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Full Length Research

Effect of PGPR on growth and performance of *Zea mays*

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The present study was aimed to investigate the effect of hormones producing plant growth promoting rhizobacteria on Maize variety EV-5098. The plant growth promoting rhizobacteria were isolated from root and rhisospheric soil of wheat infected with rust and powdery mildew. These plant growths promoting rhizobacteria produced high level of Indole acetic acid and Gibberellic acid in culture. The efficiency of these isolates were checked (evaluated) as bioinoculant on maize. Leaves and roots samples were collected 28 days after treatments. Single inoculation of plant growth promoting rhizobacteria resulted in an increase in proline, sugar, chlorophyll, root length, shoot length and salicylic acid. Among the treatments, co-inoculation was found much effective for protein contents and activities of superoxide dismutase, peroxidase and catalase. The present work revealed that co-inoculated treatments have accumulated heavy metals such as Cr^{+3}, Co^{+3}, Mn^{+3}, Ni^{+3}, Pb^{+4} and Cd^{+4}. Therefore these bacterial isolates can be used in bioremediation of heavy metals and can be used as bioinoculant. PGPR offer an environmentally sustainable approach to increase crop production and health. The application of molecular tools is enhancing our ability to understand and manage the rhizosphere and will lead to new products with improved effectiveness.

Keywords: Maize variety EV-5098, rust, powdery mildew, heavy metals.

INTRODUCTION

Maize being the highest yielding cereal crop in the world is of significant importance for countries like Pakistan, where rapidly increasing population has already out stripped the available food supplies. Several biotic and abiotic problems may affect the maize yields depending on many factors i.e. soils, climate and other natural factors. The major and most important stress problems which cause an economic loss to maize crop in Pakistan have been identified as; Maize stem borer, Stalk rots disease, Leaf blight, Drought and moisture Stress and Water logging.

The mechanisms by which PGPRs promote plant growth are not fully understood, but are thought to include: the ability to produce phytohormons (Bashan and Lavanony., 1999). Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been reported (Cattelan *et al*., 1999; Chanway *et al*., 1989; Ciccillo *et al*., 2002). *Azospirillum*, *Pseudomonas* and *Azotobacter* strains could affect seed germination and seedling growth (Cook, 2002). Inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of cereals (Chanway *et al*., 1989). Thus it has been shown that *Azospirillum* and *Pseudomonas* had the potential for agricultural exploitation and could use as natural fertilizers (Garland, 1996; Germida, 1998). Similarly, promotion in growth parameters and yields of various crop plants in response to inoculation with PGPR were reported by other workers (lynch and Whipps, 1991; Mansouri *et al*., 2002; Baudoin *et al*., 2002).

MATERIALS AND METHODS

Plant material and growth conditions

Initially, experiments on maize variety EL-5089 were

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conducted in pots at the Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Seeds of maize were soaked in distilled water for 24 hrs. Prior to sowing each pot was filled with soil and were placed under natural climatic conditions. When maize crop reached late vegetative stage (28 days after sowing) plants were harvested and separated into leaves, stems and roots. They were dried, ground and stored.

Sterilization of seeds

Seed were surface sterilized with 95% ethanol for 1 minute followed by sterilization in 10% chlorox (commercial grade) with shaking for 2-3 minutes, followed by successive 8-9 times washings with sterilized water.

Preparation of inoculums and its application

Fresh cultures of isolates were prepared by inoculating pikoviskya media by inoculating with bacterial isolates and shaken at 30°C for 48 hrs in an orbital shaker at 120 rpm. Then 48 hrs old bacterial cultures were centrifuged at 3000 rpm for 15-20 mins., when the OD of cultures at 600nm for 1. The sterilized seeds were soaked in distilled water in case of un-inoculated control. The rest of sterilized seeds were soaked in broth cultures of isolates for 4-5 hrs prior to sowing.

Elemental analysis of root and shoot of maize plants

For the determination of all the aforementioned elements, different stock solutions were made. 100 ppm stock solution of the K, Mg, Ca, Na, Fe, Co, Mn, Cu, Cr, Zn, Ni, Li, Pb, Cd, were prepared by dissolving required amount of salts in distilled water. The availability of different elements in all plants samples was determined by Perchloric-acid digestion method (Allen, 1974).

Leaf protein contents

Protein content of leaves was determined following the method of Lowery *et al*. (1951) using BSA as standard.

Phosphate Buffer (Stock Solution)

a. Monobasic sodium phosphate: 27.6g was dissolved in distill water (1000ml)
b. Dibasic sodium phosphate: 53.6g was dissolved in 1000ml

Monobasic sodium phosphate (16ml) and dibasic sodium phosphate (84ml) was mix together to get the desire pH (7.5) of phosphate buffer.

Reagent A: 2g sodium carbonate (Na_2CO_3) 0.4g NaOH (0.1 N) and 1g Na-K tartrate was dissolved in100 ml of distilled water?
Reagent B: $CuSO_4.5H_2O$ (0.5g) was dissolved in 100ml of distill water.
Reagent C: Solution A (50ml) and Solution B (1ml) were mixed.
Reagent D: Folin phenol reagent was diluted with distill water in 1:1 ratio.

Procedure

Fresh leaves of 0.1g were ground with the help of mortar and pestle in 1ml of phosphate buffer pH 7.5 and, centrifuged for 10 min at 3000rpm. The supernatant (0.1ml) of given sample containing unknown amount of protein was poured in the test tubes and total volume of 1ml was made by distilled water. 1ml of reagent C was added. After shaking for 10min, 0.1ml of reagent D was added. The absorbance of each sample was recorded at 650nm after 30 min incubation. The concentration of protein contents was determined with the reference to standard curve made by using standard BSA (Bovine Serum Albumen). The BSA of different concentration viz 20,40,60,80,320, and 640mg was prepared. The absorbance of BSA was recorded at 650 nm.

