 ± 0.02 a	1.49 ± 0.01 a	4.861 ± 0.01 a	0.391 ± 0.01 a	0.82 ± 0.03 c	14.20 ± 0.16 a
BS_0	2.75 ± 0.02 c	1.35 ± 0.01 c	4.433 `± 0.03 c	0.326 ± 0.04 c	1.04 ± 0.01 a	10.35 ± 0.04 c
BS_1	2.96 ± 0.01 a	1.48 ± 0.00 a	4.824 ± 0.00 a	0.387 ± 0.02 a	0.86 ± 0.01 c	14.04 ± 0.07 a
P_0	2.76 ± 0.02 c	1.36 ± 0.03 c	4.441 ± 0.01 c	0.326 ± 0.03 c	1.05 ± 0.02 a	10.36 ± 0.02 c
P_1	2.96 ± 0.02 a	1.48 ± 0.01 a	4.825 ± 0.02 a	0.388 ± 0.03 a	0.87 ± 0.02 c	14.11 ± 0.10 a
C_0	2.73 ± 0.01 c	1.34 ± 0.00 c	4.399 ± 0.02 c	0.324 ± 0.00 c	1.05 ± 0.01 a	10.30 ± 0.08 c
C_1	2.88 ± 0.01 b	1.43 ± 0.01 b	4.667 ± 0.02 b	0.357 ± 0.01 b	0.94 ± 0.00 b	12.31 ± 0.25 b

Mean values followed by the different letter (s) in the same column are statistically different ($p \leq 0.05$)

Carrier based multi−strain PGPR inoculation also induced a marked and progressive increase in crude protein concentration. High concentrations of ethylene are known to inhibit protein synthesis while PGPR used in this study decreased ethylene production due to their ACC−deaminase activity. In plants proline serves as a non−toxic protective osmolyte and its accumulation is an indication of stress tolerance. It regulates osmotic adjustment and protects intracellular macromolecules from dehydration and also functions as a hydroxyl radical scavenger (Han & Lee, 2005; Ahmad *et al*., 2014; Talaat & Shawky, 2014).

In this study, it was found that proline concentration significantly decreased when the salt−stressed plants were inoculated with multi−strains of PGPR. This may imply that PGPR made a significant contribution to plant growth promotion under salinity stress by increasing various metabolic defense strategies (Ahmad *et al*., 2014, Talaat & Shawky, 2014). Moreover, accumulation of compatible solute is an energy consuming process in addition to the already existing metabolic costs. This may imply that PGPR reduced the severity of salt stress on plant and thus proline concentration in the leaves was decreased (Han & Lee, 2005).

However, incorporation of multi−strain inoculum into different carriers further enhanced the performance of PGPR which significantly improved biochemical attributes of wheat growth. This might be due to application of carriers that serves as growth medium for PGPR by providing optimal conditions (Malusa *et al*., 2012). Carriers are non−pollutant and rich source of various macro and micro nutrients that are involved in various metabolic process of PGPR which in turn improved microbial efficiency. They also have better C: N ratio to support increasing microbial count in stressed environmental conditions (Pandey & Maheshwari, 2007). Furthermore, carriers have adjusted pH to neutrality, more water holding capacity, greater porosity and aeration that help in the establishment of dense microbial colonies in the competitive environment (Brahmaprakash & Sahu, 2012).

Table 3. Ionic characteristics of wheat.

