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RESEARCH ARTICLE

The stimulatory effects of L-tryptophan and plant growth promoting rhizobacteria (PGPR) on soil health and physiology of wheat

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Abstract

During the present study, *Pseudomonas moraviensis* and *Bacillus cereus*, were isolated from rhizosphere soil of halophytic weed (*cenchrus ciliaris* L.) of Khewra salt range, and used as bioinoculants. The plant growth promoting rhizobacteria (PGPR) were applied to wheat (*Triticum aestivum*) by seeds soaking, and aqueous solution of tryptophan was added to the rhizosphere soil at 1ug/L, after seed germination. Experiment was conducted at Quaid-e-Azam University Islamabad both in pots (filled with sterilized soil) under axenic condition and in field under natural condition, for two consecutive years. The inoculation of *Pseudomonas moraviensis* and *Bacillus cereus,* significantly increased the organic matter, P, K, Ca, and NO_3-N availability of soil. The inoculation of these PGPR positively enhanced growth and physiology of treated plants, and this affect was further augmented in the presence of tryptophan. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* increased the fresh weight, proline contents and activities of antioxidant enzymes significantly over control. Added tryptophan with both PGPR, improved the number of plants at yield and seeds establishment by improving number of seeds/spike and spike length. Effects of PGPR inoculation alone and with tryptophan were more pronounced in pots grown plants. It is inferred from the results, that tryptophan addition is a competent source for increasing potential of PGPR, thereby improving wheat growth, and physiology.

Keywords: PGPR and wheat, *Bacillus cereus, Pseudomonas*, antioxidants, L-tryptophan

1. Introduction

One of the most important aspects of PGPR is phytohormones production for improving growth and physiology of corps. Among these phytohormones, indoleacetic acid (IAA) commonly known as auxin is of prime importance. Agriculturally important PGPR are screened out on the basis of their potential to produce IAA. The production of auxin depends upon response of plant seedling and types of applied microbial inoculants. Microbial strains having ability to produce higher and lower amount of IAA, or indoleacetamide (IAM) have resulted increase growth and yield of wheat (Tsavkelova *et al*., 2007). L-tryptophan, the precursor of IAA, is naturally present in root exudates of plants (Villareal *et al*., 2012). It is also synthesized by hydrolysis of proteins of dead cells (Patten and Glick 1996), and

is converted into indole acetic acid by the activity of plant growth promoting rhizobacteria (Sasirekha *et al*.,2012).

Application of L-tryptophan in soil has proved very fruitful for increasing growth of many vegetables as well as crops like chickpea (Abbas *et al*., 2013) and wheat (Mohite, 2013). It is believed that about 80% of bacterial isolates from rhizosphere soil are capable of synthesizing IAA (Idris *et al*., 2004)

Microbial bioinoculants have ability to improve the availability of soil nutrients and modulation of phytohormones (Hayyat *et al*., 2013). Auxin biosynthesis in bacteria is affected by a number of factors, including environmental stress, pH, osmotic and matrix stresses, carbon starvation, and the composition of the root exudates.

Bacillus cereus has the potential to increase the yield, growth and nutrition of broccoli plant under organic growing conditions. It is efficient phosphate solubilizer and a bio pesticide against the fungal pathogens that attack the plants during the nodulation stage of *Pigeon Pea* (Rani *et al*., 2011). The use and effectiveness of *Bacillus cereus* as PGPR has previously been proved (Zhao *et al*., 2011).

Pseudomonas spp and their consortium with other microbial strains improved growth and yield of wheat (Rosas *et al*., 2009). Similarly, inoculation of *Pseudomonas fluorescence* increased roots and shoots mass in sugarcane (Mehnaz *et al*., 2009). *Pseudomonas aeruginosa* has been used for promoting growth of cow pea (Sasirekha *et al*., 2012). *Pseudomonas* inoculation on winter wheat depends upon development phase of wheat as well as on population size of *Pseudomonas* (Wachowska *et al*., 2006).

This paper reports the effects of tryptophan alone and in association with two plant growth promoting bacteria, *Bacillus cereus* and *Pseudomonas moraviensis* on the growth and physiology of wheat under pots and field conditions. The work highlights the effectiveness of these PGPR and tryptophan contribution in modulation of nutrient status of soil and their accumulation in the leaves.

