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BACILLUS CEREUS: A COMPETENT PLANT GROWTH PROMOTING BACTERIUM OF SALINE SODIC FIELD

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Abstract

The effects of *Bacillus cereus* were investigated on wheat in the presence or absence of L-tryptophan, in a saline sodic field. An aqueous solution of L-tryptophan was added to the rhizosphere soil at 1 µg/L, after 8d of seeds germination with irrigated water. The survival efficiency measured as colony forming unit revealed that *B. cereus* was salt tolerant to rhizosphere soil filtrate and in NaCl. Bio-inoculation of *B. cereus* significantly decreased Electrical conductivity (EC), Na and Cl contents by 35%, and increased K, NO_3-N, P, and organic matter by (25%) over control. Tryptophan addition assisted *B. cereus* to further decrease Na, Cl, sodium absorption ratio (SAR) and Na/K by 80%. Inoculation of *B. cereus* alone and with tryptophan significantly increased proline, antioxidant enzymes, phytohormones and yield attributes. The results revealed that tryptophan addition augmented the potential of *B. cereus* in improving crop growth and productivity which was mediated by the salinity alleviation.

Key words: *B. cereus;* Saline sodicity; Soil phytohormones; L-tryptophan.

Introduction

Indoleacetic acid (IAA) plays vital role in all aspects of plant growth and developmental processes (Abel, 2007). Under abiotic stresses plant signalling is controlled by IAA (Park *et al.*, 2007). IAA not only enables plants to enhance nutrient uptake, but also constructs root architecture to cope with nutrient starvation which often prevails under stresses (Kazen, 2013).

L-tryptophan (the precursor of IAA) is a natural and important constituent of root exudates of plants (Villareal *et al.*, 2012). Supplementation of L-tryptophan to soil has been documented to improve growth of many crops and vegetables (Frankenberger & Arshad; 1991; Sarwar & Frenkenberger; 1994; Arshad *et al*., 1995).

Salt affected lands are present in every part of the world. High salinity imparts damaging consequences on cereals as it reduces yield by many fold (Shafi *et al*., 2010; Singh *et al.*, 2011). Different physical and chemical measures have been adopted to improve the productivity of wheat under high salinity. Among these measures, application of Plant Growth Promoting Rhizobacteria (PGPR) has been proved effective (Lugtenberg & Kamilova; 2009).

PGPR have the ability to improve growth and yield of crops by improving nutrient availability and acquisition, synthesis of plant hormones and alleviation of the deleterious effects of abiotic stresses (Ahemad & Kibret; 2014). Isolation of indole acetic acid producing PGPR and their applications on crops is a promising way to increase soil fertility and plant production (Vejan *et al.*, 2016). Little is known about the performance of PGPR for tryptophan conversion and its impact on crop physiology under saline sodic field condition (Idris *et al.*, 2007).

Among PGPR, *Bacillus pumillus* and *Bacillus subtilis* are well characterized strains for IAA production in recent years (Tiwari *et al*., 2011; Zhao *et al*., 2011). The modulation of IAA is correlated with availability of tryptophan in soil (Zaidi *et al*., 2009). This goal may be achieved by supplementing tryptophan with PGPR in culture media or directly in soil (Spaepen & Vanderleyden; 2011).

B. cereus roles as efficient phosphate solubilizer and bio-pesticide against the fungal pathogens of Pigeon Pea have been documented previously (Yildirim *et al*., 2006; Rani *et al*., 2011). The ability of *B. cereus* to synthesize IAA and to promote growth has previously been demonstrated (Mohite, 2013; Hassan & Bano; 2015).

Kazen (2013) documented that leaf litter and dead decaying plants are important sources of the amino acids like tryptophan, and decaying of these materials plays fundamental role under salt stress. Additionally, the role of IAA as plant growth promoting hormone along with abscisic acid (ABA) also reveals mechanism of salt tolerance in plants (Wani *et al*., 2016).

The aim of the present study was to unveil the role of *B. cereus* as PGPR under saline sodic field condition, and its ability to modulate phytohormone production, especially IAA in presence of L-tryptophan. The combined effect of *B. cereus* and tryptophan were assessed for improving physiology and yield of wheat and salinity reclamation under natural field condition.

Material and Methods

Plant material and growth conditions: Isolation of *B. cereus* was made from the halophytic weed *Cenchrus ciliaris,* growing in Khewra salt range of Pakistan). *B. cereus* (accession # LN714048) was selected on the basis of IAA production and salt tolerance potential. *Triticum aestivum* L. cv. Inqlab 91 was grown in Soil Salinity Research Institute Pindibhattian (EC = 4.7 dS m^{-1}, SAR = 17.5). The experiment was conducted for two consecutive years i.e., 2011 and 2012. Treatments included bio-inoculation of *B. cereus* alone (*B. cereus*), application of *B. cereus* with tryptophan (*B. cereus* + Trp), tryptophan application alone (Trp) and un-inoculated control (control). At early vegetative stage of wheat (57 days after sowing), plants were sampled for physiological parameters and maturity plants for yield parameters.

