International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 4, No. 2, p. 30-39, 2014

INNSPUB

RESEARCH PAPER OPEN ACCESS

Role of plant growth promoting rhizobacteria and L-tryptophan on improvement of growth, nutrient availability and yield of wheat (*Triticum aestivum)* under salt stress

Tamoor-ul-Hassan*, Asghari Bano

Department of Plant Sciences Quiad-i-Azam University, Islamabad 45320, Pakistan

Article published on February 25, 2014

Key words: PGPR, L-tryptophan, nutrient uptake, salt stress, *Bacillus cereus*.

Abstract

During the present study *Pseudomonas sp* and *Bacillus cereus* isolated from rhizospheric soil of halophytic weeds of Khewra salt range were used as bioinoculant on wheat. Aqueous solution of tryptophan was added to the rhizospheric soil @1ug/L after seed germination. Experiment was conducted at Quaid-i-Azam University Islamabad in pots under sterilized condition. Electrical conductivity (EC) was maintained to 3.7 dsm-1 by applying 150mM NaCl twice (after 7 and 14d of seed germination). PGPR inoculation significantly decreased EC, pH, SAR, Na, and Cl contents and improved K, Ca, Mg, Fe, P, NO_3 and organic matter contents of the rhizospheric soil. Tryptophan addition assisted the PGPR to further decrease the EC, SAR and improved nutrients uptake and growth. Tryptophan augmented the PGPR-induced increase in fresh weight, chlorophyll, proline and sugar contents. Superoxide dismutase and peroxidase activities of leaves of inoculated plants were also higher in the presence of tryptophan. Greater production of abscisic acid and Indole acetic acid were recorded in leaves of PGPR inoculated plants and addition of tryptophan augmented the phytohormone production in leaves of treated plants. Inoculation of PGPR alone and with tryptophan positively affected the yield of crop by improving seed establishment and number of seeds/spike.

* **Corresponding Author:** Tamoor-ul-Hassan ✉ tamoorqau80@gmail.com

Introduction

Production of plant is affected in many cases due to soil salinity because it has strong effects on physiology and nutrient uptake (Singh *et al.,*2011). Though salinity affects many crops but its major victim is wheat whose production is decreased by 65% due to mild salinity (Shafi *et al.,* 2010). It is believed that salinity directly effects on ionic imbalance of ions (Upadhyay *et al.*, 2011).

Many approaches have been made for reclamation of salt effected soil and some of them are effective also. Application of Plant Growth Promoting rhizobacteria for this purpose is regarded one of the best option. These rhizobacteria colonize the rhizosphere of many crops including wheat (Cakmakci *et al.*, 2006). Many strains of Plant Growth Promoting rhizobacteria have been proved fruitful in improving plant growth but not all bacterial strains are capable for surviving under saline condition (Gracia and Hernandez 1996).

Growth and survival of beneficial organisms is dependent on environment in which they are growing (Bull *et al.*, 1991). Salt tolerant bacterial strains are handful if they have empty niche and competing ability with indigenous microflora (Rekha *et al.*, 2007). Availability of stress tolerant strains is major limitation in reclamation of soil and crops improvement. However, screening of halophytic bacteria and their survival in different type of agroclimatic condition is broadening the scope of PGPR application.

Yield of economically important crop is increased by many folds due to the ability of PGPR for producing plant growth promoting hormones (Kohler et al., 2006).The most important hormone is auxin which has prominent and diverse role in plant physiology (Ashrafuzzaman *etal.*, 2009). Inoculation of wheat with *Pseudomonas* sp toxic uptake of toxic ions and increase IAA production for improvement of growth under salt stress (Hasnain and Sabri 1996).

Strains belonging to *Bacillus* and *Pseudomonas* are highly potent in agriculture and agronomic yield improvement (Talik *et al.*, 2006). These bacteria along with some other bacterial strains are important root colonizing bacteria of wheat (Cakmakci *et al.*, 2006). Plant growth and health is improved by PGPR by providing nutrients (P, N, Fe and Zn) to them as well as plant growth promoting substances in the form of phytohormones(Naveed *et al.*, 2008).

Naturally occurring auxin (Indole acetic acid) is synthesized by Plant Growth Promoting rhizobacteria which exploit L-tryptophan, a precursor of Indole acetic acid (Spaepan *et al.*, 2007). Root exudates of plants and protein hydrolysis are the major sources of naturally occurring L-tryptophan. (Rajesh *et al.*, 2005) (Patten and Glick 1996). Effect of L-tryptophan on allelochemical activities and plant growth has been documented (Brazani and Friedman 2000). Conversion of L-tryptophan into indole acetic acid in which Plant Growth Promoting rhizobacteria act like catalyst has been described by many conversion pathways but in abiotic stresses our knowledge is limited (Idris *et al.*, 2007).