Treatments	Na %	K %	K/Na	N in straw %	P in straw %	N in grains %	P in grains %	K in grains %
PM_0	0.67 ± 0.01c	1.32 ± 0.01 c	1.99 ± 0.01 d	1.25 ± 0.00 d	0.23 ± 0.00 d	1.73 ± 0.01 c	0.47 ± 0.01 c	1.45 ± 0.02 c
PM_1	0.44 ± 0.01 a	1.46 ± 0.01 a	3.29 ± 0.09 b	2.07 ± 0.04 b	0.31 ± 0.00 b	2.20 ± 0.04 a	0.72 ± 0.02 a	1.75 ± 0.04 a
CM_0	0.65 ± 0.11 c	1.34 ± 0.00 c	2.07 ± 0.04 d	1.27 ± 0.00 d	0.23 ± 0.00 d	1.75 ± 0.00 c	0.48 ± 0.01 c	1.46 ± 0.03 c
CM_1	0.41 ± 0.01 a	1.47 ± 0.01 a	3.57 ± 0.07 a	2.13 ± 0.06 a	0.32 ± 0.01 a	2.23 ± 0.04 a	0.73 ± 0.02 a	1.77 ± 0.04 a
BS_0	0.66 ± 0.01 c	1.33 ± 0.01 c	2.02 ± 0.05 d	1.26 ± 0.00 d	0.23 ± 0.00 d	1.73 ± 0.01 c	0.47 ± 0.01 c	1.45 ± 0.02 c
BS_1	0.44 ± 0.01 a	1.46 ± 0.01 a	3.36 ± 0.11 ab	2.10 ± 0.05 b	0.31 ± 0.00 a	2.22 ± 0.04 a	0.72 ± 0.02 a	1.76 ± 0.04 a
P_0	0.65 ± 0.01 c	1.33 ± 0.01 c	2.04 ± 0.66 d	1.26 ± 0.01 d	0.23 ± 0.00 d	1.74 ± 0.01 c	0.47 ± 0.01 c	1.45 ± 0.03 c
P_1	0.42 ± 0.01 a	1.47 ± 0.01 a	3.48 ± 0.07 ab	2.11 ± 0.04 ab	0.32 ± 0.01 b	2.22 ± 0.05 a	0.73 ± 0.03 a	1.76 ± 0.05 a
C_0	0.67 ± 0.01 c	1.32 ± 0.01 c	1.96 ± 0.02 d	1.24 ± 0.02 d	0.22 ± 0.01 d	1.72 ± 0.02 c	0.46 ± 0.02 c	1.44 ± 0.04 c
C_1	0.51 ± 0.01 b	1.40 ± 0.00 b	2.73 ± 0.06 c	1.69 ± 0.01 c	0.26 ± 0.00 c	1.98 ± 0.02 b	0.61 ± 0.02 b	1.61 ± 0.03 b

Mean values followed by the different letter (s) in the same column are statistically different ($p \leq 0.05$)

Table 4. Growth and yield characteristics of wheat.

Treatments	Plant height (cm)	No of tillers per plant (m^{-2})	Spike length (cm)	No of grains per spike	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	No of spikelets per spike	1000 grain weight (g)
PM_0	59.23 ± 1.05 de	286.33 ± 4.35 cd	07.65 ± 0.18 c	24.00 ± 1.15 d	1.94 ± 0.01 c	2.92 ± 0.03 cd	12.00 ± 0.57 d	21.45 ± 0.50 de
PM_1	73.60 ± 1.15 b	353.00 ± 2.64 a	10.53 ± 0.30 a	41.33 ± 1.76 b	3.25 ± 0.07 a	4.92 ± 0.04 a	20.66 ± 0.88 b	40.00 ± 0.85 b
CM_0	61.46 ± 1.18 d	286.00 ± 6.74 c	07.95 ± 0.14 c	25.33 ± 0.66 d	2.00 ± 0.07 c	3.00 ± 0.07 c	12.67 ± 0.33 d	22.68 ± 1.10 d
CM_1	77.03 ± 0.72 a	365.00 ± 2.88 a	11.03 ± 0.23 a	45.33 ± 1.76 a	3.35 ± 0.06 a	5.11 ± 0.07 a	22.67 ± 0.88 a	43.00 ± 0.92 a
BS_0	60.28 ± 1.11 de	286.33 ± 4.63 cd	07.75 ± 0.18 c	24.67 ± 1.76 d	1.96 ± 0.02 c	2.95 ± 0.05 cd	12.33 ± 0.88 d	21.93 ± 0.83 de
BS_1	74.73 ± 1.01 ab	357.00 ± 3.46 a	10.80 ± 0.46 a	41.33 ± 0.66 b	3.28 ± 0.07 a	4.98 ± 0.05 a	20.66 ± 0.33 b	40.77 ± 0.84 ab
P_0	58.43 ± 0.82 d	299.00 ± 6.65 cd	07.85 ± 0.18 c	24.66 ± 1.33 d	1.98 ± 0.06 c	2.97 ± 0.06 c	12.33 ± 0.66 d	22.35 ± 0.96 de
P_1	75.95 ± 0.63 ab	361.00 ± 2.64 a	10.85 ± 0.35 a	43.33 ± 1.33 ab	3.31 ± 0.06 a	5.04 ± 0.07 a	21.66 ± 0.66 ab	41.98 ± 1.07 ab
C_0	58.00 ± 0.79 e	285.33 ± 4.05 d	07.50 ± 0.18 c	23.33 ± 0.66 d	1.91 ± 0.02 c	2.70 ± 0.04 d	11.66 ± 0.33 d	19.91 ± 0.06 e
C_1	67.23 ± 0.70 c	331.33 ± 5.48 b	11.03 ± 0.20 b	34.66 ± 0.66 c	2.70 ± 0.01 b	4.16 ± 0.14 b	17.33 ± 0.33 c	32.06 ± 0.84 c