2. Material and Methods

2.1. Isolation of endophytic microbes and determination of colony forming unit

The Buffle grass (*Cenchrus ciliaris* L.), a naturally growing halophyte was uprooted when the plants were 13-15 cm high. Roots were washed with tap water followed by washing with autoclaved water. Grinded roots (1 g) was suspended in 9 ml autoclaved distilled water and an aliquot (100 µl) from decimal dilution was used to inoculate LB culture media. The culture plates were incubated for 24-72 h at 27 ^{0}C. The number of viable cell counts at 10^7 dilution were calculated following the formula.

Viable cell count (CFU/g) = (number of colonies/ volume of inocula) x dilution factor

a) DNA extraction

Extraction of genomic DNA of bacterial strain was carried out by using the Gen Elute Bacterial Genomic DNA Kit.

b) PCR amplification

The genomic DNA of PGPR was amplified by the method as described by Weisburg (1991). The polymerase chain reaction (PCR) was carried out by using forward (fd1) primer having nucleotide sequence AGAGTTTGATCCTGGCTCAG and reverse (rd1) primer (AAGGAGGTGATCCAGCC). After denaturation at 95 ^{o}C for 2 min, 30 rounds of temperature cycling (94 ^{o}C for 30 sec, 55 ^{o}C for 30 sec

and 72 °C for 2 min) were followed by incubation at 72 °C for 10 min. Then, 5µl of amplified PCR products were electrophoresed on 1.2% (w/v) agarose gel, in 1 X TBE buffer at 80 V and then stained with ethidium bromide (0.01g/ml). Gel was visualized under UV transilluminator lamp (S. N. 76S/64069, Bio RAD, Italy) and photographed.

c) Sequencing for 16S rRNA

Sequencing was done using Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA) and the sequencing products were resolved on sequencer (ABI 3730 x l DNA Analyzer (Applied BioSystems, USA) at the Macrogen, Inc Seoul, Korea. The results were compared at NCBI site using BLAST algorithm (www. ncbi.nlm.nih.gov/BLAST. The isolates were identified as *Pseudomonas moraviensis* (Accession No. LN714047) and *Bacillus cereus* (Accession No. LN714048) and data was submitted to gene bank.

d) Plant materials and growth conditions

Seeds of *Triticum aestivum* L. variety Inqlab 91 were obtained from National Agriculture Research Council Islamabad (NARC) and were grown at Quaid-e-Azam University, Islamabad (maximum average temperature = 21.9 ^{0}C, rainfall =8.98mm. and relative humidity = 67.16%) both in pots under axenic condition (EC 0.30 dSm-^{1}) and in field under natural condition. For field experiment, field measuring 10 x 10 m^2 was prepared with 36 cm row to rows distance. Seeds were sown by hand drill method in field soil having silt: clay: sand in 14:11:75. Simultaneously, earthen pots measuring 17 x 20 cm^2, containing 8 Kg soil/ pot were filled with autoclaved soil and mixed with sand in 3:1 ratio. No chemical or organic fertilizer was added in the soils. In each pot 10-15 seeds were sown and five plants/ pots were maintained till maturity.

Treatments include inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with and without addition of tryptophan. Uninoculated plants were taken as control while tryptophan without any PGPR was also applied. Plants sampling was done at early vegetative stage (57 DAS) for physiological parameters and at maturity (159 DAS) for yield parameters.

Prior to sowing seeds were surface sterilized with 70% ethanol for 5 min followed by soaking the seeds in 10% chlorox and successively washed with autoclave distilled water. The sterilized seeds were soaked in 7d old microbial cultures having 10^{6}cell/ ml. After shade drying seeds were sown under field condition. The RCBD design was used in field while CRD was followed for pots grown plants. After 7d of germination of seeds aqueous solution of L-tryptophan 1ug/L was applied in rooting zone of seedlings.

e) Sampling and chemical analysis of rhizosphere soil

The rhizospheric soil samples of wheat were collected at early vegetative stage (57 DAS) below 7-10 cm from surface. Soil samples were homogenized, and sieved through 2 mm sieve and processed for the isolation of rhizobacteria and determination of physico-chemical properties.

f) Soil organic matter

Soil organic matter was determined by method of Walkley and Black, 1934.