The sterilized seeds of wheat were soaked for 30 min in 7d old microbial culture of *B. cereus* having 10^8cell/ml. After shade drying of 45 min, seeds were sown in field. The Randomized Complete Block Design (RCBD) was followed and for each treatment five replicates were made. Tryptophan 1µg L^{-1} was made and 50 ml of this solution was applied to the rooting zone of wheat seedlings, after eight days of seed emergence.

Isolation of *B. cereus* and amplification for PCR: *B. cereus* was grown on LB media (10% tryptone, 5% yeast extract and 5% NaCl pH 7.4). For the isolation of genomic DNA Ez-10 spin column genomic DNA kit (Bio basic Inc. Ontario Canada) was used. PCR amplification, the initial denaturation was done at 94°C for 3 min followed by 35 cycles of temperature profile: denaturation at 95°C for 20 sec, annealing at 45°C for 60 sec, extension at 72°C for 3 min plus additional cycles of chain elongation at 72°C for 10 min.

Amplified PCR products were purified by using EZ-10 spin column PCR purification kit (Bio basic Ontario Canada) and were sequenced by Quintara bio, San Francisco.

Physicochemical analysis of rhizosphere soil

Soil pH, electrical conductivity (EC) and organic matter: The pH of rhizosphere soil was measured by preparing 1:1(soil: water) suspension (McKeague, 1978; Mclean, 1982). Organic matter of soil was measured by method of Walkley & Black; 1934.

Macronutrients analysis of rhizosphere soil

Nitrate-N (NO_3-N) and phosphorus (P): The extractions of Nitrate-N (NO_3-N), chloride, Phosphorus (P) and bi-carbonate were made following the method of Reitemeier (1943).

Proline contents of leaves: Bates *et al.,* (1973) method was followed for the determination of proline.

Extraction and activity for peroxidase: Antioxidants activities were measured by method of Vetter *et al*., (1958). The enzyme extract was prepared by homogenizing fresh leaves (5g) with 15 ml of 0.05 N phosphate buffer (P^H 7.0) containing 10% polyvinyl poly pyrrolidone and 0.1 M Ethylene diamine tetra acetic acid (EDTA).

Assay for peroxidase activity: The assay mixture contained 0.1 ml enzyme extract, 1.35ml 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 485nm/min/ mg.

Assay for superoxide dismutase activity (SOD): SOD activity was determined by measuring inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Beauchamp & Fridovich (1971).

Salt tolerance potential of PGPR: Salt tolerance potential of PGPR isolates was determined in vitro using a filtrate of saline sodic soil from the field as explained by Naz *et al*., (2009). Soil samples were collected 6 inches from the upper surface of the rhizosphere soil in the farm field, at the Soil Salinity Research Institute Pindibhatian. A composite soil sample (50 g) was suspended with 250 ml autoclaved water, stirred for 2 h on magnetic stirrer, and filtered using Whatman No. 42 filter paper. The EC of the filtrate was determined. From the stock solution of soil filtrate 4 dilutions were made having EC 1, 1.4, 3.5 and 4dSm^{-1} respectively. The soil filtrate (250 ml) was added to 1L of culture media. Media containing autoclaved water was treated as control. An aliquot (20 µl) of inoculum from 10^8 cell/ml was streaked on plates. The plates were incubated at 30°C for 24 h. The tolerance of *B. cereus* was measured by counting their colony forming units, using the formula:

$$\text{Viable cell count (CFU/g soil)} = \frac{\text{Number of colonies}}{\text{Volume of inoculum}} \text{ x Dilution factor}$$

Determination of ABA and IAA from soil: For the extraction of phytohormones (IAA and ABA) from rhizosphere soil of wheat method demonstrated by Frankenberger & Brunner (1983) was followed.

Determination of ABA and IAA from leaves: Kettner & Doerffling (1995) method was followed for extraction of ABA and IAA of treated leaves.

Statistical analyses: For Statistical analyses, analysis of variance (ANOVA) was applied using Statistix program (version 8.1). Four replicates were taken for field experiment in each year of the trial. The experiment was laid out according to RCBD design in the field, while for laboratory experiments Complete Randomized Design (CRD) was followed. Mean values were compared according to Steel & Torrie, (1980) by least significant difference (LSD) at $p = 0.05$.

Results

B. cereus inoculation decreased the electrical conductivity (EC) of the rhizosphere soil (Table S1) as compared to that of un-inoculated (saline) soil, irrespective of the presence and absence of tryptophan. *B. cereus* inoculation decreased EC by 21% over control ($p = 0.05$). The observed increase in organic matter was 29% when *B. cereus* was inoculated with tryptophan. The Percent decrease in EC and SAR was higher during 2nd year of experiment.