Sugar estimation

Sugar estimation of fresh leaves was done following method of Dubios *et al*. (1949).

Procedure

Fresh plant material (0.5g) was homogenized with 10ml of distilled water in a clean mortar and then centrifuged at 3000 rpm for 5 min. In 0.1ml of supernatant 1ml of 80% (w/v) phenol was added, after incubation at room temperature, 5ml concentrated sulphuric acid was added. The sample is incubated for 4 hrs and ten absorbance of each sample was recorded at 420 nm. The concentration of unknown sample was calculated with reference to standard curve made by using glucose.

Chlorophyll contents

Leaf chlorophyll content was recorded by 'SPAD-502' chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan (Arenas *et al*., 2002) before harvesting when plants were 28 days old. To record reading displayed in pre-calibrated SPAD units, leaf was inserted at about half way from the leaf tip and collar and about middle point between the leaf midrib and leaf margin. The area of measurement chamber had a range of 2 × 3mm. The

young fully expanded leaf which had an exposed collar was chosen for the measurement (Noguchi and Hansen, 1999).

Proline content of leaves

The proline contents of leaves were measured by the method of Bates *et al*. (1973). Fully expanded fresh leaves were sampled. Purified praline was used to standardize the procedure for quantifying sample values. Reagent acid Ninhydrin was prepared by warming 1.25g ninhydrin in 30ml glacial acetic acid and 20ml 6 M phosphoric acid, with agitation, until dissolved. Kept cool (store at 4℃), the reagent remains stable for 24 hrs. Approximately 0.5g of plant material was homogenized in 10ml of 3% aqueous sulphosalicylic acid and homogenate filtered with Whatman No. 42 filter paper. 2ml of filtrate was reacted with 2ml acid ninhydrin and 2ml of glacial acetic acid in a test tube for 1 hr at 100℃ and reaction is terminated in ice bath. The reaction mixture was extracted with 4ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warm to room temperature and absorbance read at 520nm against toluene as blank. The proline concentration was determined from standard curve and calculated on fresh weight basis as follow:

[(ug proline /ml x ml toluene)/ 115.5ug/umol]/ [(g sample)/5] = µ mol proline/g of fresh weight material.

Extraction for peroxidase

Fresh leaves (5g) were homogenized with 15ml of 0.05N phosphate buffer (PH 7.0) containing 10% poly vinyl poly pyrrolidore (PVPP) and 0.1 M Ethylene diamine tetra acetate (EDTA). Homogenate was centrifuged at 15,000 rpm for 15 min at 4℃. Supernatant was used for superoxide dismutase (SOD) and peroxidase (POD) assay.

Assay for peroxidase activity (POD)

POD activity was determined by the method of Vetter *et al*. (1958) as modified by Gorin and Heidema (1976). POD activity was measured following 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 for 3min with spectrophotometer. 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). The reaction mixture (3ml) was composed of 13mM methionine, 0.075 mM NBT, 0.1 mM EDTA, 0.002mM riboflavin and 0.1ml of enzyme extract in 50mM phosphate buffer (pH 7.8). The mixture in tube was placed below light chamber for 15 min. The absorbance was read at 560nm with spectrophotometer. One unit of enzyme activity was taken as that quantity of enzyme, which reduced the absorbance reading to 50 in comparison with tube lacking enzyme.

Assay for ascorbate peroxidase activity (APOX)

Ascorbate peroxidase (APX) activity was determined according to Asada and Takahashi (1987). The reaction mixture (1ml) contained 50 Mm of potassium phosphate buffer (pH 7.0), 0.5Mm of ascorbic acid, 0.1Mm of H2O2, and 200 µl of enzyme extract. The absorbance was read as the decrease at 290nm against the blank, correction was done for the low, none enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient: 2.9 Mm-1Cm-1).The enzymatic activity was expressed in U mg-1 protein (U=change in 0.1 absorbance $min^{-1}mg^{-1}$ of protein).

Assay for catalase activity (CAT)

Catalase (CAT) was measured according to Chandlee and Scandalios (1984) with modification. The essay mixture contained 2.6 ml of 50 mM potassium potassium phosphate buffer (pH 7.0), 0.4ml of 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_20_2 was followed by the decline in absorbance at 240nm.The enzyme activity was expressed in U mg $^{-1}$ protein (U=1Mm of H_2O_2 reduction min^{-1} mg^{-1} 0f protein).

Assay for polyphenol oxidase activity (PPO)

The activity of polyphenol oxidase was determined by the method of Kar and Mishra (1976) with some modification. The 3 ml reaction mixture contained 25 mM phosphate buffer (pH 6.8), 0.1 mM pyrogallol, 0.1 ml enzyme extract and blank without pyrogallol. The absorbance of the purpurogallin formed was recorded at 420 nm, and activity was calculated using the extinction coefficient [2.47 mM-1cm-1] for purpurogallin.

Determination of phytohormones

Salicylic acid (SA)

Salicylic acid was extracted and purified according to the method of Enyedi *et al.* (1992) and Seskar *et al*. (1998) with some modifications. The plant leaves (1g) were

grounded in 80% methanol, at 4°C with an antioxidant butylated hydroxy toluene (BHT). The homogenate was vortexed for 10 min and filtered with Whatman No. 42 paper. The residues in the flask and filter paper were rinsed three times with aliquots of MeoH-BHT and two times with 100% methanol. The extracts were pooled together, mixed and used for the purification of SA. The extract was concentrated to an aqueous residue by rotary flask evaporator (RFE) at 40°C. The flask used for RFE was rinsed with 10 ml n-hexane. Partitioning was done with n-hexane at pH 8 followed by partitioning with n-hexane at pH 3. The pH was then adjusted to 2.8 and centrifuged for 15 min at 13,000 rpm. The supernatant was again partitioned three times with 10 ml diethylethet-BHT. Each 10 ml portion of ether was partitioned against 1 mM HCl. Three fractions of partitioned ether were combined and dried down by RFE. The residue was immediately dissolved in 500 µl 80% ice cold methanol and kept in 1.5 ml Eppendorf tube. These samples were stored overnight and then recentrifuged at 25,000 rpm for 10 min. The supernatant was collected and subjected to HPLC for analysis. Samples were analyzed on HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller) using U.V. detector and C-18 column. For identification of hormones, samples filtered through 0.45-millipore filters were injected into column. The elution system contain 100% methanol:1% acetic acid (52:48 v/v) as solvent; run with 1.10 ml/min flow rate at 40°C. Detection of salicylic acid was done at 280 nm by co-chromatography. The standards were also prepared by following the same method.