g) Nitrate-N (NO_3-N) and Phosphorus (P)

Nitrate-N (NO_3-N) and Phosphorus (P) were extracted from rhizosphere soil following the method of Reitemeier (1943).

h) Chlorophyll contents of leaves

The chlorophyll contents of leaves were determined by chlorophyll meter (SPAD 502 plus)

2.2 .Proline content

Proline contents were measured by the method of Bates *et al*., (1973) and activity for peroxidase was measured by method of Vetter *et al*., (1958). Fresh leaves (5 g) were homogenized with 15 ml of 0.05N phosphate buffer (pH 7.0) containing 10% polyvinyl poly pyrrolidone and 0.1 M Ethylene diamine tetra acetate (EDTA).

2.3. Assay for Peroxidase activity (POD)

The assay mixture contained 0.1ml enzyme extract, 1.35 ml of 100 mM MES buffer (pH 5.5), 0.05% H_2O_2 and 0.1% phenylenediamine. Change in absorbance was recorded at 485 nm with spectrophotometer (UV-120-01, Shimadzu). The activity of POD was presented as ΔOD 485 $nmmin^{-1}$ mg^{-1}.

2.4. Assay for superoxide dismutase activity (SOD)

SOD activity was determined by measuring inhibition of photochemical reduction of nitrobluetetrazolium (NBT) using method of Beauchamp and Fridovich (1971).

2.5. Statistical analyses

The statistical analyses of the data were conducted using analysis of variance (ANOVA) in Statistix program, version 8.1. In field experiments Randomize Block Design (RCBD) was followed and for pots experiment Complete Randomized Design (CRD) was applied. Mean values were separated (p=0.05) and represented by different letters both in tables and figures along with ±standard error.

3. Results and Discussion

The endophytic bacteria, residing in the roots of plants, are the tools of extensive studies. The application of these endophytes as Plant growth promoting bacteria (PGPB) and their role in crop improvement is widely documented. *Bacillus cereus* and *Pseudomonas moraviensis* has also been isolated and tested for their growth promoting potential previously (Zhao *et al*., 2011; Yadav *et al*., 2013).

3.1. Soil nutrients

Inoculation of *Bacillus cereus* and *Pseudomonas moraviensis,* exhibited 35% higher soil organic matter in rizosphere of pots grown wheat plants, and a further 25% increase was observed both in pot and field grown plants, when tryptophan was added.

The PGPR bioinoculants increased the availability of phosphorous and enriched the rhizophere with NO_3 – N. Increase in NO_3 –N (Table 1) was 24% and 45% when *Pseudomonas moraviensis* or *Bacillus cereus* were applied separately in fields and plant grown plants respectively. The PGPR resulted further 24-30% increase in NO_3 –N in the presence of tryptophan. Increase in P contents of rhizosphere soil was 25% and 16% higher over control in pots and field grown plants. Tryptophan addition further increased P contents of pots grown plants by 28%. Similarly, tryptophan addition with PGPR enhanced the K contents of rhizosphere soil by 35% over control, both in pots and field grown plants. PGPR application improved Ca and Mg contents of rhizosphere soil of wheat by 20% over control (Table 1). Application of tryptophan with PGPR showed further 10% increase in pots and field grown plants. Similarly, increase in

Mg contents was 16% higher in pots and 40% in field when PGPR were added with tryptophan.

The contribution of PGPR in improving plant growth is directly correlated with the mechanism by which they enhance the availability of nutrients (N, P and K) (Shaharoona *et al*., 2008). The increased phosphorus, NO_3-N contents in treated soil, with tryptophan and PGPR might be attributed to phosphate solublization and N-fixing ability of *Pseudomonas moraviensis* and *Bacillus cereus* (Schoebitz *et al* 2013; Yadav *et al*., 2013). Tryptophan addition to soil stimulated the ability of applied PGPR in improving N, P, K, and other nutrients. Improvement in K, Ca, Mg, and Fe attributed the ability of PGPR in balancing nutrients. The increase in nutrients uptake of PGPR treated plants is in agreement with previous findings of Minaxi *et al*., (2013). (Table 2)

3.2. Leaves nutrients

Table 1. Effects of PGPR on organic matter (%), macro and micronutrients (mg/Kg) of rhizosphere soil. Measurements were made at 57 DAS (2-3 leaf stage). Values are mean of four replicates.