In 2010-2011, P and NO_3- N were improved by 20-27% in the soil inoculated with *B. cereus.* Tryptophan addition augmented *B. cereus* effect and increased NO_3-N by 40% over control (Table 1). *B. cereus* inoculation improved K and Mg content of rhizosphere soil by 30% and 40%, respectively over control. Tryptophan addition with *B. cereus* further increased 14% K and Mg. The increase in Ca content was 25% higher over control when *B. cereus* was applied with tryptophan. Similar trend was observed during 2011-2012 for all the treatments (Table 2). However, the percent increases in NO_3- N and Mg were higher in *B. Cereus*+ Trp treatment.

Table S1. Effects of PGPR on electrical conductivity, pH, organic matter and Sodium Absorption ratio (SAR) of rhizospheric soil. Measurement were made at 57 DAS of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	2010-2011				2011-2012			
	EC (dSm^{-1})	pH	SAR	O.M (%)	EC (dSm^{-1})	pH	SAR	O. M (%)
*Control	4.48ab	8.41a	25.72a	0.846b	4.55ab	8.4a	26a	0.834b
	(±0.04)	(±0.06)	(±0.11)	(±0.02)	(±0.06)	(±0.12)	(±0.28)	(±0.07)
B. cereus	3.64c	7.89a	20.26a	0.916b	3.85c	8.01a	20.56a	0.917b
	(±0.14)	(±0.08)	(±0.61)	(±0.02)	(±0.11)	(±0.18)	(±0.42)	(±0.08)
B. cereus + tryp	3.48c	7.88a	18.04a	1.295a	3.11d	7.9a	18.23a	1.3a
	(±0.07)	(±0.17)	(±0.22)	(±0.04)	(±0.08)	(±0.15)	(±0.36)	(±0.09)
tryp	4.36ab	8.4a	24.68a	0.855b	4.67ab	8.24a	24.9a	0.88b
	(±0.11)	(±0.18)	(±0.36)	(±0.07)	(±0.12)	(±0.17)	(±0.29)	(±0.06)
LSD	1.17	1.29	3.65	1.06	2.08	3.46	4.45	1.62

* = Un-inoculated control, O.M = organic matter, EC = Electrical conductivity, SAR, sodium absorption ratio, Tryp = tryptophan. Values followed by different letters in a column are significantly different (p = 0.05)

Table 1. Effects of PGPR application on organic matter (%) and nutrients contents (g/kg) of soil in saline sodic field. Measurements were made after 57 d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	2010-2011						
	NO_3-N	P	K	Ca	Mg	Na	Cl
Control	22.22e	7.7b	130.58ab	36.48b	7.66c	130.19a	198.06a
	(±0.24)	(±0.12)	(±2.95)	(±0.24)	(±0.12)	(±2.43)	(±3.43)
B. cereus	28.27b	8.28b	163.75a	40.2b	11.55a	106.58a	153.06ab
	(±0.33)	(±0.16)	(±3.11)	(±0.36)	(±0.16)	(±2.77)	(±3.14)
B. cereus + tryp	30.76a	9.49a	173.43a	45.1a	11.95a	94.23b	133.66c
	(±0.45)	(±0.33)	(±3.14)	(±1.12)	(±0.16)	(±2.23)	(±3.14)
tryp	24.82d	8.08b	133.72ab	37.03b	9.23b	102.84a	189.5a
	(±0.45)	(±0.43)	(±4.44)	(±0.99)	(±0.16)	(±3.15)	(±2.14)
LSD	3.92	3.44	4.15	3.39	6.12	8.88	6.34

* = Untreated un-inoculated control, O.M= organic matter, *P. moraviensis = Pseudomonas moraviensis, B. cereus = Bacillus cereus,* tryp = tryptophan. Values followed by different letters in a column are significantly different at (p = 0.05). Values represented in parentheses are standard error of mean

Table 2. Effects of PGPR application on organic matter (%) and nutrients contents (g/kg) of soil in saline sodic field. Measurements were made after 57 d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	2011-2012						
	NO_3-N	P	K	Ca	Mg	Na	Cl
Control	21.16e	7.86b	128.19de	37.02b	8.01d	133.32a	199.6a
	(±0.65)	(±0.18)	(±3.31)	(±0.33)	(±0.16)	(±2.77)	(±3.21)
B. cereus	28.3b	8.26b	163.72c	38.97b	11.27b	103.83a	159.09ab
	(±0.52)	(±0.17)	(±2.88)	(±0.61)	(±0.2)	(±2.06)	(±3.32)
B. cereus + tryp	30.78a	9.5a	182.90ab	45.2a	13.03a	95.7b	130.82b
	(±0.59)	(±0.66)	(±5.77)	(±0.93)	(±0.19)	(±2.02)	(±3.07)
tryp	24.8d	7.88b	138.58d	37.24b	9.18c	106.27a	180.39a
	(±0.48)	(±0.64)	(±3.24)	(±0.86)	(±0.24)	(±3.44)	(±2.55)
LSD	2.11	3.61	6.66	2.95	1.55	7.12	6.04