Extraction, purification and quantification of ABA, IAA, GA from bacterial culture

Extraction, purification and quantification of phytohormones from bacterial culture were made to understand the mechanism of growth promotion of these microbes used as inoculants. Pikoiskya broth media without tryptophan (10 mg/100 ml) was inoculated with 24 h old bacterial cultures and kept on a shaker (ECELLA E24, USA) at 100 rpm for 5 d. Thereafter, the bacterial cells were harvested by centrifugation at 10,000 rpm for 15 min at 4ºC and supernatant (cell-free liquid culture medium) was used for extraction of phytohormones following the method described by Tien *et al*. (1979). The samples were analyzed on HPLC (Agilent 1100) equipped with variable UV detector and C18 column (39 × 300 mm) (BondaPack Porasil C-18, 37/50 µm, Waters, Eschborn, BRD). Methanol and water in the ratio of 30:70 v/v were used as mobile phase @ of 1500 µl/min. with a run time of 20 min/sample. For identification of hormones, 100 µl of sample filtered through a 0.45 millipore filter, were injected into column. The growth hormones were identified on the basis of retention time of phytohormone standards (commercially grade, Sigma Chemical USA Company). IAA was eluted at 280 nm wave length while GA3 and ABA were eluted at 254 nm respectively. The culture media without microbial inoculation were taken as blank and processed for phytohormones extraction as earlier described.

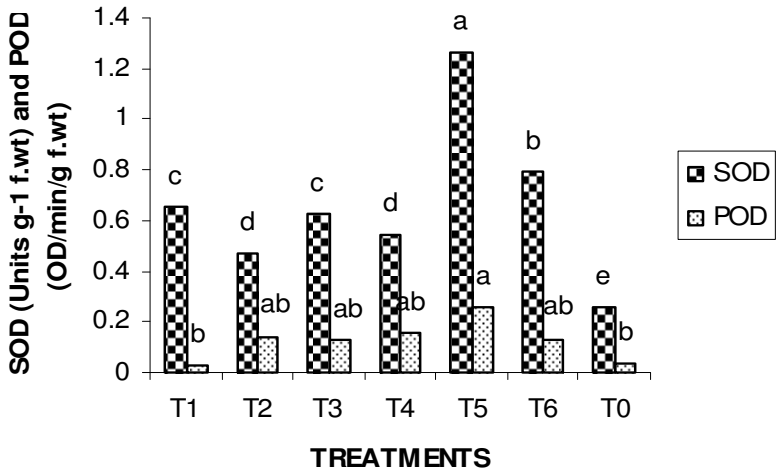
Statistical analysis

The data were analyzed statistically by Analysis of Variance technique (Steel and Torrie, 1980) and comparison among treatment means was made by Duncan's Multiple Range Test (DMRT) (Duncan's, 1955).

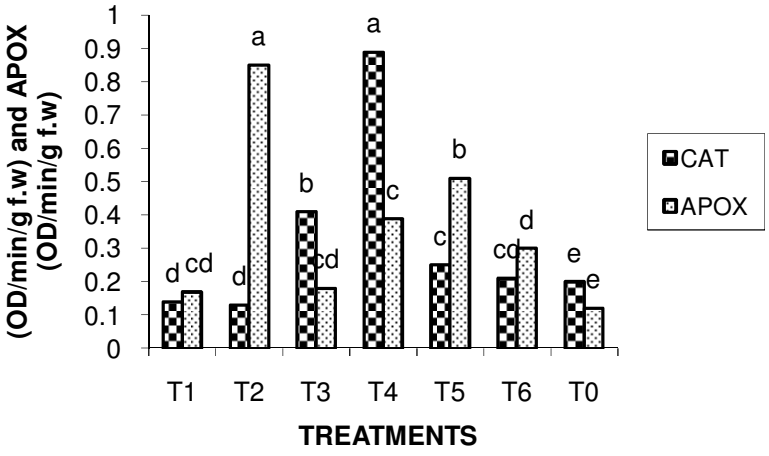
RESULTS

Result in Figures 1 indicated that significant increase was found in SOD activity of leaves of maize variety (EL 5098) inoculated with PGPR compared to un-inoculated control plant. The SOD was found maximum of all the antioxidant enzymes, while no significant result was found between root and soil isolates, whereas co-inoculation treatments resulted in significant inoculation. The SOD was found maximum in T5 (Co-inoculation with PGPR isolate from root of wheat infected with rust and isolate from rhizospheric soil of wheat infected with powdery mildew) resulted in 79% increase in SOD activity as compared to control plants whereas T1 (PGPR isolated from root of wheat infected with rust stem) and T3 (PGPR isolated from rhizospheric soil of wheat infected with powdery mildew) were found with no significant variation. Similarly maximum POD activity was found in T5 (Co-inoculation with PGPR isolated from root of wheat infected with rust and rhizospheric soil of wheat infected with powdery mildew) and resulted in 98% increase as compared to control plants whereas T2, T3, T4 and T6 have no significant variation in POD activity. The CAT activity was maximum in T4 (co-inoculation of PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew) resulted in 96% increase as compared to control plants, whereas T1 showed non-significant variation from control plants. In APOX maximum activity was found in T2 resulted in 85% increase as compared to control plants. As compared to control plants, all the treatments showed significant increase in the CAT activity of leaves over control and similar was the case with APOX.