Mean values followed by the different letter in the same column are statistically different ($p \leq 0.05$)

Ionic parameters: Ionic parameters viz. leaf Na^+ and K^+ concentrations and N, P and K contents of grain and straw are presented in Table 3. Multi strain inoculation either sole (C_1) or in combination with carriers (CM_1, P_1, BS_1 and, PM_1) significantly improved ionic parameters. Effect of alone use of carriers (CM_0, P_0, BS_0 and, PM_0) was observed non−significant with un−inoculated control (C_0). Maximum improvement in ionic parameters was obtained from treatment CM_1 followed by P_1, BS_1 and PM_1. Inoculated compost (CM_1) substantially increased K/Na ratio (30.77%) by increasing K (5.00%) and decreasing Na (19.61%) contents as compared to inoculated control (C_1). CM_1 enhanced the uptake of N and P contents of straw by 26.00 and 23.07% over inoculated control (C_1). Furthermore, improvement in N, P and K contents of grain by CM_1 was upto 12.63, 19.67 and 9.94% as compared to inoculated control (C_1) (Table 3).

It is generally assumed that salinity stress causes nutritional imbalance in plants that leads to stunted growth (Han & Lee, 2005, Yue *et al*., 2007). It is evident from the findings of different researchers that salinity causes an increase in Na^+ uptake and thus, decrease in K^+ and Ca^{2+} contents of plant (Marulanda *et al*., 2010; Tank & Saraf, 2010) and results in low K^+/Na^+ ratio. Both these ions compete for entrance into root cells of plant. In saline soils, where concentrations of Na^+ is greater than that of K^+ such competition can have negative effects on plant growth. The results of present study indicated that carrier based multi−strain inoculation of wheat significantly reduced the accumulation of Na^+ and increased the affinity of plants for K^+ uptake in saline soils which resulted in higher K^+/Na^+ ratio. The cause of restricted uptake of Na^+ and increased acquisition of K^+ resulting in higher K^+/Na^+ ratio might be due to better growth and increased surface area of roots to explore more soil by the ACC−deaminase activity of PGPR. Furthermore, PGPR may contribute to the production of exopolysaccharides (EPSs) which have ability to bind Na^+ making it unavailable for plant uptake in salt stressed conditions (Upadhyay *et al*., 2012).

Under saline soils, press−mud, compost, peat and biogas slurry as carrier material for multi−strain inoculation significantly suppressed the Na^+ uptake and enhanced K^+ absorption by plant roots as compared to liquid inoculum and un−inoculated control. This might be due to plant growth promoting characteristics of microbes along with beneficial effects of carriers. Carriers maintained sufficient amount of microbial colonies by

their shielding effect against environmental stresses and toxic compounds present on seed coat (Temprano *et al*., 2002). Moreover, porous nature of carriers supported more microbial colonies through better aeration. Different scientists like Hansen (1994), Kyei *et al*. (2001) and Espiritu (2011) highlighted the importance of carrier materials to improve the plant microbe interaction.

In the current study, results related nitrogen (N), phosphorus (P) and potassium (K) concentrations in straw and grain of wheat indicated that carrier based multi−strain inoculation performed better as compared to un−inoculated control and liquid inoculum under salt affected field conditions. This enhanced concentration of NPK in straw and grain might be due to improved root growth, increased N_2−fixation, phosphorus solubilization and productions of plant growth promoting hormones and other unknown factors in the response of PGPR inoculation (Ahemad & Kibret, 2014).