* = Untreated un-inoculated control, O.M= organic matter, *P. moraviensis = Pseudomonas moraviensis, B. cereus = Bacillus cereus,* tryp = tryptophan. Values followed by different letters in a column are significantly different at (p = 0.05). Values represented in parentheses are standard error of mean

Tryptophan addition with *B. cereus* decreased Na contents by 30% over control. Similarly, *B. cereus* decreased Cl content by 39% of rhizosphere soil. In the presence of tryptophan, this decrease was 50% (Table 1). The decrease in Na was also higher during 2011-2012.

It was observed that accumulation of Na was significantly decreased in the leaves of treated plants. This decrease was better in 2nd year of experimentation (2011-2012) (Table 3). The stimulatory effects of tryptophan on accumulations of NO_3-N, P and K in the rhizosphere soil and plant leaves were noteworthy. Tryptophan addition improved 15% K and Mg as compared to the PGPR application alone. The accumulations of K and Mg in the leaves of treated plants were higher during 2011-2012.

Na/K ratio (Table S2) in rhizosphere soil was 50% less than un-inoculated control soil. Tryptophan addition with *B. cereus* decreased 41% Na/K over single inoculation.

Table S2. Na/k and Na/Ca ratio of soil and leaves and Biological accumulation coefficient (BAC).

Treatments	2010-2011							2011-2012						
	Na/K		Na/Ca		BAC			Na/K		Na/Ca		BAC		
	Soil	Leaves	soil	Leaves	Na	K	Ca	Soil	Leaves	soil	Leaves	Na	K	Ca
Control	0.97	0.88	3.27	1.98	0.15	0.15	0.34	1.04	1.05	3.59	1.8	0.16	0.16	0.39
B. cereus	0.65	0.57	2.65	1.5	0.14	0.21	0.55	0.63	0.99	2.67	1.47	0.18	0.23	0.52
B. cereus + tryp	0.52	0.43	2.08	0.76	0.13	0.27	0.64	0.54	0.41	2.13	0.79	0.15	0.3	0.6
Tryp	1.07	0.76	2.75	1.7	0.14	0.16	0.34	1.09	0.6	2.86	1.73	0.12	0.2	0.38

* = Un-inoculated control, Tryp = tryptophan. Values followed by different letters in a column are significantly different (p = 0.05)

Table 3. Effects of PGPR application on leaves nutrients contents (g/kg) of wheat grown under saline sodic field condition. Measurements were made after 57 d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	2010-2011				2011-2012			
	Na	K	Ca	Mg	Na	K	Ca	Mg
Control	16.27a	15.23d	8.37c	16.22d	16.87a	15.92d	9.36d	15.02d
	(±0.43)	(±0.31)	(±0.24)	(±0.12)	(±0.52)	(±0.31)	(±0.24)	(±0.2)
B. cereus	14.25c	22.15b	9.11c	22.15c	14.5c	29.25b	9.83c	31.25b
	(±0.77)	(±0.14)	(±0.33)	(±0.15)	(±0.33)	(±0.54)	(±0.44)	(±0.18)
B. cereus + tryp	12.25e	26.11a	16.19a	24.15a	13.13e	31.88a	16.71a	34a
	(±0.23)	(±0.43)	(±0.33)	(±0.12)	(±0.26)	(±0.43)	(±0.3)	(±0.14)
tryp	15.05b	16.98d	8.9c	17.35c	15.35b	25.43d	8.84cd	27.84c
	(±0.51)	(±0.64)	(±0.21)	(±0.23)	(±0.29)	(±0.39)	(±0.27)	(±0.39)
LSD	0.22	1.12	0.72	0.61	0.47	1.87	0.91	0.31

* = Untreated un-inoculated control, *P. moraviensis* = *Pseudomonas moraviensis*, *B. cereus* = *Bacillus cereus*, tryp = tryptophan. Values followed by different letters in a column are significantly different at (p = 0.05). Values represented in parentheses are standard error of mean

B. cereus increased the plant height (Table 4) of plants by 89% over control, and further 6-10% increase was observed by tryptophan addition. *B. cereus* increased chlorophyll contents of leaves by 25% and tryptophan addition resulted in further 6-20% increase (p = 0.05). The proline contents were 50% greater over control following the inoculation of *B. cereus*. Tryptophan addition with *B. cereus* increased the proline content by 20% over single inoculation. The observed increases in both years of application on growth and physiology of wheat were similar, but overall effects of *B. cerues* with and without tryptophan were more pronounced (5-10% higher) during 2011-2012.