Polyphenol oxidase enzyme (PPO) helps in defense mechanism of plants. It converts toxic phenolic compounds into quinones which are not harmful to plants. Polyphenol oxidase enzyme (PPO) was found highest in T1 (PGPR isolated from root of wheat infected with rust stem) resulted in 57% increase as compared to control plants. In T3 (PGPR isolated from rhizospheric soil of wheat infected with powdery mildew) and T4 (PGPR isolated from root of wheat infected with rust and



(a)



(b)

Figures 1. Effect of PGPR inoculation on SOD, POD, CAT and APOX activity of Maiz. T1: Inoculated with PGPR isolated from root of wheat infected with rust stem. T2: Inoculated with PGPR isolated from root of wheat infected with powdery mildew. T3: Inoculated with PGPR isolated from rhizospheric soil of wheat infected with powdery mildew. T4: Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew. T5: Co-inoculation with PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew T6: Co-inoculation with PGPR isolated from root of wheat infected with powdery mildew and rhizospheric soil of wheat infected with powdery mildew T0: Control.

root of wheat infected with powdery mildew) treatments no significant variation was between them, whereas T2 (PGPR isolated from root of wheat infected with powdery mildew) and T5 (PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew) treatments were found with no significant variation from control plants. No significant variation existed between root and soil isolates. Result in Figures 2 and 3 **i**ndicated that significant increase was found in sugar content of leaves inoculated with PGPR as compared to un-inoculated plants. The root isolates performed significantly greater than the soil isolates. The sugar content was found higher in T2 resulted in 51% increase in sugar content as compared to control. Whereas T1 (PGPR isolated from root of wheat infected with rust stem and T3 (PGPR isolated from rhizospheric soil of wheat infected with powdery mildew) treatments have found no significant variation. Proline content was found highest in T1 resulted in 19% increase as compared to control. Result in Figure 4 indicated that

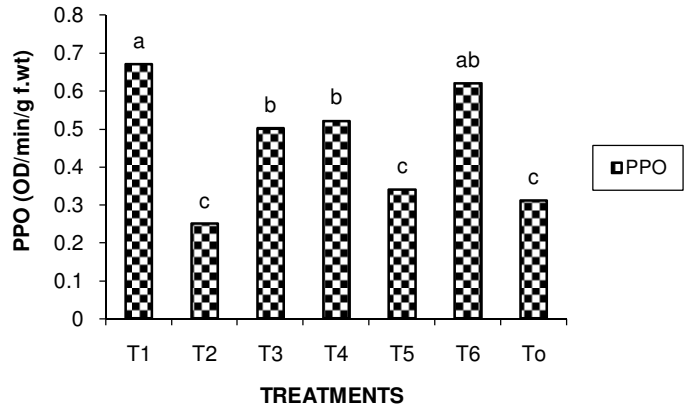


Figure 2. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on PPO (OD/min/g f.wt) of maize variety EL 5098, Measurement were made 28 D after treatment.

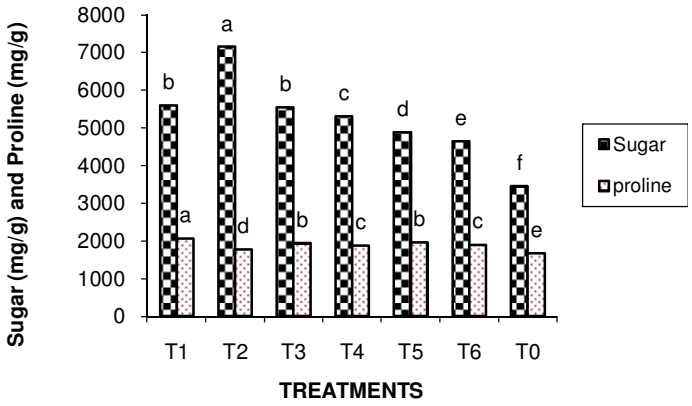


Figure 3. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on Sugar and Proline (mg/g) of maize variety EL 5098, Measurement were made 28 D after treatment.

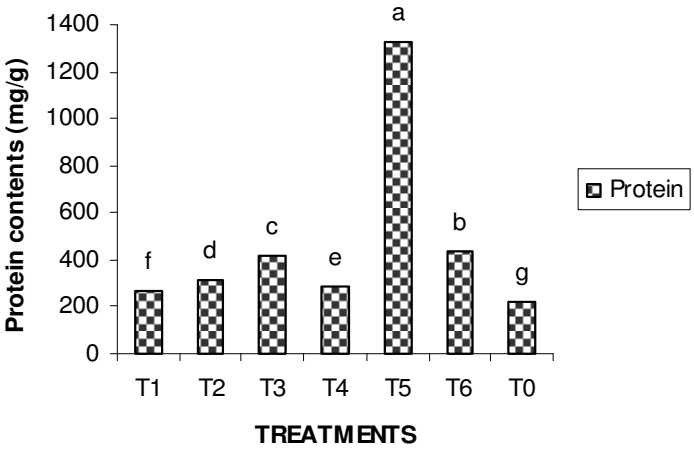


Figure 4. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on Protein (mg/g) of maize variety EL 5098, Measurement were made 28 D after treatment.

