RESEARCH ARTICLE

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Synergetic effect of indigenous PGPR consortium on growth and yield of cultivated wheat (*Triticum aestivum*) in the Saurashtra region of India

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ABSTRACT
This study was carried out to create a microbial consortium from native species of wheat rhizosphere co-inoculated with exotic *Azotobacter chromococcum* to understand their effects on wheat (*Triticum aestivum* L.) growth and crop yield. Seven bioactive bacterial strains were isolated from three district of the semi-arid region of Saurashtra (21.867°N, 70.8120°E), Gujarat, India. The results of the study showed that the PGPR consortium with or without *Azotobacter chroococcum* had a significant impact on wheat yield and quality parameters in relation to the control. Microbial consortia of identified strains *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Acinetobacter sp*., and *Erwinia sp* give the significant result in terms of tillers number (32.5%, 9.5%), shoot height (8.4%, 5.2%), dry shoot weight (38.6%, 4.11%), flower cone number (34%, 16%) at 60 days after showing (DAS). This consortium significantly impacts 1000 seeds weight (31.44%, 25.6%), spikelets spike-1 (29.26%, 14.6%), spike plant-1 (14.79%, 13%) grain yield (24.4%, 11.28%) yield parameters of wheat.

Key words: Rhizobacteria, wheat, consortium, *Pseudomonas*

Introduction

Wheat (*Triticum aestivum* L.) is the most important staple food in India contributing protein, vitamins, dietary fiber, and phytochemicals to the consumers. Wheat mostly cultivated in temperate zones and is in increasing demand in developing countries due to urbanization and industrialization. Wheat production in 2020-21 estimated at 109.24 million tons, the highest ever. Wheat production gets reduced due to fertility loss, drought condition, microbial attack and many more abiotic factors. Chemical fertilizers and pesticides effect on the fertility and rhizospheric microbial community (Huang *et al.* 2021). Now It is necessary to adapt few ways to overcome this problem.

Plant growth promoting rhizobacteria (PGPR) are mutually inhabited around the rhizosphere and rhizoplane area to improve plant health. Majority of the PGPR group belongs to genera *Acinetobacter, Agrobacterium, Arthobacter, Azotobacter, Azospirillum, Bacillus Burkholderia, Bradyrhizobium, Frankia, Pseudomonads, Rhizobium, Serratia and Thiobacillus* according to Glick, 1995 and Vessey, 2003.

PGPR influence the growth and yield of many cereals through direct or indirect mechanism i.e IAA production, phosphate solubilisation, zinc solubilisation, etc. Micronutrient density in staple foods such as wheat play an important role in improving human nutrients on global scale. PGPR help plants in mineral solubilisation and immobilization from the rhizospheric area of plants.

A bacterium does not necessarily possess all the traits necessary for the development of the plant, so it is necessary to prepare mixture of a bacteria with such traits (consortia). The microbial consortia are the mixtures of two or more microbial species, living together symbiotically and performs better than the inoculum of single microbial species (Behera *et al.* 2021). Colonization of microbial consortia around the rhizosphere influence on the shoot growth, root growth, and grain yield of wheat after inoculation of microbial consortia (Cortivo *et al.* 2018).

In the present study, we report isolation, characterization and plant growth promotional activities of all 48 isolates from rhizospheric area of wheat cultivated lands in Saurashtra area, Gujarat, India. All the isolates were tested on wheat cultivar for their plant growth promotional activities. Soil fertility damaged in this area due to uncontrolled use of

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chemical fertilizers in place of natural one. Cow dung and PGPR having a property to restore the bioavailability of nutrients and increase the plant growth. Along with that we analyses the effect of microbial consortium of selected PGPR on wheat growth and development.

Materials and Methods

Sampling site and sample collection

In this study, three geographically distinct areas were selected near to the Junagadh district, Gujarat, India. Cultivated wheat (*Triticum aestivum* L.) were surveyed for the isolation of rhizospheric bacteria. There were 9 samples collected from all geographical locations in September to December 2017 from cultivated wheat. Three sampling sites were selected from each geographical location (Table 1). Soil samples were collected from the rhizosphere area of healthy wheat plants at 15 ± 2 cm depth area of the cultivated wheat containing roots and root-adhered.