However, carrier based multi−strain inoculation had significant influence on chemical parameters (N, P and K) of wheat plant under salty field conditions. It was observed that carrier based multi−strain inoculation enhanced nutrient concentration of N, P and K in the treatments where press mud, compost, peat and biogas slurry were used as a carrier material for multi−strain inoculum. Increase in nutrient concentration might be due to superior physico−chemical properties of carriers like increased porosity, greater density and more water retention capacity and lower C: N ratio of carriers which might have enhanced microbial shelf life and performance by providing suitable micro−environment (Pandey & Maheshwari, 2007; Malusa *et al*., 2012).

Growth and yield parameters: Results presented in Table 4 further justified that sole (C_1) and combined use of multi−strain inoculation with carriers (CM_1, P_1, BS_1 and, PM_1) substantially increased growth and yield parameters of plant as compared to un−inoculated control (C_0) and un−inoculated carriers (CM_0, P_0, BS_0 and, PM_0) under salt affected filed conditions. Treatments (CM_0, P_0, BS_0 and, PM_0) where carriers were applied without inoculation, the increase in growth and yield parameters was statistically non−significant as compared to un−inoculated control (C_0). Better growth and yield was observed in case of liquid inoculation (C_1) as compared to alone carriers (CM_0, P_0, BS_0, PM_0) and un−inoculated control (C_0). Again, inoculated compost (CM_1) showed promising results by increasing plant height, no. of tillers per plant, no. of spikelets per spike, spike length, no. of grains per spike, grain yield, straw yield, 1000 grain weight upto 32.82, 27.92, 94.29, 47.11, 94.29, 75.39, 86.04 and 115.90%, respectively, as compared to un−inoculated control (C_0) while this increase in plant height, no. of tillers per plant, no. of spikelets per spike, spike length, no. of grains per spike, grain yield, straw yield, 1000 grain weight was upto 14.57, 10.16, 30.77, 16.75, 30.76, 23.77, 22.74 and 34.09%, respectively, as compared to inoculated control (C_1).

In the present experiment, carrier based multi−strain inoculation significantly improved growth and yield parameters of wheat viz. plant height, no. of tillers, spike length, spikelets, no. of grains per spike, grain and straw yield and 1000 grain weight. This increment in growth and yield parameters might be due to increased activity of plant growth promoting rhizobacteria (PGPR) through multiple mechanisms of action i.e. biological fixation of atmospheric nitrogen, solubilization of mineral nutrients, production of plant growth promoting hormones, secretion of metabolites and enzymes which make the deficient element available to the plant, reduction of high ethylene levels and oxidative damage and induction of biochemical changes (accumulation of proline, betains, anti−oxidants), favoring colonization by other beneficial soil microorganisms, mycorrhizal fungi and reducing the growth of plant pathogens (Upadhyay *et al*., 2012, Ahemad & Kibret, 2014).

However, formulation of multi−strain inoculum in different carrier materials showed remarkable response. Number of workers reported about the improvement in bacterial performance when incorporated into carrier materials as compared to liquid inoculum application (Rice *et al*., 2000; Kyei *et al*., 2002; Albereda *et al*., 2008). Press mud, compost, peat and biogas slurry based inoculants performed better for enhancing plant height, root and shoot growth and all other growth and yield parameters as compared to liquid inoculum. The improvement in the performance of multi−strain inoculum might be attributed to the fact that the micro−environment of different carrier materials is differentially suited for the physiological activities of plant growth promoting bacteria (Roy *et al*., 2010). Moreover, carriers have better porosity, neutral pH, high water holding capacity and improved nutrient status, which maintain better colonization and efficiency of inoculant (Ahmad *et al*., 2014).

Conclusion

In the present study, results showed that carrier based multi−strain inoculation significantly enhanced wheat production by improving gas exchange, biochemical, ionic, growth and yield attributes as compared to liquid inoculation, alone un−inoculated carriers and un−inoculated control under salt affected field conditions.

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