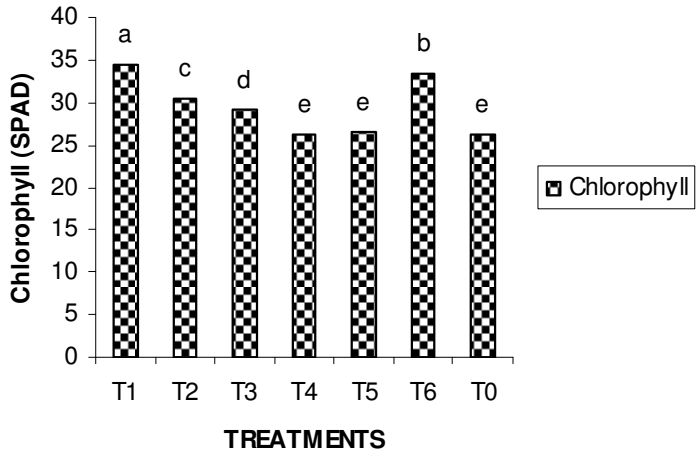


Figure 5. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on Chlorophyll content (SPAD) of maize variety EL 5098, Measurement were made 28 D after treatment.

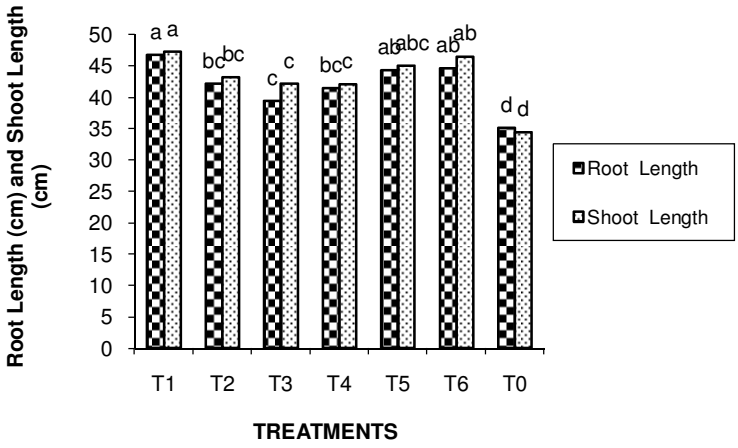


Figure 6. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on root and shoot length of maize variety EL 5098, Measurement were made 28 D after treatment.

protein contents was found to have the highest in T5 (co-inoculation of PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew) resulted in 83% increase as compared to control.

Result in Figure 5 indicated the chlorophyll content, no significant result was found between root and soil isolates. Results showed that chlorophyll content were found highest in T1 (PGPR isolated from root of wheat infected with rust stem) resulted in 33% increase in chlorophyll content whereas no significant variation was found among T4 (Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew) and T5 (Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew) treatments. Analysis of data in Figure 6 indicated that application of PGPR to plants showed significant increase in root length and shoot length as compared to untreated plants. In root length significant effect was found in root isolate as compared to soil isolate. The results showed that T1 have highest root length 24% as compared to control plants. In shoot length significant result was found in root isolate as compared to soil isolate. T1 have highest shoot length 27%, whereas T3 and T4 treatment have no significant variation among each other. All the treatments significantly increase root and shoot length over the control. The % increase in shoot length was found higher

Table 1. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.) on R/S Ratio and Leaf Area (cm) of maize variety EL 5098, Measurement were made 28 D after treatment.

Treatment	R/S Ratio (cm)	Leaf Area (cm)
T1	0.91 a	29 b
T2	0.93 a	39.66 a
T3	0.94 a	27 bc
T4	0.94 a	25 c
T5	0.92 a	37.66 a
T6	0.90 a	36.66 a
T0	0.91 a	19 d
LSD	**0.03**	**2.04**

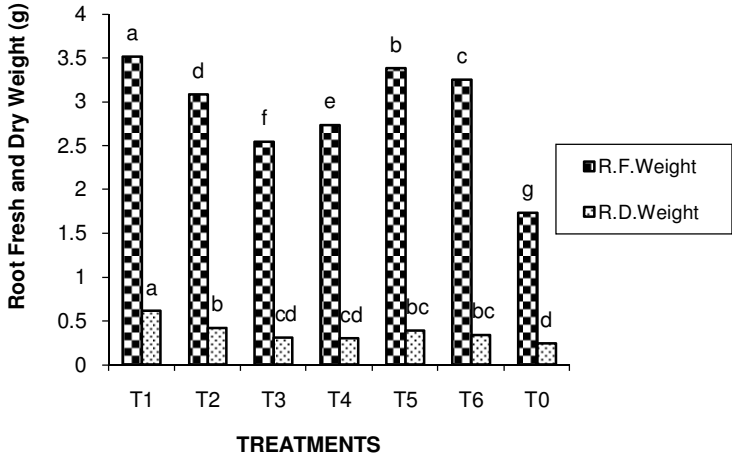


Figure 7. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on root fresh and dry weight (mg/g) of maize variety EL 5098, Measurement were made 28 D after treatment.

than the root length.

Table 1 showed root/shoot ratio and leaf area and it was concluded that no significant variation was found in root/shoot ratio in all the treatment as compared to control plants. Significant result was found in root isolate and in co-inoculation treatments. Maximum leaf area was found in T2, T5 and T6 treatments and all these treatments have no significant variation among each other. The maximum leaf area is about 57% greater as compared to control plants.

Data in Figure 7 revealed that PGPR application resulted in significant increase in root, shoot fresh and dry weight as compared to untreated (control) plants. Root fresh weight was found maximum in T1 resulted in 50% increase as compared to control plants; this was followed by T5 treatment. Root dry weight was found highest in T1 resulted in 60% increase as compared to control plants, whereas T3, T4, T5 and T6 treatments have no significant variation among each other. Among all the treatments single inoculation with PGPR was found to be more effective.

Result in Figure 8 indicated shoot fresh and shoot dry weight. Shoot fresh weight was found highest in T2 (PGPR isolated from root of wheat infected with powdery mildew) which resulted in 34% increase as compared to control plants. Also shoot dry weight was increased significantly in T2 (PGPR isolated from root of wheat infected with powdery mildew) resulted in 45% increase as compared to control plants.T4 and T6 treatments were better than T5 among co-inoculation treatments. No significant difference was observed among single inoculation or co-inoculation.