Isolation and purification of rhizobateria from wheat (Triticum aestivum L.)

Rhizospheric bacteria were isolated from 1 g soil tightly adhering to the root by serial dilution plating on pseudomonas agar plates and King's B medium as described by Somasegaran *et al.* 1994. The plates were incubated at 28 ± 2 °C for 48 hrs for colony formation. Morphologically unique colonies appeared after 24 hrs were sub-cultured for purification and store at -20°C in nutrient broth medium containing 40% glycerol.

Detection and quantification of plant growth promoting attributes

Most prospective isolates for wheat growth and yield enhancement are screened using IAA generation, phosphate solubilisation, zinc solubilisation, and seed germination assay. The synthesis of indole acetic acid (IAA) was evaluated by culturing rhizobacterial isolates in tryptophan broth and incubating them in the dark at 30 ± 2°C for two days. The amount of IAA produced by bacteria was determined using the Salkowsky reagent (Sherathia *et al.* 2016). Phosphate solubilisation was evaluated by spotting isolates on Pikovskaya agar medium plates containing tri-calcium phosphate($Ca_3(PO_4)_2$). Plates were incubated at 30 ± 2°C for seven days for phosphate solubilisation property. Zone of solubilisation around colonies indicate the positive result for phosphate solubilisation activity (Verma *et al.* 2001). Phosphate solubilisation index was calculated as described by Dey *et al.* 2004.

Wheat PGPR identification using 16S rRNA sequence analysis

The selected wheat PGPR strains were identify by partial sequencing of the 16S rRNA gene. The 16S rRNA gene fragment were amplify from the total genomic DNA by polymerase chain reaction (PCR) using universal primer, 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Galkiewicz & Kellogg 2008) in thermocycler (Master Cycler Gradient 3113, Eppendorf). Using BLAST, the sequencing data were aligned and analyzed to determine the closest neighbours (NCBI, USA). Most related sequences and their accession numbers were retrieve form the GenBank database, National Center for Biotechnology Information (NCBI). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei & Kumar, 2000). Evolutionary analyses were conducted in MEGA11 (Tamura *et al.* 2021).

Field trial design

Microbial consortia formed by mixing seven potential isolates viz *Pseudomonas putida* 13JN, *Pseudomonas aeruginosa* 17JN, *Pseudomonas putida* 1GS, *Pseudomonas aeruginosa* 4GS, *Acinetobacter sp.* 13GS, *Erwinia sp.* 10JM, 11JN. The effect of PGPR consortium with or without of *Azotobacter chroococcum* on wheat var. Lok 1 on field experiments were conducted during rabi season of 2020-21. Each treatment had three randomized independent replications with a single plot of 12 feet × 8 feet in area. Cow dung manure given to each replication was in standard concentration and format.

Experiments were set up in triplicate in a fully randomized design (RCBD). Seeds were treated with microbial inoculum during sowing. After germination (2 weeks after seeding), further treatments of microbial formulations in liquid were applied to the soil. All seven isolates with equal proportion mixed together and applied in field experiment (Table 2).

Effect of consortium on wheat growth and yield

All selected potential isolates were tested for the compatibility with each other so as to develop a consortia formulation with broad spectrum of action and growth promotion. Growth and developmental parameters like total chlorophyll content, shoot length, root length, tiller numbers, plant dry weight, spike per plant, and spikeletes were measured at 30DAS, 45DAS, 75DAS. Various yield parameters like dry shoot weight, average shoot height, number of spikelets per plant and spikes per spikelet, and 1000 seeds weight were recorded at the time of harvesting.

Table 1. *Geographical locations of sampling sites in several districts of Saurashtra region, Gujarat, India*.