Application of L-tryptophan in soil has shown marked increase in growth of many vegetables and economically important crops like maize (Frankenberger and Arshad, 1991), (Sarwar and Frenkenberger, 1994) and (Arshad *et al.*, 1995).

Research on precursor inoculums interaction have already been documented using tryptophan as precursor for IAA (Zahir *et al.*, 2007) but information is lacking on the role of tryptophan under stresses.

This paper demonstrate the role of tryptophan on the production of phytohormones by the PGPR in culture as well as in wheat when used as bioinoculant. This aims to elucidate the role of PGPR also to modulate the level of phytohormones in general and IAA in particular in presence of tryptophan. Since tryptophan is economically more feasible to be used along with PGPR bioinoculant to combat stress.

Material and methods

Plant material and growing conditions

Triticum aestivum L. variety was collected from National Agriculture Research Council Islamabad. Seeds were surface sterilized with 70% ethanol for 5 min and were rinsed with autoclaved water. Seeds were soaked in 7d old culture of PGPR for 15-20 min.

After shade drying seeds were sown in sterilized soil filled in earthen pots. The salt (NaCl) aqueous solution was applied to the pots prior to sowing. After 7d of germination of seeds L-tryptophan solution (0.001 g/pot) was applied in rooting zone of plants.

Plant material and growth condition

During the present study two Isolates *Pseudomonas* sp and *Bacillus cereus* from the halophytic weed *Chrysopogan aucheri* and *Cenchrus ciliaris* were used as PGPR. Seeds of *Triticum aestivum* L. variety Inqlab 91 were obtained from National Agriculture Research Council Islamabad and were grown in Quaid-i-Azam University Islamabad. 150mM NaCl was applied with irrigation water to the sterilized soil (EC 3.7 ds/m). Treatments include inoculation of *Pseudomonas* sp and *Bacillus cereus* with and without addition of tryptophan. Uninoculated plants were taken as control. Plant sampling was done at early vegetative stage (57 days after sowing) for physiological parameters and at maturity 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 rhizobial culture having 10^6 cell/ml. After shade drying seeds were sown under field condition. The CRD design was used. After 7d of germination of seeds aqueous solution of L-tryptophan 1ug/L was applied in rooting zone of seedlings.

CHEMICAL ANALYSIS OF RHIZOSPHERIC SOIL

Soil organic matter

soil organic matter was determined by method of Walkley-Black(1934).

Macronutrients analysis of rhizospheric soil

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

NITRATE-N (NO_3-N), Phosphorus (P), were extracted from rhizospheric soil following the method of Reitemeier (1943).

Proline content of leaves was measured by the method of Bates *et al.*, (1973).

Antioxidant Assays

Extraction and activity for antioxidants was measured by method of Vetter *et al.*, (1958). Fresh leaves (5g) were homogenized with 15ml of 0.05N phosphate buffer (p^H 7.0) containing 10% polyvinyl poly pyrrolidone and 0.1 M Ethylene diamine tetra acetate (EDTA).

Assay for Peroxidase activity

The assay mixture contained 0.1ml enzyme extract, 1.35ml of 100mM MES buffer (pH 5.5), 0.05% H_2O_2 and 0.1% phenylene diamine. 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 protein.

Assay for Superoxide Dismutase Activity (SOD)

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

Determination of ABA and IAA from soil

The extraction and purification for ABA and IAA from rhizospheric soil was made following the method of Frankenberger and Brunner (1983).

Determination of ABA and IAA from leaves

The extraction and purification for ABA and IAA from the plant leaves were made following the method of Kettner and Doerffling (1995).

Statistical analyses of the data were conducted using analysis of variance (ANOVA) in statistix program, version 8.1. Since year wise treatments interaction was not significant in most of evaluated parameters, mean of four replicates of each year and combined data of two years were presented. Mean values were separated according to LSD test P=0.05 with ±SE.

Results and Discussion

Survival efficiency of PGPR

Colony Forming Unit (cfu) (Fig 1) of *Pseudomonas* sp was 20% higher in rhizospheric soil of wheat after 57d of sowing as compared to *Bacillus cereus*. Addition of tryptophan further increased cfu of *Pseudomonas* sp and *Bacillus cereus* by 4-9% over *Pseudomonas* sp and *Bacillus cereus* inoculated alone.