Significant increase was found in leaves salicylic acid by application of PGPR to maize plant variety (EL 5098). Highest salicylic acid was found in T1 (PGPR isolated from root of wheat infected with rust stem) resulted in 88% increase as compare to control plants, whereas in T5 (Co-inoculation with PGPR isolated from root of wheat

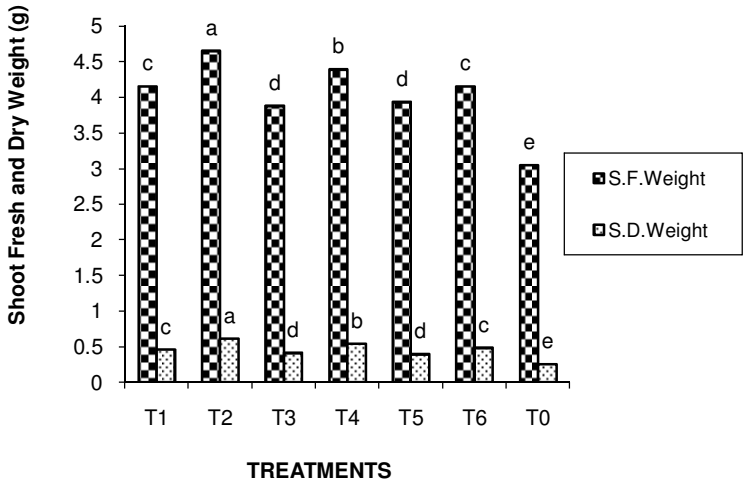


Figure 8. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on root fresh and dry weight (mg/g) of maize variety EL 5098, Measurement were made 28 D after treatment.

infected with rust and rhizospheric soil wheat infected with powdery mildew) treatments no significant concentration was found.

Macronutrients accumulation in root and shoot

Result in Table 2 indicated that significant concentration of nutrient was found in root and shoot of maize plants treated with PGPR as compared to untreated plants. Na^+ content in root was found higher in T2 (PGPR isolated from root of wheat infected with powdery mildew) which resulted in 24% increase as compared to control plants. While higher Na^+ content was found in shoot of T5 which resulted in 48% increase in Na^+ content of shoot as compared to control plants. Result indicated that K^+ content was found highest of all the macronutrient. There were significant result found in root and soil isolates whereas co-inoculation treatment was found to have highest K^+ content. In root highest K^+ content was found in T4 (Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew) which showed 45% increase as compared to control plants, whereas in T1 and T6 treatments as well as T3 and T5 treatments do not differ significantly. In case of shoot K^+ was found with no significant variation in all the treatment but found 92% significant as compared to control plants. In root Mg^{+2} content was found highest in T4 whereas in T2 and T3 treatments no significant variation was found among each other, while T1 and T6 was found non-significant from control plants. In shoot significant increase was found in root isolates as compared to soil isolate. Mg^{+2} in shoot were found highest in T5 and T1 which resulted in 46% increase as compared to control plants.

Micronutrients accumulation in root and shoot

Table 3 showed that among Fe^{+2} accumulations was found higher of all other micronutrient, whereas no significant variation was found between root and soil isolates. In root highest Fe was found in T3 (PGPR isolated from rhizospheric soil of wheat infected with powdery mildew) which resulted in 53% increase as compared to control plants while T2 and T4 do not differ significantly. In case of shoot highest Fe^{+2} was found in T2 and T6 which resulted in 88% increase as compared to control plants. In root Zn^{+2} was found highest in T2 and T6 which resulted in 91% increase as compared to control plants, whereas between T1 and T3 no significant variation was found .In shoot Zn^{+2} content was maximum in T6 which resulted in 64% increase as compared to control plants, whereas between T1 and T5 treatments no significant variation was found. In root Cu^{+3} was found maximum in T6 (Co-inoculation with PGPR isolated from root of wheat infected with powdery mildew and rhizospheric soil of wheat infected with powdery mildew) which resulted in 68% increase as compared to control plants, whereas T1 and T3 treatment as well as T2 and T4 treatment were found with no significant variation among each other. In shoot highest Cu^{+3} was found in T4 and T5 which resulted in 86% increase as compared to control plants, whereas T1 and T6 treatments were found with no significant variation between them.

Heavy metal accumulation in root and shoot

Table 4 showed that $Cr^{+3,}$ Co^{+3}, Mn^{+2} ion accumulations in root and shoot maize of plants. In root Cr^{+3} was found higher in T4 (Co-inoculation with PGPR isolated from

Table 2. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on Na^{+}, K^{+} and Mg^{+2} content of Root and Shoot (ug/g), of maize variety EL 5098, Measurement were made 28 D after treatment.

Treatment	Na (µg/g)			K (µg/g)			Mg (µg/g)		
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
control	2934e	3007c	5941	6747d	1667b	8414	2849d	2658d	5507
T1	3721b	4738b	8459	11305b	21064a	32368	3475cd	4999a	8474
T2	3863a	4459b	8322	11518ab	20899a	32417	4407b	4035c	8442
T3	3859a	5055ab	8914	9673c	20793a	30466	4392b	4544abc	8936
T4	3474b	4479b	7953	12373a	21061a	33434	5066a	4887b	9953
T5	3707b	5792a	9499	9403c	21651a	31054	3502c	5003a	8505
T6	3256d	5227ab	8483	10679b	21231a	31910	3328cd	4121bc	7449
LSD	50.83	824.8	875.63	639.7	933	1572.7	415.3	541.10	956.40

T1: Inoculated with PGPR isolated from root of wheat infected with rust stem. T2: Inoculated with PGPR isolated from root of wheat infected with powdery mildew. T3: Inoculated with PGPR isolated from rhizospheric soil of wheat infected with powdery mildew. T4: Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew. T5: Co-inoculation with PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew T6: Co-inoculation with PGPR isolated from root of wheat infected with powdery mildew and rhizospheric soil of wheat infected with powdery mildew T0: Control.