	O.M		P		NO_3-N		K^+		Ca^+		Mg^+	
	(%)		(mg/kg)									
Treatments	Field	Pots	Field	Pots	Field	Pots	Field	Pots	Field	Pots	Field	Pots
control*	0.567e	0.45d	4.71d	0.561c	16.43c	14.42e	77.41f	105.21c	27.5c	32.89F	4.56e	7.666e
	(±0.08)	(±0.08)	(±0.22)	(±0.08)	(±0.44)	(±0.23)	(±2.90)	(±2.23)	(±0.76)	(±0.76)	(±0.21)	(±0.89)
P.moraviensis	0.687b	0.62c	5.89b	0.623b	21.75c	21.12b	88.8c	111.54c	32.01ab	40.22c	5.65c	9.67d
	(±0.02)	(±0.06)	(±0.12)	(±0.02)	(±0.76)	(±0.39)	(±3.98)	(±1.21)	(±0.77)	(±1.11)	(±0.12)	(±0.49)
B. cereus	0.683b	0.67c	5.66b	0.648b	20.53b	20.23b	87.5c	112.21c	32.17ab	37.76d	5.85c	10.5c
	(±0.03)	(±0.1)	(±0.04)	(±0.03)	(±0.47)	(±0.54)	(±2.22)	(±1.6)	(±0.11)	(±0.79)	(±0.18)	(±0.64)
P.moraviensis+tryp	0.786a	0.772a	6.26a	0.781a	25.43a	23.31a	103.7a	121.56a	38.97a	45.45a	6.31a	12.7b
	(±0.06)	(±0.05)	(±0.04)	(±0.1)	(±0.43)	(±0.11)	(±5.48)	(±1.11)	(±0.47)	(±0.56)	(±0.19)	(±0.43)
B.cereus+tryp	0.807a	0.733b	6.28a	0.809a	23.45a	22.42a	98.29b	124.49ab	34.05a	44.11b	6.36a	12.922a
	(±0.07)	(±0.07)	(±0.06)	(±0.08)	(±0.45)	(±0.56)	(±3.42)	(±1.18)	(±0.88)	(±0.43)	(±0.16)	(±0.93)
tryp	0.609c	0.48c	5.01c	0.611b	20.24b	17.11c	80.46d	106.58c	28.48c	30.1g	5.05d	8.28d
	(±0.04)	(±0.02)	(±0.09)	(±0.11)	(±0.24)	(±0.44)	(±6.66)	(±1.76)	(±0.78)	(±0.32)	(±0.14)	(±0.77)
LSD	0.59	0.99	2.11	1.44	3.09	2.98	1.39	1.72	3.31	2.88	1.23	1.09

*= untreated uninoculated control, O.M= organic matter, *P.moraviensis = Pseudomonas moraviensis, B.cerues = Bacillus cereus,* tryp = tryptophan**.** Values followed by different letters are significantly at (P<0.05). Values represented in parenthesis are standard error of means.

Table 2. Effects of PGPR application on leaves nutrients contents (mg/kg). Measurements were made at After 57 DAS (2-3 leaf stage).

Treatments	Ca		K		Mg	
	Field	Pots	Field	Pots	Field	Pots
control*	16.46c	12.22c	13.38c	14.11c	15.95c	9.05c
	(±0.17)	(±0.78)	(±0.15)	(±0.66)	(±0.54)	(±0.98)
P.moraviensis	19.51b	15.63b	17.63b	21.21b	21.46b	13.23b
	(±0.11)	(±0.92)	(±0.14)	(±0.44)	(±0.98)	(±0.65)
B. cereus	19.9b	16.46b	18.77b	19.34b	20.54b	12.49b
	(±0.14)	(±0.65)	(±0.12)	(±0.55)	(±0.13)	(±0.43)
P.moraviensis+tryp	22.09a	19.13a	19.14a	25.56a	22.67a	14.81a
	(±0.12)	(±0.88)	(±0.41)	(±0.89)	(±0.33)	(±0.92)
B.cereus+tryp	21.96a	20.87a	18.78a	24.43a	23.02a	15.5a
	(±0.15)	(±0.56)	(±0.31)	(±1.11)	(±0.16)	(±1.02)
tryp	16.86e	13.88c	13.48c	14.33c	16.24c	10.06c
	(±0.18)	(±0.45)	(±0.36)	(±1.65)	(±0.14)	(±0.45)
LSD	3.33	4.41	2.97	5.09	2.22	4.67

*= untreated uninoculated control, *P.moraviensis = Pseudomonas moraviensis, B.cerues = Bacillus cereus,* tryp = tryptophan**.** Values followed by different letters are significantly at (P<0.05). Values represented in parenthesis are standard error of means.