Soil and leaf nutrient contents

The PGPR bioinoculants increased the availability of P and enriched the rhizosphere with NO^-_3 – N (Table

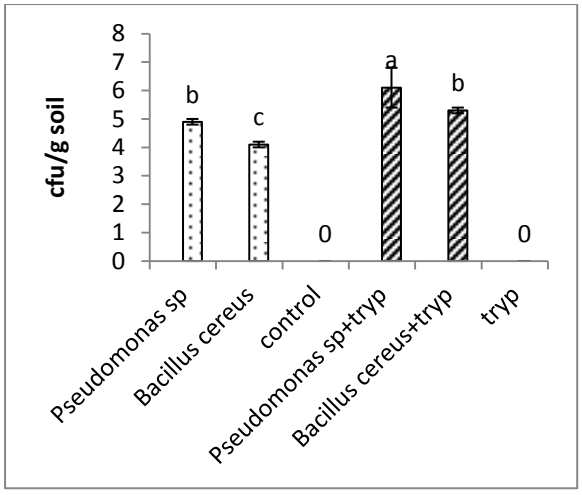

Fig 1. Colony forming units of inoculated PGPR from rhizospheric soil after 57d of sowing. Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (P<0.05) using Statistix 8.1 version.

2). Maximum increase in NO^{-}_{3} was 47% and 37% due to *Bacillus cereus* and *Pseudomonas sp* respectively which showed further 6-20% increase in presence of tryptophan. Etesami *et al*., 2009 also reported PGPR induced increase in N, P and K which was further augmented in presence of tryptophan. *Azospirillum* enhanced uptake of N, P and K in presence of Ag+ ion and L-tryptophan (Tien *et al.,* 1979).

The PGPR decreased the electrical conductivity, pH and SAR(Table 1) value which was further reduced by addition of tryptophan. Maximum decrease 29% and 49% in EC and sodium absorption ratio (SAR) was observed when tryptophan was added with *Bacillus cereus*. Addition of tryptophan with *Bacillus cereus* and *Pseudomonas* sp improved organic matter by 30% and 23% respectively.

The PGPR induced decrease in Na contents (Table 2) of soil and it was further augmented by tryptophan addition. This decrease was 25% when *Bacillus cereus* with tryptophan was applied and 21% when *Pseudomonas* sp with tryptophan was applied. PGPR application improved Fe, K, Ca and Mg contents (Table 2). *Bacillus cereus* with tryptophan showed significantly higher (125%) Mg over control. The Ca and K of soil were 25% and 51% when *Pseudomonas* sp with tryptophan was applied. Improvement of K, Ca, Mg, Fe attributed to the ability of PGPR in balancing nutrients (Cakmakci *et al*., 2007)

The Na contents of leaves (Table 3) were significantly decreased by 51% over control when *Pseudomonas* sp was applied with tryptophan. Highest increase 29% and 48%, 51% and 42% in leaf K, Mg, Ca and N were observed when *Pseudomonas* sp with tryptophan was applied.

Table 1. Effect of PGPR on electrical conductivity (ds/m^{-1}),pH, organic matter(%) and Sodium Absorption ratio(SAR) of rhizospheric soil after 57d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	EC dsm-1	pH	SAR	Organic matter(%)
control*	3.7	8.8	13.26	0.884
	±0.08	±0.08	±0.07	±0.44
***Pseudomonas* sp**	3.4	8.5	9.94	0.999
	±0.06	±0.02	0.03	±0.76
Bacillus cereus	3.5	8.4	9.49	0.987
	±0.1	±0.03	±0.13	±0.47
***Pseudomonas* sp+tryp**	3.2	8.02	8.64	1.09
	± 0.05	±0.06	±0.18	±0.43
Bacillus cereus+tryp	2.87	8.05	8.88	1.145
	±0.07	±0.07	±0.12	±0.45
tryp	3.66	8.6	12.59	0.887
	±0.02	±0.04	±0.21	±0.24

Values followed by different letters in a column are significantly different (P<0.05) using statistix 8.1 version.

Table 2. Effect of PGPR application on soil nutrients contents (mg/kg) After 57 d of sowing (2-3 leaf stage).

Treatments	P	N	K	Na	Ca	Mg	Cl
control*	2.87C	13.22E	110.56C	70.11A	24.24C	4.42E	8.18A
	±0.08	±0.23	±3.08	±0.44	±0.23	±0.23	±0.43
***Pseudomonas* sp**	3.35B	18.12B	140.617B	61.77C	28.12B	7.12B	7.07AB
	±0.06	±0.39	±4.02	±0.76	±0.39	±0.39	±0.57
Bacillus cereus	3.8B	19.33B	150.64B	62.54C	29.22B	7.23B	7.11B
	±0.1	±0.54	±5.03	±0.47	±0.54	±0.54	±0.45
***Pseudomonas* sp+tryp**	4.6A	21.31A	173.786A	58.44D	31.31A	8.31A	6.45B
	±0.05	±0.11	±0.06	±0.43	±0.11	±0.11	±0.61
Bacillus cereus+tryp	4.87A	20.12A	166.807A	56.45D	30.44A	9.02A	6.07B
	±0.07	±0.56	±0.07	±0.45	±0.56	±0.56	±0.93
tryp	3.01C	14.19C	113.6B	68.23B	24.87C	5.11C	6.07B
	±0.02	±0.44	±0.04	±0.24	±0.44	±0.44	±0.83

Table 3. Effect of PGPR application on leaves nutrients contents (mg/kg) After 57 d of sowing(2-3 leaf stage).