Sample ID	Sampling sites	Longitude and latitudes	CFU gm^{-1}	pH
JN1	Dhandhusar Village	21°32'50.1"N 70°20'29.8"E	1.2×10^6	8.01
JN2	Ambaliya Village	21°34'40.5"N 70°21'07.1"E	1.6×10^6	8.25
JN3	Umatvada Village	21°32'07.8"N 70°24'31.8"E	1.5×10^6	8.75
GS1	Gir-gadhada Town	20°54'51.4"N 70°54'59.5"E	2.5×10^6	7.45
GS2	Jankhiya Village	20°55'15.5"N 70°54'02.3"E	2.4×10^6	7.66
GS3	Vadviyala Village	20°53'05.8"N 70°57'52.6"E	2×10^6	7.58
JM1	Gingani Village	21°52'52.6"N 70°04'16.7"E	0.9×10^6	7.92
JM2	Sidsar Village	21°52'00.6"N 70°06'36.4"E	1.3×10^6	7.96
JM3	Hariyasan Village	21°50'58.8"N 70°07'33.5"E	1.2×10^6	7.88

Table 2. *List of bacterial treatments used in the field trial.*

Designation	Treatment detail
C (control)	Un-inoculated
T1	PGPR Consortia (1.2×10^9 CFU) + Dung compost 100 kg ha^{-1}
T2	PGPR consortia (1.2×10^9 CFU) + *Azotobacter chroococcum* (1.0×10^9 CFU) + Dung compost 100 kg ha^{-1}

CFU: colony forming unit; **ha**$^{-1}$ per hector

Statistical analysis

Significant differences among means were compare using Post hoc Tukey test (pair wise comparison at $P = 0.05$) was performed to assess the least significant difference (LSD) between the treatment means at $P \leq 0.05$. Statistical analysis and box plot were carried out in R v3.5.1 using RStudio v4.2.1.

Results

Isolation purification and preservation of rhizospheric isolates

Microbial population of rhizospheric soil represent the health of soil varied from 0.9 x 10^6 to 2.5 x 10^6 (CFU/ml) at different locations of Saurashtra region. A total 86 rhizospheric bacteria isolated from three geographical locations on the basis of their colony morphology. The colony morphology varies as flat to raised, small to large size, entire to undulated margin, transparent to opaque and creamy white, brown, green or yellow pigments. All isolates were kept at -20 °C which immerge with 35% glycerol for long term preservation.

Plant growth promoting traits screening for consortia and field trials

One possible approach is to combine all those isolates which having best plant growth promoting traits. The application of most potential PGP isolates activities in form of consortia are more beneficial in terms of growth, yield and disease resistance of plants. Selection of potential isolates for consortia was on the basis of plant growth promoting response in phosphate solubilisation, indole acetic acid (IAA) production, zinc (Zn) solubilisation, germination assay.

The phosphate solubilisation zone ranged from 13.2 mm to a maximum of 29.3 mm, after 96 hrs of incubation. Phosphate solubilizing activity index of the PGPR isolates ranged from 3.28 to 7.92. The zinc solubilisation zone ranged from 10mm to a maximum of 25.3 mm in 7GS, after 48 hrs of incubation (Figure 1). Zinc solubilizing activity index of the PGPR isolates ranged from 1.80 to 7.17. Among the PGPR isolates, 13GS, 6GS, recorded higher activity indexes of 7.17 and 6.07 respectively. The IAA production ranged from 3.15 µg ml^{-1} to 46.01 µg ml^{-1} broth among the isolates, the maximum in case of isolate 7JM (46.01 µg ml^{-1}) followed by 8GS (41.45 µg ml^{-1}), after 72 hrs of growth (Table 3).

Seed germination assay in controlled condition

Germination effect of all isolates were ranges in between 37 to 100 percentage which is validate by statistical tools. Out of 25 potential isolates, 8 isolates (4JN, 9JN, 11JN, 1GS, 4GS, 13GS, 8JM, 15JM) recorded 100 percent germination. Mean of root length and shoot length of each treatment was varies in between 7.23 cm to 10.66 cm and 9.59 cm to 12.36 cm respectively (Figure 2). Among the 25 PGPR isolates, 22 isolates recorded higher vigour index than the un-inoculated water control (Figure 3).