Bacillus Cereus and *Pseudomonas moraviensis* improved the nutrient acquisition in treated wheat leaves in presence or absence of tryptophan. The Ca contents of leaves were increased by 18% in field and 28% in pots grown plants, following the treatment of *Pseudomonas moraviensis* and *Bacillus cereus*. Tryptophan addition further enhanced Ca by 19% and 37% in field and pots grown plants. Similarly the K contents of leaves were increased significantly (30% and 50%) in field and pots grown plants, and tryptophan addition further increased K contents by 15-20% both in pots and field grown plants. The Mg contents were increased by 30-40% when PGPR were applied singly, and with tryptophan further 15-20% higher Mg was observed. Nutrient availability and uptake is reported to be enhanced by PGPR in wheat. The higher accumulation of nutrients in treated leaves might be attributed to the PGPR proficiency in improving nutrient absorption and their translocation (Aslantas *et al*., 2007).

3.3. Wheat growth

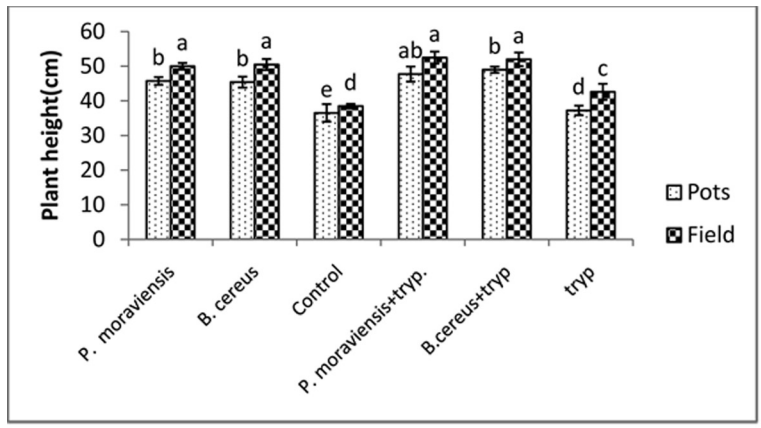

Figure 1. Plant height (cm) treated with *Pseudomonas moraviensis* and *Bacillus cereus* alone and in addition with tryptophan. Control= untreated uninoculated plants, *P.moraviensis = Pseudomonas moraviensis, B.cerues = Bacillus cereus* and tryp= tryptophan alone. Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (P<0.05).

Both *Pseudomonas moraviensis* and *Bacillus cereus* increased plant height (Figure 1) in pots and field. Inoculation of *Pseudomonas moraviensis* increased the plant height of wheat grown in pots and field by 32 % and 25% respectively. Tryptophan addition with *Pseudomonas moraviensis* further increased plant height by 4% and 9% in field and pots grown plants respectively. Similarly, *Bacillus cereus* increased plant height by 31% and 23% and further 4% and 7% increase was observed in pots and field grown plants in the presence of tryptophan. The evidenced increase in plant height of inoculated wheat in the presence of tryptophan and PGPR attributed the enhanced IAA availability, which induce cell division and cell elongation. The readily available tryptophan in treated plants is converted into IAA, thereby improving plant height as documented previously (Mohite, 2013).