Treatments	P	N	K	Na	Ca	Mg	Cl
control*	4.17	9.22E	17.56C	5.44	6.24	6.77	6.67A
	±0.18	±0.37	±0.18	±0.59	±0.23	±0.23	±0.91
***Pseudomonas* sp**	5.35	10.12B	21.617B	4.11	8.12	8.18	5.59AB
	±0.26	±0.39	±0.17	±0.66	±0.39	±0.62	±0.62
Bacillus cereus	5.8	10.33B	20.43B	4.01	7.22B	8.02	5.71B
	±0.1	±0.62	±0.54	±0.47	±0.54	±0.13	±0.45
***Pseudomonas* sp+tryp**	7.6	12.01A	22.86A	3.77	8.31	10.01	5.11B
	±0.35	±0.41	±0.13	±0.53	±0.11	±0.37	±0.61
Bacillus cereus+tryp	7.87	13.12A	21.80A	3.52	9.44	9.56	4.97B
	±0.77	±0.83	±0.19	±0.31	±0.56	±0.77	±0.93
tryp	4.87	9.33C	18.06B	5.12	6.87	7.11	6.29A
	±0.12	±0.21	±0.34	±0.14	±0.44	±0.34	±0.83

Plant height and Fresh weight

Both *Pseudomonas* sp and *Bacillus cereus* increase plant height (Fig 2). Inoculation with *Pseudomonas* sp increased plant height by 29 % and 33% and addition of tryptophan further increased plant height by 14% and 35%. Increase in PGPR+tryp induced increase in plant height may be attributed to IAA induced cell division, cell elongation (Joo *et al.*, 2004) and increase conversion of tryptophan into IAA by PGPR resulted in improvement of plant height (yasmin *et al.*, 2007).

The *Pseudomonas* sp increased the fresh weight (Fig 3) by 44% and 63% over control respectively. Addition of tryptophan further increased 50-57% fresh weight on *Pseudomonas* sp and *Bacillus cereus* inoculation respectively. The observed increase in fresh weight may be attributed to IAA induced water and nutrient uptake (Spaepen *et al.,* 2007) and proliferation of root system. Inoculation with *Pseudomonas* sp and *Bacillus cereus* increased chlorophyll contents(results not presented) (8-16%) and addition of tryptophan with *Pseudomonas* sp showed 13% increase in chlorophyll.

Proline and antioxidants

Pseudomonas sp and *Bacillus cereus* increased proline content (Fig 4) by 50% over control. Addition of tryptophan with *Pseudomonas* sp and *Bacillus cereus* further increased the proline contents of plants

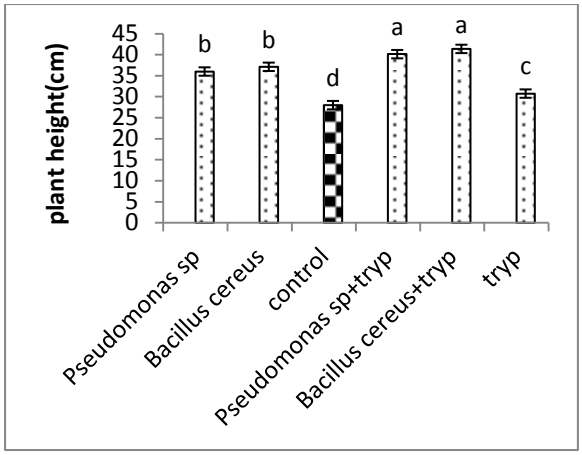

Fig 2. Plant height (cm) treated with *Pseudomonas* sp and *Bacillus cereus* alone and in addition with tryptophan, control= untreated uninoculated and tryp= tryptophan alone. Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (P<0.05) using Statistix 8.1 version.

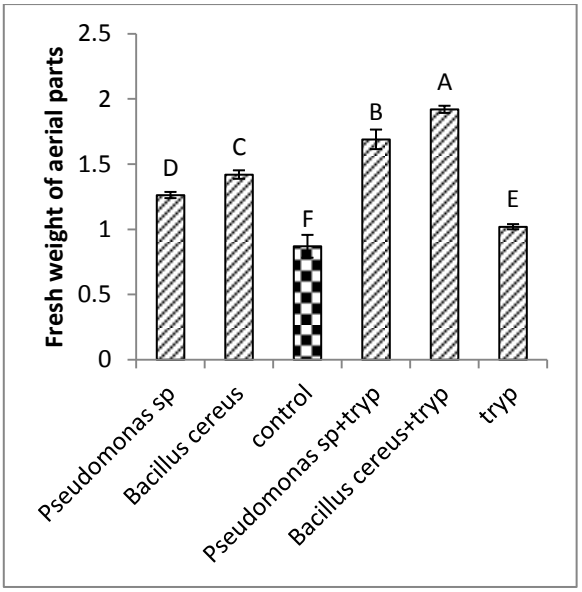

Fig 3. Fresh weight (g) of leaves after 57 days of sowing.

by 10-15%. Proline acts as a source of organic nitrogen reserve as well as osmoprotectant and antioxidant in such cases (Meloni *et al.*, 2005 ; Ali *et al.*, 2013).