Superoxide dismutase (SOD) and peroxidase (POD) activities (Table 4) were 100% and 130% higher over control, respectively. Addition of tryptophan with *B. cereus* increased SOD by 16% and POD by 19% over single inoculation. The increase in antioxidant activities were 5-10% higher during 2011-2012.

B. cereus increased number of plants and seeds/spike by 32% and 28%, respectively. Addition of tryptophan with *B. cereus* increased number of plants, and seeds/spike by 58% and 60% over control. Increase in seed weight by 22% following the inoculation of *B. cereus* (Table 5). The increases in yield attributes were 3-5% higher during 2nd year of experiment.

The survival efficiency of *B. cereus* as measured by colony forming unit (cfu) (Fig. 1) showed a linear decrease with increasing salt concentrations of rhizosphere soil. The % increase in survival efficiency was higher in NaCl as compared to mixture of salt, indicated at 1.5 dS m^{-1} in soil filtrate and 1.4 dS m^{-1} in NaCl. *B. cereus* showed 87% decrease over control at same EC.

The sensitivity of *B. cereus* to NaCl was greater (66% decrease) over control (2 dS m^{-1}NaCl) (Fig. 2). The result indicated that mixture of salt was inhibitory for survival of rhizobacteria. The Colony Forming Unit of *B. cereus* was declined by 4 fold in mixture of salt having EC 4.7 dSm^{-1}.

Addition of tryptophan to the culture media increased the growth of *B. cereus* as compared to control measured as O.D at 660 nm. The maximum growth of *B. cereus* was at 10^{-5} M of tryptophan where the O.D of *B. cereus* was 54% higher over control (data not shown).

Rhizophere soil of plants inoculated with *B. cereus* contained 47% higher IAA over un-inoculated control soil. Treated leaves had 30% higher IAA over control (Fig. 3). Addition of tryptophan with *B. cereus* enhanced 56% IAA in the rhizosphere soil and 45% in leaves. *B. cereus* increased 22% higher GA in soil and 30-35% in leaves (Fig. 4). Tryptophan addition resulted 15% further GA in soil and leaves.

ABA content of soil inoculated with *B. cereus* was higher than that of IAA (Fig. 5). ABA contents in rhizosphere soil and leaves were 35% higher. ABA content of soil were by 80%, 45% in leaves when tryptophan was added with *B. cereus*.

Table 4. Effects of PGPR application on physiological parameters of rhizosphere soil and leaves of wheat grown in saline sodic field.
Measurements were made after 57 d of sowing. Values are mean of four replicates.

Treatments	2010-2011						2011-2012					
	Plant height (cm)	F. Wt (g)	chlorophyll ($\mu g/cm^2$)	Proline ($\mu g/g$)	SOD (Unit g^{-1})	POD (OD min^{-1} g^{-1})	Plant height (cm)	F. Wt (g)	chlorophyll ($\mu g/cm^2$)	Proline ($\mu g/g$)	SOD (unit g^{-1})	POD (OD min^{-1} g^{-1})
Control	27b (±1.08)	2.2c (±0.04)	30.36b (±0.88)	311d (±8.9)	2.65c (±0.08)	2.48c (±0.04)	23.5b (±1.12)	2.28c (±0.03)	28.19c (±0.73)	314.5d (±8.21)	2.76c (±0.1)	2.44c (±0.03)
B. cereus	36.11ab (±1.44)	2.65b (±0.04)	37.14c (±1.02)	341.5c (±6.12)	5.43ab (±0.04)	3.34bc (±0.1)	33.13ab (±1.4)	2.7b (±0.04)	35.83b (±1.11)	356.5c (±6.44)	5.53ab (±0.06)	3.46b (±0.05)
B. cereus + tryp	40a (± 1.6)	3.03a (±0.03)	42.25a (±1.09)	565a (±8.88)	6.12a (±0.06)	4.71a (±0.13)	38.38a (±1.11)	3.15a (±0.05)	41.06a (±1.3)	555a (±6.32)	6.05a (±0.06)	4.48a (±0.11)
tryp	29b (±1.77)	2.29c (±0.08)	34.27b (±1.43)	251d (±9.23)	3.6c (±0.14)	2.89bc (±0.11)	24.13b (±1.52)	2.69c (±0.04)	31.08c (±1.51)	313.75c (±7.76)	3.48c (±0.05)	2.9bc (±0.16)
LSD	1.65	3.13	5.39	7.93	7.85	2.2	2.92	3.11	5.72	7.84	7.33	1.08

***** = Un-treated un-inoculated control, *P. moraviensis* = *Pseudomonas moraviensis*, *B. cereus* = *Bacillus cereus*, tryp = tryptophan. F.Wt = fresh of aerial parts of single plant. Values followed by different letters in a column are significantly different at (p = 0.05). Values represented in parentheses are standard error of mean

Discussion

The isolation *B. cereus* was made from the halophytic weed *Cenchrus ciliaris* which have been previously characterized for the salt tolerance potential and PGPR under axenic condition (Hassan & Bano, 2014). The increased growth of *B. cereus* in culture media containing NaCl or salt mixture; supplemented with tryptophan is possibly due to better salt tolerance potential of strain (Hassan & Bano; 2015).