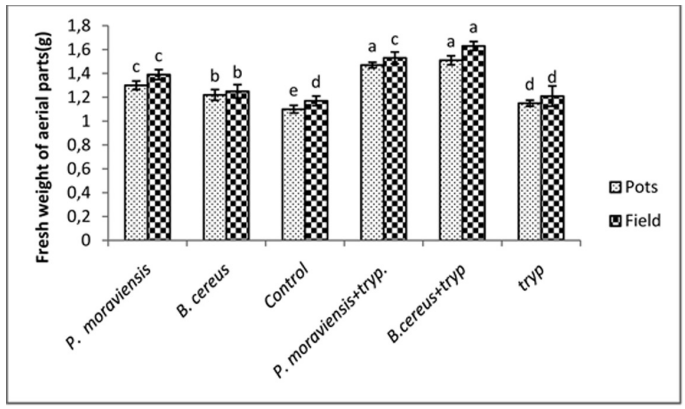

Figure 2. Fresh weight (g) of aerial parts of single plant treated with *Pseudomonas moraviensis* and *Bacillus cereus* alone and with tryptophan. Control= untreated uninoculated plants, *P.moraviensis = Pseudomonas moraviensis, B.cerues = Bacillus cereus and* tryp= tryptophan alone. Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (P<0.05).

The *Pseudomonas moraviensis* increased the fresh weight (Figure 2) by 19% and 18% in field and pots respectively. Addition of tryptophan further increased 11% fresh weight in field and 16% in pots. *Bacillus cereus* application with tryptophan exhibited 30-35% over control. The observed increase in fresh weight may be attributed to IAA induced water, nutrient uptake, and proliferation of root system (Mohite, 2013). Increase in plant height and fresh weight of PGPR treated plants, in the presence or absence of tryptophan also insinuate toward the role of PGPR in enhancing nutrients availability as well as improved organic matter in the soil (Sasirekha *et al.,* 2012).

3.4. Wheat physiology

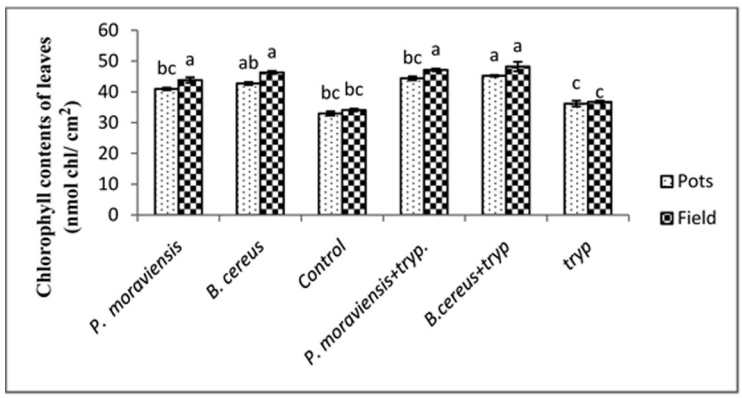

Figure 3. Chlorophyll contents **(**nmolchl/ cm^2**)** of leaves treated with *Pseudomonas moraviensis* and *Bacillus cereus* alone and with tryptophan. Control= untreated uninoculated plants, *P .moraviensis = Pseudomonas moraviensis, B.cerues = Bacillus cereus* andtryp= tryptophan alone. Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (P<0.05).

Inoculation with *Pseudomonas moraviensis* increased chlorophyll contents (Figure 3) equally 24-26% in pots and field. Addition of tryptophan with *Pseudomonas moraviensis* further increased 8-12% chlorophyll contents both in pots and field. *Bacillus cereus* exhibited significantly higher (26-35%) chlorophyll content in pots and field. Addition of tryptophan with *Bacillus cereus* further increased 11% chlorophyll in field and 8% in pots. The improved chlorophyll contents might be attributed to the ability of associated PGPR, residing there in to assist water and mineral absorption (Bashan *et al*., 2004).

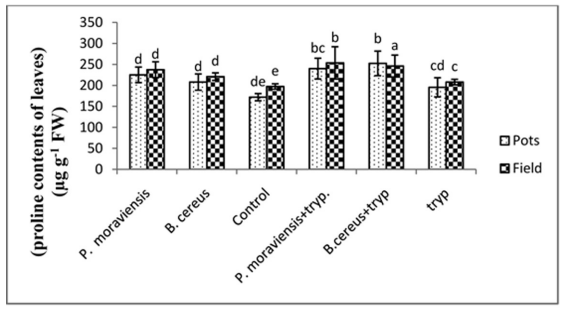

Figure 4. Proline.contents of leaves **(µg g^{-1})** of leaves treated with *Pseudomonas moraviensis* and *Bacillus cereus* alone and with tryptophan. ontrol= untreated uninoculated plants, *P moraviensis* = *Pseudomonas moraviensis, B.cerues* = *Bacillus cereus* andtryp= tryptophan alone. Values given are mean of four replicates with ± SE. Values followed by different letters heading the bars are significantly different (P<0.05).