Increase in sugar content (Fig 4) was 55% and 74% higher over control when treated with *Pseudomonas* sp and *Bacillus cereus* respectively and addition of tryptophan further increased sugar contents by 60% and 40% over control. The decrease in sugar and chlorophyll contents (Fig 4) in tryptophan treated plants over that of PGPR inoculated plants minus tryptophan could be attributed to difference in the osmotic regulation (Prado *et al.*, 2000).

Plants treated with *Pseudomonas* sp showed 57-59% higher SOD and POD activities (Fig 5). In presence of tryptophan *Pseudomonas* sp exhibited 26% more

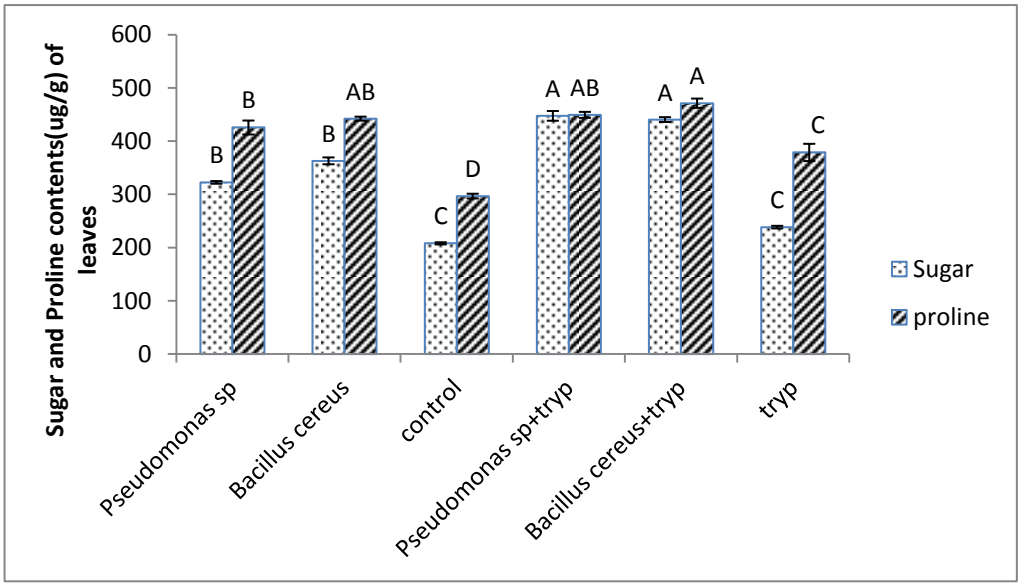

Fig. 4. Proline and sugar contents of leaves (ug/g), treated with *Pseudomonas* sp and *Bacillus cereus* alone and in addition with tryptophan, control= untreated uninoculated and tryp= tryptophan alone.

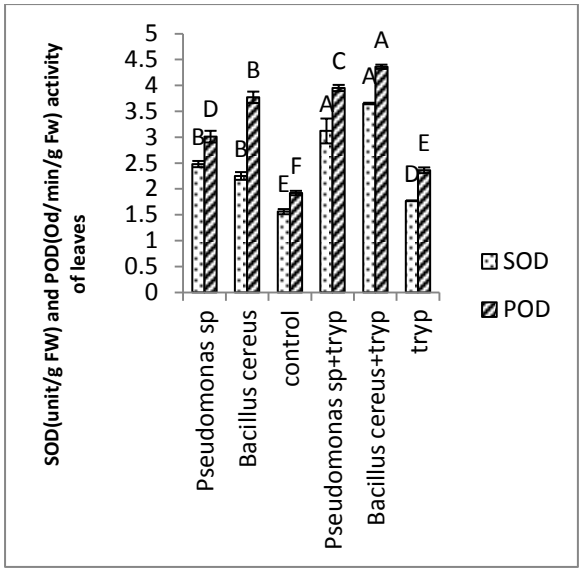

Fig 5. SOD activity (units/g FW) and POD activity (OD/min/g FW) treated with *Pseudomonas* sp and *Bacillus cereus* alone and in addition with tryptophan, control= untreated uninoculated and tryp= tryptophan alone

increase in SOD while POD activity was 30% higher. *Bacillus cereus* exhibited 44% higher SOD and 96% higher POD activity. Addition of tryptophan with *Bacillus cereus* exhibited further 55% higher SOD and 16% higher POD activities. Antioxidant activities increased by PGPR application because proline acts as ROS scavenger (Ghorbanpour *et al*., 2012).