Table 3. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum L.)* on Fe^{+2}, Zn^{+2} and Cu^{+3} content of Root and Shoot (ug/g) of maize variety EL 5098, Measurement were made 28 D after treatment.

Treatments	Fe (µg/g)			Zn (µg/g)			Cu (µg/g)		
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
control	987 e	219 f	1206	12 f	89 c	101	4.66 d	3.4 e	8.06
T1	1477 d	1008 d	2485	98 d	192 b	290	8 c	11 d	19
T2	1815 b	1954 a	3769	148 a	150 cd	298	14 ab	12.6 d	26.6
T3	2137 a	1825 b	3962	88 d	156 c	244	8 c	19 bc	27
T4	1920 b	1573 c	3493	113 c	128 d	241	13 ab	26 a	39
T5	1514 cd	565 e	2079	138 b	197 b	335	11 b	24 a	35
T6	1605 c	1940 a	3545	147 a	223 a	370	15 a	9.6 d	24.6
LSD	36.36	64.4	100.76	4.4	15.5	19.9	1.8	3.4	5.2

T1: Inoculated with PGPR isolated from root of wheat infected with rust stem. T2: Inoculated with PGPR isolated from root of wheat infected with powdery mildew. T3: Inoculated with PGPR isolated from rhizospheric soil of wheat infected with powdery mildew. T4: Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew. T5: Co-inoculation with PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew T6: Co-inoculation with PGPR isolated from root of wheat infected with powdery mildew and rhizospheric soil of wheat infected with powdery mildew T0: Control

root of wheat infected with rust and root of wheat infected with powdery mildew) which resulted in 83% increase as compared to control, among T2 , T3 and T5 no significant variation was found, while in shoot Cr^{+3} was found higher in T2 (PGPR isolated from root of wheat infected with powdery mildew) which resulted in 91% increase as compared to control plants, whereas T4 treatment was found to have no significant variation from control. In case of Co^{+3} maximum concentration in root was found in T6 (Co-inoculation with PGPR isolated from root of wheat infected with powdery mildew and rhizospheric soil of wheat infected with powdery mildew) and increase was found to be 78% as compared to control plants, whereas no significant variation was found among T3 and T4 treatments. In shoot maximum Co^{+3} was found in T6 (Co-inoculation with PGPR isolated from root of wheat infected with powdery mildew and rhizospheric soil of wheat infected with powdery mildew) which resulted in 60% increase as compared to control plants, whereas among T1 and T3 treatments no significant variation was found as compared to control plants. Result indicated that significant increase in Mn^{+2} concentration was found in T4 (Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew) which resulted in 65% increased as compared to plants, whereas no significant variation was found between T1 and T6 treatments. In case of shoot maximum Mn^{+2} concentration was found in T3 and increase was found 70% as compared to control plants, while T4, T5, and T6 treatments as well as T1 and T2 treatments was found with no significant variation among each other.

Table 4. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum L.)* on Cr^{+3} , Co^{+3} and Mn^{+2} content of Root and Shoot (ug/g) of maize variety EL 5098, Measurement were made 28 D after treatment.

Treatment	Cr (µg/g)			Co (µg/g)			Mn (µg/g)		
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
control	15 e	17 e	32	2.6 d	9 d	11.6	151 e	185 d	336
T1	74 bc	120 bc	194	5.3 cd	10 d	15.3	350 c	546 b	896
T2	62 c	204 a	266	8.6 b	16 bc	24.6	424 ab	402 b	826
T3	71 c	79 c	150	7.3 bc	10 d	17.3	402 b	621 a	1023
T4	88.3 a	21 e	109.3	7.6 bc	16 c	23.6	437 a	396 c	833
T5	62 c	87 c	149	10 ab	19 b	29	314 d	376 c	690
T6	87 b	48 d	135	12.3 a	23 a	35.3	358 c	401 c	759
LSD	**8.92**	**5.74**	**14.66**	**1.9**	**2.1**	**4.0**	**204.1**	**21.3**	**225.4**

T1: Inoculated with PGPR isolated from root of wheat infected with rust stem. T2: Inoculated with PGPR isolated from root of wheat infected with powdery mildew. T3: Inoculated with PGPR isolated from rhizospheric soil of wheat infected with powdery mildew. T4: Co-inoculation with PGPR isolated from root of wheat infected with rust and root of wheat infected with powdery mildew. T5: Co-inoculation with PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew T6: Co-inoculation with PGPR isolated from root of wheat infected with powdery mildew and rhizospheric soil of wheat infected with powdery mildew T0: Control.

Table 5. Effect of PGPR isolated from rhizospheric soil sample and roots of rust and powdery mildew infected wheat (*Triticum aestivum* L.*)* on Ni^{+3}, Pb^{+4} and Cd^{+4} content of Root and Shoot (ug/g) of maize variety EL 5098, measurement were made 28 D after treatment.