Pseudomonas moraviensis increased proline contents by 17% in field and 31% in pots. Addition of tryptophan with *Pseudomonas moraviensis* further increased the proline contents of plants by 8-10% both in pots and field. *Bacillus cereus* increased proline content by 12% and 21% over control in pots and field respectively. This increase was 24% and 49% in *Bacillus cereus* + tryp treatment. The observed increase in chlorophyll and proline contents reflects the role of PGPR in stomatal conductance, osmoregulation, and photosynthesis (Prado *et al*., 2000). Proline acts as a source of organic nitrogen reserve, osmoprotectant and antioxidant under stress and PGPR application imparted stimulatory effects on proline accumulation (Ali *et al*, 2013).

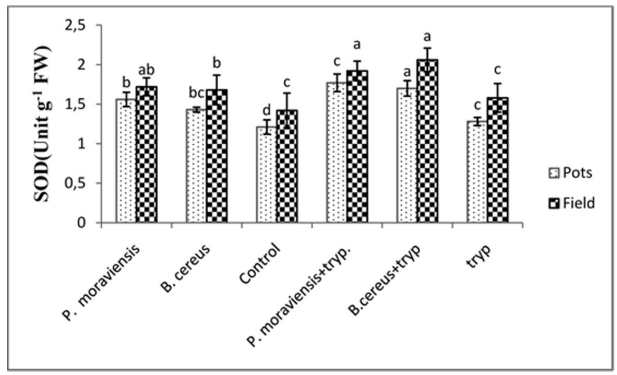

Figure 5. SOD activity (unit g^{-1} FW) of leaves treated with *Pseudomonas moraviensis* and *Bacillus cereus* alone and with tryptophan. Control= untreated uninoculated plants, *P .moraviensis* = *Pseudomonas moraviensis, B.cerues* = *Bacillus cereus* andtryp= tryptophan alone. Values given are mean of four replicates with ± SE. Values followed by different letters heading the bars are significantly different (P<0.05).

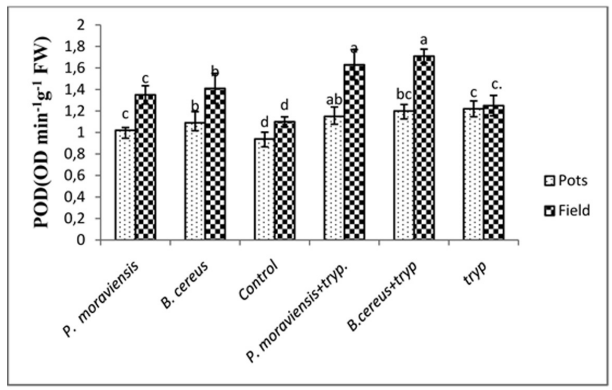

Figure 6. POD activity (OD min^{-1}g^{-1} FW) of leaves treated with *Pseudomonas moraviensis* and *Bacillus cereus* alone and with tryptophan. Control= untreated uninoculated plants, *P .moraviensis = Pseudomonas moraviensis, B.cerues = Bacillus cereus* andtryp= tryptophan alone. Values given are mean of four replicates with ± SE. Values followed by different letters heading the bars are significantly different (P<0.05).

Plants treated with *Pseudomonas moraviensis* showed 20% higher SOD (Figure 5) and POD (Figure 6) activities in field. This increase was 29% and 9% higher over control in pots grown plants. In the presence of tryptophan *Pseudomonas moraviensis* exhibited further 14% higher SOD and 26% higher POD activity in field, and 17% and 13% in pots. *Bacillus cereus* showed 11% higher SOD and 28% higher POD in field and 18% in pots respectively. Addition of tryptophan exhibited further 27% higher SOD and 34% higher POD activities in field. The increase in SOD and POD was 22% and 12% over control in pots. The observed increase in antioxidant activities, may be attributed to the positive role of PGPR on detoxification of reactive oxygen species (Faize and Burgos, 2011).