Phytohormones in soil and leaves

Rhizopheric soil of wheat inoculated with *Pseudomonas* sp and *Bacillus cereus* contained 50% and 89% higher IAA respectively over uninoculated control soil (Fig 6) whereas leaves of inoculated plants contained 17-18% higher IAA over control. Tryptophan addition with *Pseudomonas* sp and *Bacillus cereus* increased IAA contents by 2fold in rhizospheric soil and 20% higher than control in leaves. Zahir *et al*., 2007 reported increased wheat growth with addition of PGPR and tryptophan. Increase in the level of IAA in the rhizospheric soil and leaves following inoculation with PGPR in presence and absence of tryptophan demonstrate the PGPR induced modulation of IAA level in the inoculated plants and several genra of *Bacillus and Pseudomonas* are reported to be involved (Saharan and Nehra *et al*., 2011).

The *Bacillus cereus* produced higher ABA than that of *Pseudomonas* sp. Addition of tryptophan to rhizospheric soil significantly augmented ABA production both in rhizospheric soil and plant leaves. The ABA content (Fig 6) of leaves treated with both PGPR was 13-16% higher than that of IAA. The ABA contents of *Bacillus cereus* was 30% higher in

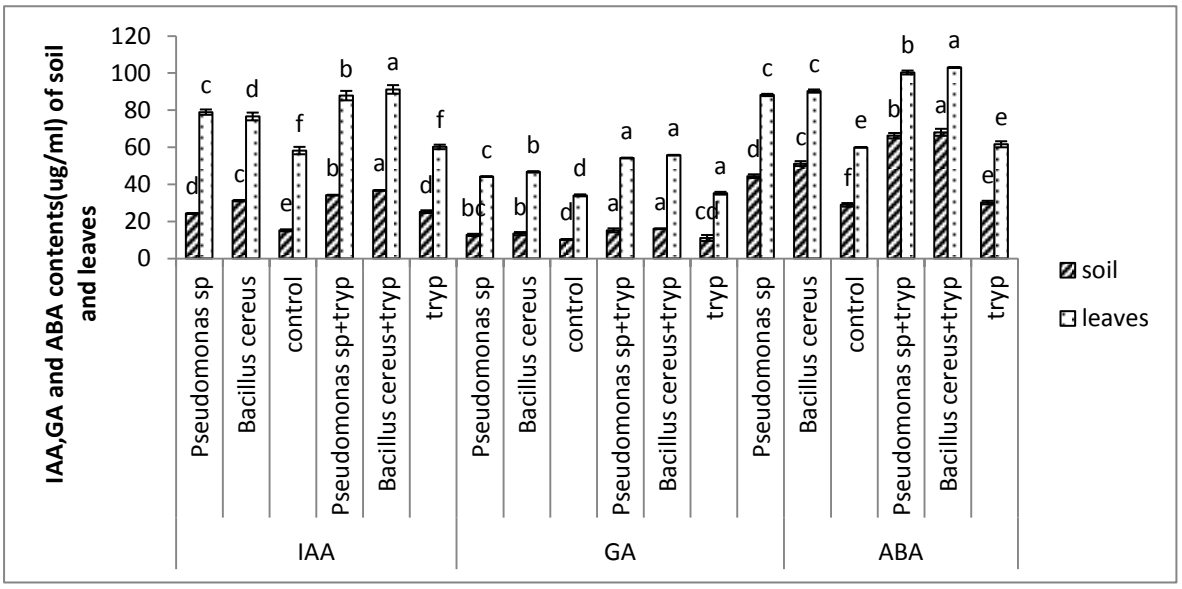

Fig 6. Measurement of Indole acetic acid(IAA), Gibberellic acid GA) and Abscisic acid(ABA) (ug/ml) production after 57d of inoculation in rhizospheric soil and leaves of wheat

Control = uninoculated plants and soil

rhizospheric soil than that of uninoculated control soil. As compared to uninoculated control ABA contents in the leaves were 80-84% higher in *Pseudomonas* sp and *Bacillus cereus* inoculated plants respectively. Addition of tryptophan to plants increased the ABA content of soil further by 30-50% whereas in leaves 12-15% more ABA were observed over uninoculated control.

Bacillus cereus produced 6-8% higher Gibberelic acid (Fig 6) than that of *Pseudomonas* sp. Leaves of the plant had 35% and 29% higher GA and 24-31% higher GA contents were recorded in rhizospheric soil over uninoculated control. Tryptophan addition had resulted 50% more GA in rhizospheric soil and 62% higher GA in leaves of PGPR inoculated plants. Plant growth and development is modulated by the enzymes and phytohorhormones and PGPR based mechanism is involved in direct synthesis of Indole acetic acid, Gibberelic acid and Abscisic acid (Gray *et al.*, 2005).