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Molecular characterization of wheat PGPR isolates

The 16S ribosomal DNA of all seven isolates were PCR amplified and gel purified and subsequently the amplicon of 1486 bp was sequenced by sangers sequencing method. Thereafter, the entire sequence was used to perform blastn at NCBI site for identifying maximum match with the available 16S rDNA sequence database (Table 4). Thus, on the basis of the similarity with the available database of NCBI, these isolates were identified as *Pseudomonas putida* (13JN, 1GS), *Pseudomonas aeruginosa* (17JN, 4GS), *Acinetobacter sp.* (13GS), *Erwinia sp.* (10JM). The phylogenetic tree showed that strains were separated into different clades (Figure 4).

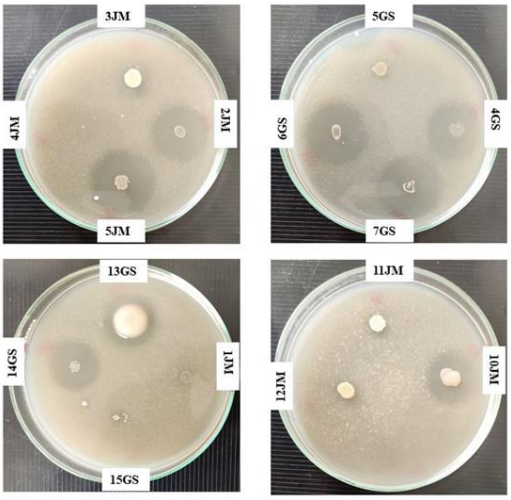

Figure 1. *Zinc solubilisation on modified pikovaskya's agar plate by few PGPRs.*

Figure 2. *Germination effect of wheat seeds in the presence of bacterial isolates during potted condition.*

Effect of PGPR consortia on growth and yield of wheat (Triticum aestivum L.)

PGPR consortia treated plants showed significantly higher values for total chlorophyll content than the control. The total chlorophyll (12.10 µg g^{-1} plant fresh weight) were recorded in the treatment T1 followed by T2 (9.06 mg g^{-1}) at 45DAS (Figure 5C).

The treatment of potential microbial consortia with *Azotobacter chroococcum* (T1) enhance the plant height by 72.57 cm and tiller number 4.42 at 75DAS (Figure 5F). Tiller number increase up to 24.53% in treatment T1 (PGPR Consortia + *Azotobacter chroococcum*) at 75DAS as compare to control (Fig. 5D). Shoot length increase up to 10.75% in treatment T1 at 60DAS followed by 8.31% in T2 (PGPR Consortia) as compare to control. The treatment T1 recorded maximum dry shoot weight (6.85 gm) and dry root weight (4.48 gm) which was higher than treatment T2 (Figure 5A, Figure 5B).

Effect of PGPR consortium on wheat yield

There was a significant effect of microbial consortia with or without *Azotobacter chroococcum* on different yield parameters. Microbial formulation of various potential isolates was significantly increases yield index, grain yield, 1000 seeds weight. However, yield index was high in case of control condition with respect to microbial consortia treatments. The grain yield was 4.20 kg $plot^{-1}$ in treatment T1, and 3.76 kg $plot^{-1}$ in treatment T2.