Application of PGPR with tryptophan improved soil organic matter. PGPR are capable of improving organic matter and this effect was further improved by carbon sources and tryptophan addition (Hassan & Bano; 2015; Abbas *et al*., 2013). Increase in organic matter in the presence of tryptophan suggested the role of PGPR in maintaining soil structure and nutrient storage by activating carbon sources (Ghosh *et al*., 2003).

The observed decreases in EC and SAR of rhizosphere soil in *B. cereus* + tryp may be attributed to the corresponding decrease in Na contents, and concomitant increases in K, NO_3-N and P (Rana *et al*., 2012). Upadhyay *et al*., (2012) reported that *Bacillus subtilis* and *Artherobacter* species improved P and K and decreased Na under salt stress. *B. cereus* resulted in greater accumulations of K, NO_3 and P in soil, tested under axenic condition (Hassan & Bano, 2014).

The observe decrease in Na following the addition of tryptophan with *B. cereus* insinuate the potential of applied PGPR in reclamation of soil. Ashraf *et al*., (2004) ascribed that microbial synthesis of exopolysacchrides decreased Na contents in soil. Similarly, decrease in K/Na also indicates that *B. cereus* inoculation ameliorated the deleterious effects of salinity. Giri *et al.*, (2007) also revealed that PGPR associated with plants could efficiently alter K/Na ratio and decrease salinity stress.

Increase in organic matter and soil nutrients and decrease in EC and SAR are correlated with better rainfall in 2011-2012. Possibly, this change in moisture content of soil augmented microbial activities (Eilers *et al*., 2012).

The observed increase in plant height following addition of tryptophan may be attributed to the PGPR induced conversion of tryptophan into indole acetic acid (Majeed *et al*., 2015). IAA being growth promoter induced cell division and cell elongation (Pin-Ng *et al*., 2015). Increase in growth of wheat could be attributed to enhanced physiological responses of crop with added PGPR and tryptophan (Zahir *et al*., 2007).

The improved effects of PGPR on growth physiology of wheat during 2nd year of experimentation are associated with lower EC and SAR of soil. Zahir *et al*., (2012) also observed better affectivity of PGPR in the soil with less EC and SAR.

B. cereus inoculation increased chlorophyll, proline and antioxidant enzymes activities which were augmented by the addition of tryptophan. The observed stimulation in chlorophyll, proline and antioxidant enzymes (SOD, POD) activities in inoculated plants indicated the positive role of PGPR on osmoregulation, stomatal conductance and photosynthesis (Hayat *et al*., 2012; Fan *et al*., 2012; Sharma *et al*., 2012). Ghorbanpour *et al*., (2013) revealed that strong antioxidant system in plants not only scavenged, but also provided tolerance to plants under stresses.

Table 5. Effects of PGPR application on yield parameters of wheat grown in field under saline sodic condition. Measurements were taken at maturity at 159 DAS. Values are mean of four replicates.

Treatments	2010-2011				2011-2012			
	Plant/m^2	spike length (cm)	seed/spike	seed weight (g)	Plant/m^2	spike length (cm)	seed/spike	seed weight (g)
Control	215c	6.9b	45d	40.57c	222.5c	7.3	41.5c	41.42c
	(±4.33)	(±0.05)	(±1.12)	(±1.23)	(±4.11)	(±0.02)	(±1.08)	(±1.11)
B. cereus	287.5b	8.4a	60.75ab	48.14a	286.75b	8.55	61.25ab	47.12ab
	(±3.02)	(±0.07)	(±0.98)	(±0.69)	(±2.13)	(±0.02)	(±0.76)	(±0.75)
B. cereus + tryp	348a	8.8a	66.5ab	55.35a	356.55a	9	64.5a	55.45a
	(±6.67)	(±0.05)	(±2.12)	(±1.14)	(±5.93)	(±0.04)	(±1.22)	(±1.62)
tryp	226c	7b	48cd	41.24b	228.12c	7.82	50bc	42bc
	(±7.13)	(±0.01)	(±2.22)	(±1.04)	(±6.18)	(±0.06)	(±2.06)	(±1.12)
LSD	5.56	2.22	8.88	4.08	6.06	3.11	2.23	5.05