3.5. Wheat yield

Table 3. Effects of PGPR application on yield parameters of wheat at maturity. Measurements were made at 159 DAS.

Treatments	Plants/m^2		spike length (cm)		seeds/spike		seeds weight (g)	
	Field	Pots	Field	Pots	Field	Pots	Field	Pots
control*	222.5b	--	5.2c	4.4c	39b	25.25e	39.67b	37.23b
	(±7.94)	--	(±0.11)	(±0.16)	(±3.45)	(±3.73)	(±1.24)	(±0.95)
P.moraviensis	239.25b	--	6a	5.2ab	45.75a	32.25c	50.76a	43.19a
	(±6.09)	--	(±0.15)	(±0.1)	(±0.85)	(±3.5)	(±0.79)	(±0.63)
B. cereus	242.75bb	--	6.2a	5.5b	46.25a	33.75c	50.2a	44a
	(±6.29)	--	(±.09)	(±0.23)	(±1.93)	(±1.93)	(±1.23)	(±2.32b)
P.moraviensis+tryp	336a	--	6.52a	5.9b	49.25a	35.75b	51.42a	46.5a
	(±9.5)	--	(±.35)	(±0.12)	(±2.49)	(±2.48)	(±1.37)	(±1.08)
B.cereus+tryp	289.5a	--	6.47a	6.1a	48.5a	37b	53.42a	45a
	(±5.21)	--	(±0.22)	(±0.11)	(±3.23)	(±2.87)	(±1.93)	(±1.49)
tryp	232b	--	5.5b	4.32bc	42.75b	30.25d	40.29b	39.21b
	(±6.63)	--	(±0.18)	(±0.19)	(±1.41)	(±3.86)	(±1.75)	(±0.28)
LSD	7.32		1.65	2.21	3.92	4.34	5.55	2.29

*= untreated uninoculated control, *P.moraviensis = Pseudomonas moraviensis, B.cerues = Bacillus cereus,* tryp = tryptophan**.** Values followed by different letters are significantly at (P<0.05). Values represented in parenthesis are standard error of means.

Pseudomonas moraviensis and *Bacillus cereus* application with tryptophan exhibited 30% more plants/m^2 (Table 3) over control. Increase in spike length of wheat treated with PGPR was 18% and 25% in field and pots grown plants. Addition of tryptophan with *Bacillus cereus*, further increased 10-14% higher spike length over control both in pots and field grown plants. Similarly, *Pseudomonas moraviensis* increased 15% seeds/spike and 22% seed weight in field, and 28% and 16% in pots. This increase was higher (11% and 20%) in pots and field grown plants when tryptophan was added with *Pseudomonas moraviensis.*

Bacillus cereus produced 18% more seeds /spike and 21% more seed weight in field and 29% and 19% in pots. Addition of tryptophan with *Bacillus cereus* further increased 10% seed/spike and seed weight both in pots and field. Increase in spike length, grain yield and seed weight might be attributed to increase level of N, P and K in the presence of PGPR and tryptophan (Shaharoona *et al*., 2008). Addition of tryptophan stimulates the activities and efficiency of PGPR in the soil, by improving the ability to produce phytohormones (Spaepan *et al*., 2011). The higher observed increase in yield components might be attributed to the greater microbial and enzymatic activities and rapid release of the nutrients in the soil (Gryndler *et al*., 2008).

4. Conclusions

This study revealed the positive role of *Bacillus cereus* and *Pseudomonas moraviensis,* on wheat, when used as bioinoculants. Single inoculation of both strains, comprehensively increased the growth and improved the physiology of treated plants. Tryptophan addition further augmented these effects in sterilized pot or field condition. The tryptophan addition, may help the applied PGPR to improve their IAA production, and would be helpful in strengthening wheat physiology. Though, it is documented previously that different bacterial strains have different capability for utilization of tryptophan, but this study emphasized the distinctive role of tryptophan application under field condition. *Bacillus cereus* was found to be more effective than *Pseudomonas moraviensis,* either in the presence or absence of tryptophan. The exploration of such beneficial bacterial strain, and their use as biofertilizer in different agroclimatic regions could be handful in sustainable agriculture.

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