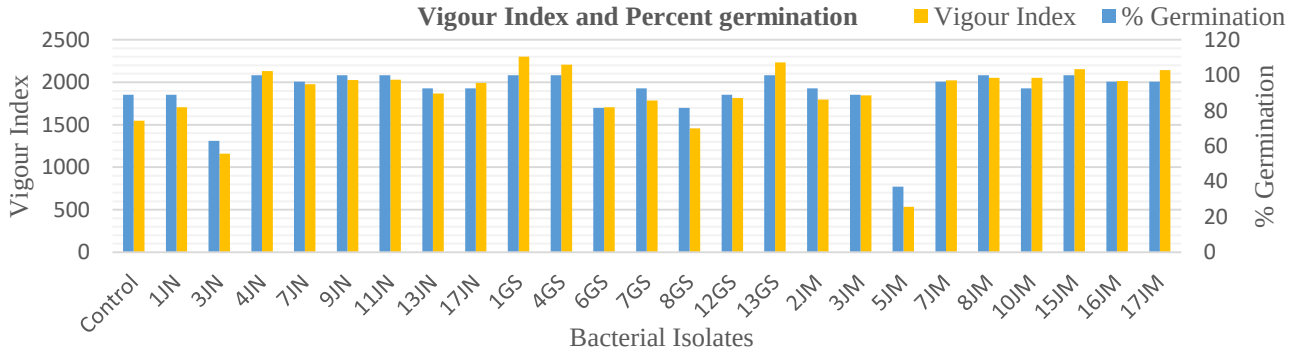

Figure 3. *Effect of PGPR treatments on seed germination, vigour index of wheat seedlings grown in pots analysis.*

Table 3. *Phosphate solubilisation, zinc solubilisation and IAA production properties of some selected potential bacteria isolated from rhizosphere of wheat (Triticum aestivum L.).*

Sr No	Isolates ID	Phosphate Solubilisation (mm)	Zn Solubilisation (mm)	IAA Production (µg ml^{-1})
1	11JN	17.33 ± 0.57	16.0 ± 0.34	12.98 ± 0.970
2	13JN	18.67 ± 1.15	22.3 ± 0.05	34.07 ± 0.774
3	17JN	18.00 ± 1.00	24.0 ± 0.00	12.38 ± 0.442
4	1GS	13.67 ± 0.57	21.3 ± 0.30	12.14 ± 0.837
5	4GS	27.50 ± 0.50	20.0 ± 0.00	17.76 ± 0.676
6	13GS	15.67 ± 1.15	24.6 ± 0.11	15.16 ± 0.632
7	10JM	18.67 ± 0.57	20.0 ± 0.00	3.33 ± 0.592

Data are mean of three replications; ± standard deviation

Table 4. *Similarity index of selected PGPR isolates with respect to 16s rRNA sequence analysis.*

Sr No	Isolates ID	16S rRNA gene (bp)	NCBI Accession Number	Closely related Taxa	Similarity
1	11JN	-	-	Not identify	-
2	13JN	963	MZ356564	*Pseudomonas putida*	100
3	17JN	534	MZ362511	*Pseudomonas aeruginosa*	99.24
4	1GS	739	MZ348541	*Pseudomonas putida*	100
5	4GS	957	MZ350069	*Pseudomonas aeruginosa*	99.37
6	13GS	494	MZ362429	*Acinetobacter sp.*	100
7	10JM	441	MZ362868	*Erwinia sp.*	100

The dry weight of 1000 seeds in treatment T1 was 47.21 gm, whereas 45.13 gm was observed in treatment T2. The yield index in T1 was 0.634, while it was 0.65 in T2 (Table 5).

Discussion

Twenty-five isolates out of forty-eight were able to synthesize IAA in vitro. After 72 hrs of growth, IAA production ranged from 3.15 µg ml^{-1} broth to 46.01 µg ml^{-1} broth which is quite wide range (Abbasi *et al.* 2011). The production of IAA by bacteria isolated from rhizosphere of different crops, i.e., peanut, maize, wheat and rice had already been reported in number of studies (Dey *et al.* 2004; Çakmakçı *et al.* 2007; Mehnaz *et al.* 2010).

The use of plant growth-promoting microorganisms and their consortia as possible bio-inoculants represents a low-cost, alternative to chemical fertilizers, with the ultimate goal of supporting plants in growing and yielding more. On the basis of selective plant growth boosting features, 48 bacterial isolates were extracted and evaluated. All of the isolates were tested for compatibility with one another and seven bacterial strains were chosen for consortium formation. Plant growth-promoting qualities were discovered in all bacterial consortia, including zinc solubilization, phosphate solubilization, indole acetic acid generation.