* = Untreated un-inoculated control, *P. moraviensis* = *Pseudomonas moraviensis*, *B. cereus* = *Bacillus cereus*, tryp = tryptophan. Values followed by different letters in a column are significantly different at (p = 0.05). Values represented in parentheses are standard error of mean

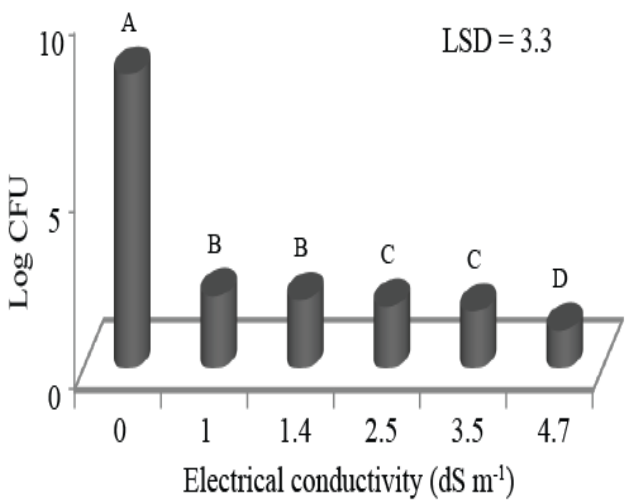

Fig. 1. Colony forming units of PGPR at different electrical conductivity (EC) of soil filtrate containing salt mixture (NaCl, CO_3- and HCO_3-).Values given are mean of four replicates. Alphabets heading the bars represent significant difference (p=0.05).

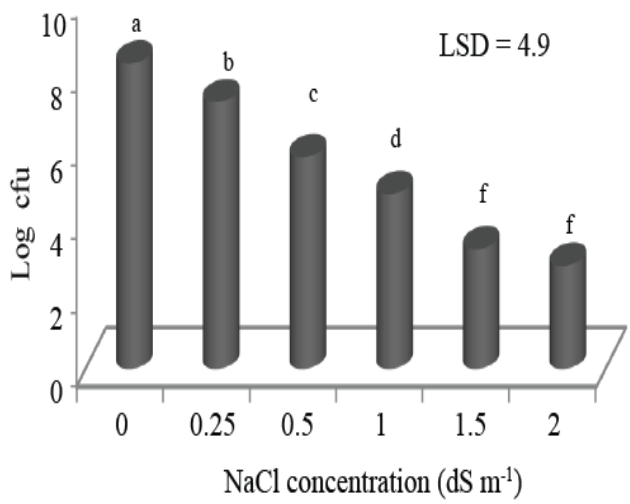

Fig. 2. Colony forming unit of PGPR at different concentration of NaCl. Values given are mean of four replicates. Alphabets heading the bars represent significant difference (p = 0.05).

Noteworthy, tryptophan addition with *B. cereus* increased phytohormone contents (IAA, GA and ABA), in soil and leaves. Results indicate that *B. cereus* has ability to modulate IAA in the rhizosphere soil and leaves and tryptophan addition augmented this ability of PGPR. The production of IAA in presence of L-tryptophan has been reported for PGPR belonging to several genera including *Bacillus and Pseudomonas* (Saharan & Nehra;. 2011; Abbas *et al*., 2013). Exogenous application of application GA has been reported to increase biomass and weight of seed tuber in *Helianthus tuberosus* L. (Ruttanaprasert *et al*., 2018).

The higher production of ABA in rhizosphere soil in *B. cereus* + Trp suggests the better adoptability of plants under salt stress. Previously application of PGPR improved ABA contents in plant (Vacheron *et al*., 2013).

B. cereus inoculation modulated the IAA/ABA ratio that was least (0.83) for rhizospheric soil of inoculated plants as compared to that in plant leaves (1.5). Exposure of plants to salinity is known to induce proportional increase in ABA concentration that is in most cases correlated with leaf or soil water potential (Zhang *et al*., 2006). Increase in ABA translocation from soil to leaves as result of PGPR inoculation and in the presence of tryptophan might be attributed for the decrease in ethylene production (Porcel *et al*., 2014).

The physiological responses of wheat assessed by proline, antioxidants activities and phytohormones accumulation was better during 2011-2012. Rainfall and moisture contents of soil directly influenced edaphic characters and nutrient availability which act as motive force for better physiological responses of plants (Zheng *et al*., 2015).

The greater % increase in seed size of *B. cereus* inoculated plants may be attributed to higher IAA/ABA ratio in leaves which may account for increased biomass. Nia *et al*., (2012) reported that PGPR inoculation increased the yield attributes of wheat by mitigating fatal effects of salinity.