Treatment	Ni (ug/g)			Pb (ug/g)			Cd (ug/g)		
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
Control	13d	22.33b	35.33	183c	238c	421	4d	5.67c	9.67
T1	26c	44.67a	70.67	161d	364a	525	10.3ab	14b	24.33
T2	29b	46.33a	75.33	183 b	362a	545	8c	28a	36
T3	16.33d	50.33a	66.66	203a	351a	554	7.3c	27a	34.3
T4	30.6b	44.67a	75.27	187b	319b	506	8.67bc	9.67bc	18.34
T5	40.3a	43.67a	83.97	201a	316b	517	11.3a	31a	42.33
T6	34b	31.67b	65.67	209a	319b	528	10.3ab	28a	38.33
LSD	**8.8**	**14.8**	**23.6**	**3.8**	**6.1**	**9.9**	**1.3**	**5.05**	**6.35**

Table 5 showed Ni^{+3}, Pb^{+4} and Cd^{+4} accumulations in root and shoot of maize plants. It was showed in Table 4 that Ni^{+3} accumulation in root was found higher in T5 (Co-inoculation with PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew) which resulted in 67% increase as compared to control plants, whereas T2, T4 and T6 was found with no significant variation among each other, while T3 was found with no significant variation from control plants. In case of shoot T1, T2, T3, T4 and T5 was found with no significant variation among each other and was found 55% as compared to control plants, whereas treatment was found with no significant variation from control plants. Pb^{+4} accumulation in root was found higher in T3 (PGPR isolated from rhizospheric soil of wheat infected with powdery mildew), T5 and T6 treatments and all these treatments was found non-significant among each other, whereas T1 treatments was found non-significant from control plants. In shoot Pb^{+4} was found maximum in T1, T2 and T3 and no significant variation was found among these treatments and increase occur 37% as compare to control plants, whereas T4, T5 and T6 treatments was found with no significant variation among each other. Cd^{+4} accumulation in root was found higher in T5 (Co-inoculation with PGPR isolated from root of wheat infected with rust and rhizospheric soil wheat infected with powdery mildew) which resulted in 64% increase as compared to control plants, whereas T2 and T3 treatments was found with no significant variation among each other. In shoot Cd^{+4} was found higher in T2, T3, T5 and T6 treatments and resulted in 79% increase as compared to control plants and all these treatments were found to be significantly indifferent.

Highest concentration of IAA was found in IPMR (isolated from root of infected powdery mildew wheat)

followed by IPMS (isolated from rhizospheric soil of infected powdery mildew wheat) while lowest concentration of IAA was found in IRR (isolated from root of infected rust wheat). ABA concentration was found in IPMS (isolated from rhizospheric soil of infected powdery mildew wheat) followed by IPMR (isolated from root of infected powdery mildew wheat) while lowest concentration was found in IRR(isolated from root of infected rust wheat). GA concentration was found higher in IRR (isolated from root of infected rust wheat) followed by IPMR (isolated from root of infected powdery mildew wheat) and IPMS (isolated from rhizospheric soil of infected powdery mildew wheat).

DISCUSSION

The present results indicated that significant increase was found in SOD activity of leaves of maize variety (EL 5098) inoculated with PGPR compared to un-inoculated control plants. According to our present findings significant increase in SOD activity was observed in leaves of maize plants and this result was also supported by (Marschner, 1995) that the content of this enzyme increase because of higher accumulation of Ca^{+2} in roots and bacterial root interaction. According to Kohler, (2008) *Pseudomonas* species increase catalase under drought stress condition which support our present findings. All these results indicated that application of PGPR to plants could produce better results as compared to untreated plants. The increased shoot growth can be attributed to the observed improvement in root growth which consequently enhanced nutrient uptake. The better root development also helped to colonize more bacterial cells which increased N2 fixation. The findings are supported by Saad *et al*. (1999) who found out that *Azospirillum* inoculation increased the grain yield and total N content in wheat and sweet potato. The findings are in agreements with Malik *et al*. (2000) found increased plant biomass by PGPR inoculation through N2 fixation in wheat and rice. Present investigation demonstrated that highest shoot fresh and dry weight and leaf area was found in T2 (Inoculated with PGPR isolated from root of wheat infected with powdery mildew). Result reported here indicated that significant concentration of nutrient was found in root and shoot of maize plants treated with PGPR as compared to untreated plants. It was found in the result that K^{+} concentration was found highest of all the macronutrient in maize plants inoculated with PGPR as compared to control plants because these bacteria can produce plant growth-promoting substances (Gyaneshwar *et al*., 2002). The present findings are supported by (Bashan, 1990) who concluded that *A. brasilense* is capable of increasing the mineral nutrient content in maize plants. The results are in agreement with (Kapulnik *et al*., 1985; Morgenstern and Okon, 1987; Sarig *et al*., 1988). Plants adaptation to environmental stresses is associated with metabolic adjustments that lead to the accumulation of several compatible organic solutes like sugars, polyamines, proline and other amino acids (Yancey *et al*., 1982). Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions. According to Stewart and Lee (1994) proline is a source of energy, carbon and nitrogen for recovery tissue. Proline can protect cells from damage resulted by stress. The increased accumulation of proline depends on the stimulation in osmotic potential of leaves as has been reported earlier by Zhu (2002). Chlorophyll content was also increased significantly in all the PGPR strain treatments as compared to control plants. According to Bashan *et al.* (2006) *Azospirillum brasilense* increased quantity of photosynthetic pigments resulting in greener plants. Result reported here investigated that chlorophyll content was increased in T1 (Inoculated with PGPR isolated from root of wheat infected with rust stem). The present findings are in agreement with Vivas *et al*. (2003), on the conclusion that Chlorophyll content was increased significantly in all the PGPR strain treatments.

The PGPR produce plant growth promoting compounds including phytohormones; auxins, cytokinins and gibberellins. IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. Isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992). The GA concentration was found highest in IRR followed by IPMR while lowest concentration found in IPMS. The present findings are in agreement with Kloepper *et al*. (1993) that PGPR synthesize phytohormone promotes plant growth at various stages.

Conclusion

It has been concluded from the present work that single inoculation of plant growth promoting rhizobacteria resulted in an increase in proline, sugar, chlorophyll, root length, shoot length and salicylic acid. Co-inoculation was found effective for protein contents and activities of superoxide dismutase, peroxidase, catalase and hyperaccumulation of heavy metals such as Cr^{+3}, Co^{+3}, Mn^{+3}, Ni^{+3}, Pb^{+4} and Cd^{+4}. Therefore these bacterial isolates can be used in bioremediation of heavy metals and can be used as bioinoculant.

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