Furthermore, all bacterial consortia were already evaluated using a wheat seed germination assay. The compatibility of the seven most promising strains was examined in order to create a consortia formulation that would play a more effective role in plant development and yield. According to the results all seven isolates were compatible with one another. The synergy of two or more than two PGPRs has been studied in the last two decades when they are inoculated in the same plant at the same time (Lally *et al.* 2017; Mpanga *et al.* 2019). In comparison to single inoculation, co-inoculation of Rhizobium sp. and *Pseudomonas* sp. in *Phaseolus vulgaris* boosted nodulation and growth metrics (Sánchez *et al.* 2014).

Field trial was done with selected bacterial consortia and the result showed the effect of the application of PGPR with cow manure fertilizers on *Triticum aestivum* L. total chlorophyll content, shoot height, root length, dry shoot weight, dry root weight and tiller number. Microbial formulations with conventional fortification were found to significantly increase all growth parameters recorded at 60DAS and 75DAS.

In few studies it was reported that in comparison to un-inoculated plants, the bacterial isolates boosted plant growth metrics (Rana *et al.* 2011).

Plant height (40.91 %), panicle weight (37 %) and root

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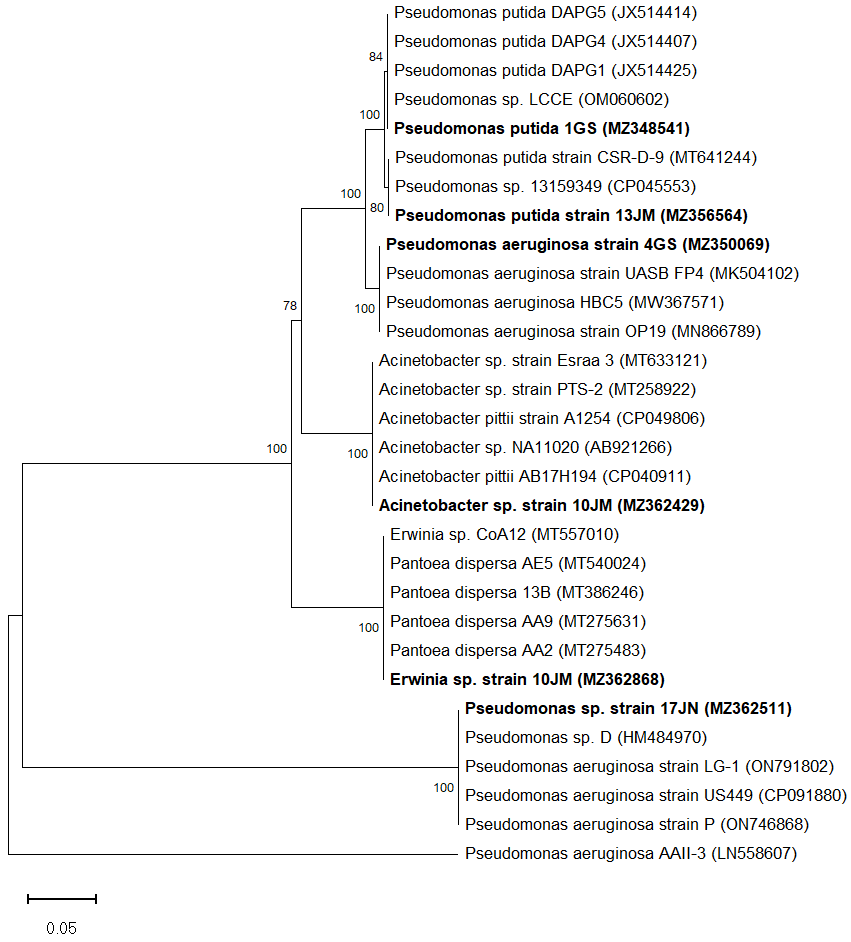