Spike length, Seeds/spike and seed weigh *Pseudomonas* sp and *Bacillus cereus* significantly increased (35% of control) spike length (Table 4). Addition of tryptophan with *Pseudomonas* sp and *Bacillus cereus* further increased the spike length by 4% and 14% respectively. *Pseudomonas* sp and *Bacillus cereus* increased 27%, 40% seeds/spike respectively and addition of tryptophan with *Pseudomonas* sp and *Bacillus cereus* further increased (11-15%) seeds/spike. Both *Pseudomonas* sp and *Bacillus* cereus increased seed weight equally (36%) and addition of tryptophan increased seed weight by 5% in both treatments.

Table 4. Effect of PGPR application on yield parameters at maturity

Treatment	spike length	Seeds/ spike	Number of seeds/spike
control	3.7D ±0.05	22D ±1	22.06C ±0.54
***Pseudomonas* sp**	5.06B ±0.04	28.63BC ±0.63	30.65A ±0.15
Bacillus cereus	5.02B ±0.13	31.37AB ±1.38	30.73A ±0.38
Pseudomonas* sp+*tryp	5.2B ±0.1	31.88AB ±0.88	31.21A ±0.21
Bacillus cereus*+*tryp	5.51A ±0.13	32.75A ±0.25	31.19A ±0.19
tryp	4.4C ±0.02	26.88C ±1.88	24.65C ±0.75

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 (Cakmakci *et al.*, 2007) and increase rate of photosynthesis (Baset-Mia *et al.*, 2010)

Conclusion

Bacillus cereus and *Pseudomonas* sp have ability to convert and utilize tryptophan. Long term survival of PGPR in sterilized soil along with added tryptophan alleviate osmotic, oxidative and dehydration stresses. Improvement in phytohormone contents of leaves and soil under salt stress due to applied PGPR and tryptophan is of great importance. Hence tryptophan application may be beneficial for plant adaptability under stress.

Acknowledgement

We are thankful to Higher Education Commission (HEC) of Pakistan for providing us support and funding for our work.

References

Arshad M, Hussain A, Shakoor A. 1995. Effect of soil applied L–tryptophan on growth and chemical composition of cotton, Journal Of Plant Nutrtion **18,** 317-329.

Ali NM, Yusof HM, Long K, Yeap SK, Ho WY, Beh BK, Koh SP, Abdullah MP, Noorjahan NB. 2013. Antioxidant and Hepatoprotective Effect of Aqueous Extract of Germinated and fermented Mung Bean on Ethanol-Mediated Liver Damage. Bio Med Research International **9,** 693-713.

Ashrafuzzaman M, Hossen FA, Ismail MR, Hoque MA, Islam MZ, Shahidullah SM, Meon S. 2009. Efficiency of plant growthpromoting Rhizobacteria (PGPR) for the enhancement of rice growth. African Journal of Biotechnology **8,** 1247-1252.

Barazani O, Friedman J. 2000. Effect of exogenously applied L–tryptophan on allelochemical activity of plant – growth promotion rhizobacteria. Journal of Chemical Ecology **26,** 343–349.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Analytical Biochemistry **44,** 276-287.

Baset-Mia MA, Shamsuddin ZH, Wahab Z, Marziah M. 2010. Rhizobacteria as bioenhancer and biofertilizer for growth and yield of banana (Musa spp. cv. 'Berangan'). Science Horticulture Amsterdam **126(2),** 80-87.

Bates LS, Waldern RP, Teare ID. 1973. Rapid determination of free proline for water status studies. Plant and Soil **39,** 205–207.

Bull CT, Weller DM, Thomashow LS. 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. Phytopathology **81,** 954-959.

Cakmakci RF, Aydın A, Sahin F. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under green house and two different field soil conditions. Soil Biology and Biochemisty **38,** 1482-1487.

Cakmakci RF, Dönmez MF, Erdoğan U. 2007. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. Turkish Journal of Agriculture and Forestry **31,** 189-199.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugar and related substances, Annals of Chemistry, **28,** 350-356.

Etesami H, Alikhani HA, Akbari A. 2009. Evaluation of plant growth hormones production (IAA) ability by Iranian soils rhizobial strains and effects of superior strains application on wheat growth indexes. World Applied Sciences Journal **6,** 1576–1584.

Frankenberger WT, Arshad M. 1995. Phytohormones in soil. Microbial production and function. In: Marcel Dekker, ed. New York: 1-13.

Frankenberger WT, Brunner W. 1983. Methods of detection of auxin indole acetic acid in soil by high performance liquid chromatography. Soil Science Society of American Journal **47,** 237-241.

Frankenberger WT, Arshad M. 1991. Yield response of watermelon and muskmelon to L-tryptophan applied to soil. Horticultural Science **26,** 35-37.