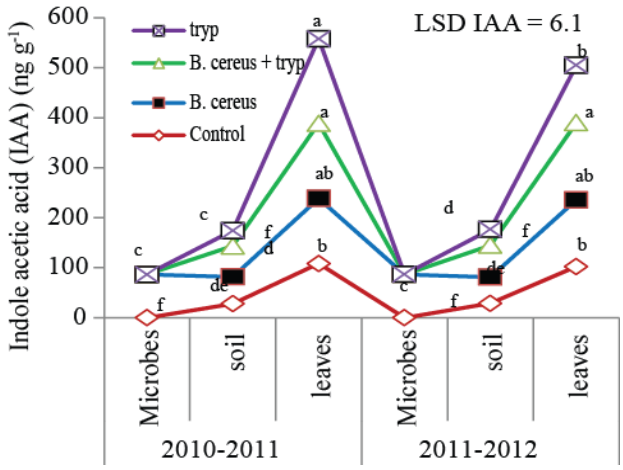

Fig. 3. Measurement of indoleacetic acid in 24h old bacterial culture, rhizoshere soil and in wheat leaves. Leaves and soil samples were collected after 57d of inoculation. Control = un-inoculated plants and soil. Values given are mean of four replicates. Alphabets heading the bars represent significant difference (p = 0.05).

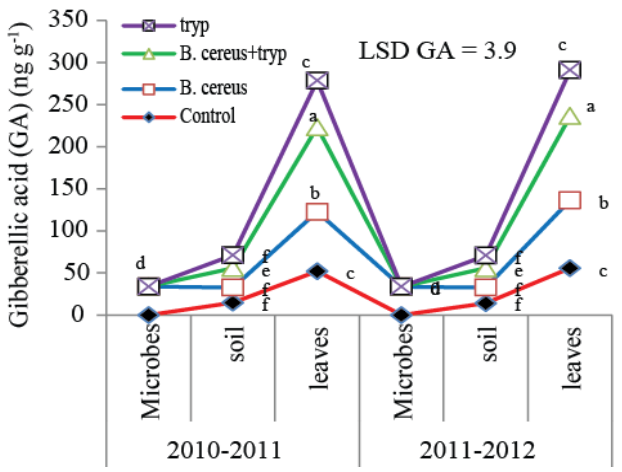

Fig. 4. Measurement of gibberellic acid production in 24h old bacterial culture, rhizoshere soil and in wheat leaves. Leaves and soil samples were collected after 57d of inoculation. Control = un-inoculated plants and soil. Values given are mean of four replicates. Alphabets heading the bars represent significant difference (p = 0.05).

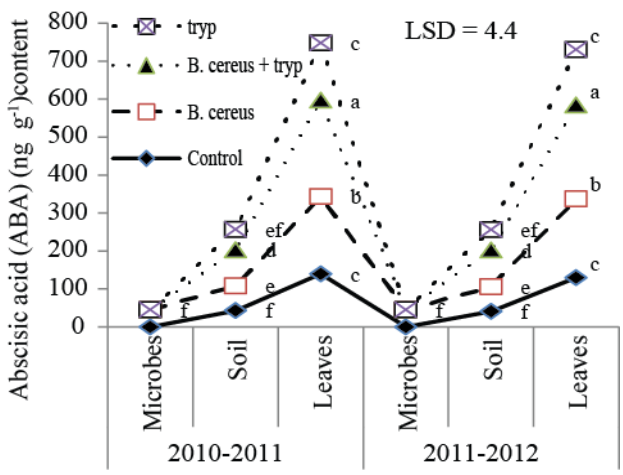

Fig. 5. Measurement of abscisic acid production in 24h old bacterial culture, rhizoshere soil and in wheat leaves. Leaves and soil samples were collected after 57d of inoculation. Control = un-inoculated plants and soil. Values given are mean of four replicates. Alphabets heading the bars represent significant difference (p = 0.05).

Conclusion

B. cereus used in the present study has the ability to produce IAA and this effect was further stimulated in the presence of IAA precursor tryptophan. *B. cereus* produced higher IAA in culture, salt stressed soil and leaves of treated plants. The higher IAA (growth promoting hormone) in the rhizosphere soil may assist the host plants interaction with the PGPR and subsequently induced tolerance against salt. The comparative effects of tryptophan alone and in the presence of *B. cereus* revealed the potential of PGPR in ameliorating the salinity and this was manifested by decreased in EC, Na/K, Na/Ca and SAR, of saline sodic soil. The accumulation of higher IAA and ABA, IAA/ABA ratio in soil and leaves of plants treated with tryptophan and grown under saline sodic field seems to be an adaptive mechanism of salt tolerance. The exploration of the other beneficial bacterial strains and their use in consortium and in the presence of tryptophan may prove beneficial for sustainable agriculture.

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