Figure 4. *Phylogenetic tree construction by the Neighbor-Joining method was performed using MEGA 11 software.*

Table 5. *Effect of microbial consortia on yield parameters of wheat (Triticum aestivum L.) grown in field trial.*

Particular	Dry shoot weight pot^{-1}	Average shoot height cm)	Spikelets plant^{-1}	Spike spikelet^{-1}	1000 seeds weight (mg)	Grain yield kg. plot^{-1}	Yield index
Control	5.05 (±0.18)^{a}	68.033 (±2.40)^{a}	3.133 (±0.21)^{a}	19.30 (±0.57)^{a}	35.92 (±0.47)^{a}	3.38 (±0.19)^{a}	0.70 (±0.01)^{a}
T1 – PGPR consortia + *Azotobacter*	7.05 (±0.07)^{b}	73.8 (±1.57)^{ab}	3.533 (±0.32)^{ab}	22.16 (±0.77)^{ab}	47.21 (±0.45)^{b}	4.20 (±0.06)^{b}	0.63 (±0.01)^{b}
T2 – PGPR consortia	5.34 (±0.10)^{b}	71.633 (±0.76)^{b}	2.733 (±0.11)^{b}	21.81 (±1.57)^{b}	45.13 (±0.80)^{c}	3.76 (±0.07)^{c}	0.66 (±0.02)^{b}

'DAS', Days After Sowing; Data are average of three replicates; (±) standard deviation; (SD), Mean with different letters in the same column indicate significantly different means (Tukey HSD post-hoc test, $p<0.05$)