Garcia C, Hernandez T. 1996. Influence of salinity on biological and biochemical activity of calciorthid soil. Plant and Soil **178,** 225-263.

Gray EJ, Smith DL. 2005. Intracellular and extracellular PGPR, commonalities and distinctions in the plant–bacterium signaling processes. Soil Biology and Biochemistry **37,** 395–412.

Hasnain S, Sabri AN. 1996. Growth stimulation of *Triticum aestivum* seedlings under Cr- stresses by non rhizospheric pseudomonad strains. Environmental Pollution **3,** 265-73.

Idris EE, Iglesias DJ, Talon M, Borriss R. 2007. Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Molecular Plant Microbe Interaction **20,** 619-626.

Joo HS, Kumar CG, Park GC, Paik SR, Chang CS. 2004. Bleach-resistant alkaline protease produced by a *Bacillus* sp. isolated from the Korean polychaeta, *Periserrula leucophryna*. Process Biochemistry **39,** 1441–1447.

Kettner J, Doerffling K. 1995. Biosynthesis and metabolism of abscisic acid in tomato leaves infected with *Botrytis cinerea*. Planta **196,** 627-634.

Kohler J, Caravaca F, Carrasco L, Roldan A. 2006. Contribution of *Pseudomonas medocina* and glomus intraradices to aggregate stabilization and promotion of biological properties in rhizosphere soil of lettuce plant under field condition. Soil Use and Management **22,** 245-252.

Meloni DA, Oliva MA, Ruiz HA, Martinez CA. 2001. Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. Journal Of Plant Nutrition **24,** 599-612.

Patten CL, Glick BR. 2002. Role of Pseudomonas putida indole acetic acid in development of the host

plant root system. Appllied Environmental Microbiology **68,** 3745-3801.

Prado FE, Boero C, Gallardo M, Gonzalez JA. 2000. Effect of NaCl on germination,growth, and soluble sugar content in Chenopodium quinoa Willd. Seeds. Botanical Bulletin of Academia Sinica **41,** 27-34.

Reitmeier RF. 1943. Semi microanalysis of saline soil solutions. In: Indus. And Engin. Chem Analyt, ed. **15,** 393-402.

Rekha PD, Lai Wa, Arun AB, Young CC. 2007. Effect of free and encapsulated Pseudomonas putida CC-FR2-4 and Bacillus subtilus CC-pg 104 on plant growth under gnotobiotic condition. Bio research Technology 98: 447-451.

Saharan BS, Nehra V. 2011. Plant growth promoting rhizobacteria: A critical review. Life Science and Medicine Research **21,** 1-30.

Sarwar M, Frankenberger WT. 1994. Tryptophan dependent biosynthesis of auxins in soil. Plant and Soil **160,** 97-104.

Shafi M, Bakhat J, Khan MJ, Khan MA, Anwar S. 2010. Effect of salinity on yield and ion accumulation of wheat genotypes. Pakistan Journal of Botany **42,** 4113–4121.

Singh JS, Pandey VC, Singh DP. 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agriculture, Ecosystem and Environment **140,** 339–353.

Spaepen S, Vanderleyden J, Remans R. 2007. Indole-3-acetic acid in microbial and microorganism plant signaling. Federation of European Microbiological Societies Microbiology Review **31,** 425-448.

Tilak KVBR, Ranganayaki N, Manoharachari C. 2006. Synergistic effects of plant-growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). European Journal of Soil Sciences **57,** 67–71.

Tien TM, Gaskin MH, Hubbel DH. 1979. Plant growth substances produced by*Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Applied Environmental Microbiology **37,** 1016–1024.

Upadhyay SK, Singh JS, Saxena AK, Singh DP. 2012. Impact of PGPR inoculation on growth and antioxidants status of wheat plant under saline condition. Plant Biology **14,** 605-611.

Vetter JL, Steinberg MP, Nelson AI.1958. Quantitive Determination of peroxidase in sweet corn. Journal of Agricultural and Food Chemistry **6,** 39-41.

Yasmin F, Othman R, Sijam K, Saad MS. 2007. Effect of PGPR inoculation on growth and yield of Sweet potato. Journal of Biological Sciences **7(2),** 421-424.

Walkley A. 1947. A critical examination of a rapid method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sciences **63,** 251-263.

Walkley A, Black IA. 1934. An examination of degtiareff method for determining soil organic matter and and a proposed modification of chromic acid titration method. Soil Sciences **37,** 29-37.

Zahir ZA, Naveed M, Zafar MI, Rehman HS, Arshad M, Khalid M. 2007. Evaluation of composted organic waste enriched with Nitrogen and L-tryptophan for improving growth and yield of wheat (*Triticum aestivum*). Pakistan Journal of Botany **39(5),** 1739-1749.