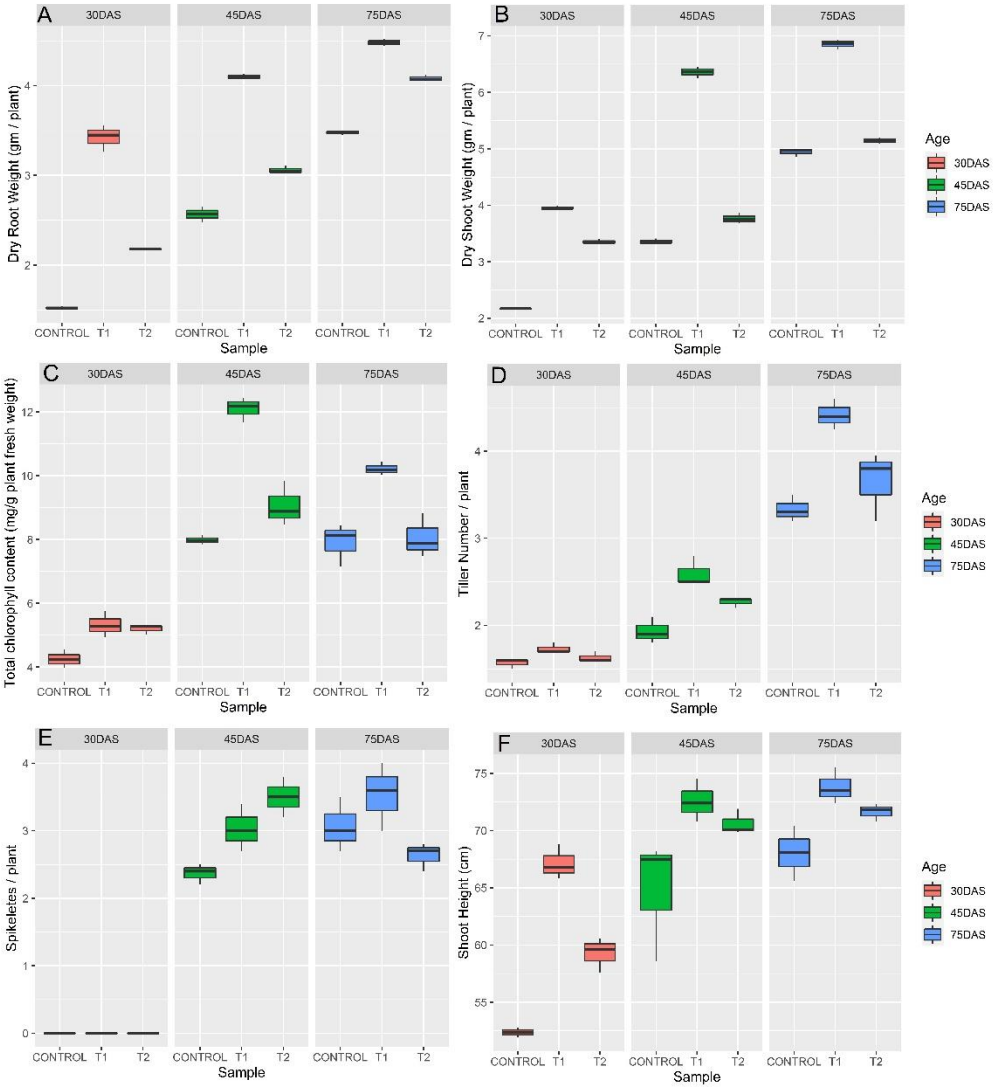

Figure 5. *Box plots showing the range in size distributions for (A) dry root weight, (B) dry shoot weight, (C) chlorophyll content, (D) tiller number, (E) spikeletes number and (F) shoot height of wheat plants grown in the field trial condition, inoculated with microbial consortia of plant growth-promoting rhizobacteria with un-inoculated control. The horizontal line inside the box is the median and the edges of the box inter-quartile ranges.*

weight (85.71 %) all rose significantly as a result of the PGPR inoculants. According to Afzal *et al.* 2005 and Sheirdil *et al.* 2019), PGPRs had a beneficial influence on the number of tillers, with an increase of up to 25% in the number of tillers in wheat following their application. Compared to T1 the other treatment T2 gave better result, it showed, maximum dry shoot weight (6.85 gm) and root weight (4.48 gm). Several studies revealed that all of the bacterial isolates increased shoot and root length, dry weight and N content in inoculated wheat seedlings. (Molina-Romero *et al.* 2017) demonstrated that a consortium of desiccation-tolerant bacteria (*Pseudomonas putida* KT2440, *Acinetobacter sp*. EMM02, *Sphingomonas sp*. OF178 and *Azospirillum brasilense* SP7) could cling to seeds and colonise the

Figure 6. *Plant of PGPR consortium on growth at 60DAS in field trial.*

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rhizosphere of maize. On different yield characteristics, microbial consortia with or without *Azotobacter* had a substantial effect.

Microbial formulation of multiple possible isolates increased yield index, grain yield and 1000 seed weight substantially. In comparison to microbial consortia treatments, however, the yield index was high in the control condition (Ozturk *et al.* 2003, Marques *et al.* 2010, Zhang *et al.* 2012).

Conclusion

In present study indicates that the application of *Azotobacter chroococcum* along with indigenous PGPR of wheat plants can synergistically impact on the growth and yield of wheat plants because of the phytohormones production, more phosphate and zinc availability. When wheat treated with *Azotobacter chroococcum* strains with the PGPR tested on wheat plant in cow manure fortified agricultural soil attained significant development as compare to its control. Under filed trial condition it was observed that the Wheat PGPR consortia of strain *Pseudomonas putida* (13JN, 1GS), *Pseudomonas aeruginosa* (17JN, 4GS), *Acinetobacter sp.* (13GS), *Erwinia* sp. (10JM) and 11JN give the adequate results in terms of tillers number, shoot height, dry shoot weight, flower cone number at 60 days after showing. This consortium significantly increases the 1000 seeds weight, spikelets spike^{-1}, spike plant^{-1}, grain yield of wheat. The positive impact of the consortium shows that the application of *Azotobacter chroococcum* with the consortium can lead to better improvements in terms of growth and yield.

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