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RESEARCH ARTICLE

Efficiency of Plant Growth Promoting Rhizobacteria (PGPR) Consortia for Modulation of Phytohormone and better Nutrient Acquisition

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ABSTRACT

Utilization of plant growth promoting Rhizobacteria (PGPR) as suitable substitute to chemical fertilizers and also in command to promote the plant growth. Five potential strains were studied for multifarious PGP traits *viz.* Indole-3-acetic acid (IAA), phosphate (P) solubilization, siderophore production, ammonia production, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), phytohormone production, exhibiting antifungal activity etc. All five strains were resulted to have multifarious PGP traits. 2 Consortium were prepared using these PGP traits and effects were tested on Mungbean. The selected isolates showed significant plant growth promotion with respect to increase in root length and number of secondary roots as compared to control. The relative gene expression analysis of three genes *ARF* (Auxin response factors), *ERF* (Ethylene-responsive factor 1), *GAI* (Gibberellic-Acid Insensitive), at a transcriptional level compared to control consortia 2 was better and also increased 5-7 folds in the juvenile root tissues of mungbean using qRT-PCR technique on 15th, 30th and 45th days of interval.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR), Modulation of Phytohormone, Mungbean

INTRODUCTION

Rhizosphere is multifaceted and hostile environment for plant–microbe interactions for exploiting vital major and minor nutrients from wading pool of nutrients. In the last few years, numerous plant growth promoting bacteria (PGPB) such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Serratia*, *Enterobacter*, *Bacillus* and *Paenibacillus* suitable for plant growth have been identified (Lei *et al*. 2008; Muresu *et al*. 2008; Gururani *et al*. 2013). Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (Bhattacharya and Pati 1999; Deshwal and Kumar 2013) like nitrogen fixation, nitrogen stabilization, phosphate (P) solubilization, siderophore production, ammonia production, production of 1-Aminocyclopropane-1- carboxylate deaminase (ACC), phytohormone production.

In legumes, pulses are imperative food crops with large protein contents and used widely in feeding masses but per acre yield is very low. During past few decades, several studies were conducted to show the effectiveness of co-inoculation of Rhizobium with PGPRs on legumes (Abbasi *et al.* 2010). A variety of PGPRs including *Bacillus* and *Pseudomonas* species are commonly associated in the rhizosphere of legume as well as non leguminous crops (Muresu *et al*. 2008). The co-inoculation of some *Bacillus* strains with effective *Bradyrhizobium* enhanced nodulation and growth of green gram (*Vigna radiata* L.) (Korir *et al*. 2017). Few studies have confirmed that the inoculation of seed with mixed cultures have tremendous positive effects on plant growth compared to the single strain inoculation. (Xavier and Germida, 2002).

Auxin response factors (*ARF*) are transcription factors that bind with TGTCTC-containing auxin response elements (*AuxREs*) found in promoters of primary/early auxin response genes and mediate responses to the plant hormone auxin. *ERF1* (*ethylene response factor*) activated by the binding of EIN3/ EIL in the primary ethylene response element (PERE) present in the promoter of *ERF1* which is involved in ethylene signal transduction pathway and acts as a positive regulator of ethylene response in rice (Abbasi *et al.* 2010). With increasing awareness about inorganic fertilizer based agricultural practices, it has become imperative to explore for region specific impending microbial inoculants to convene desired crop production (Ramesh *et al*. 2014). Application of microbial inoculants is an extensively accepted strategy in intensive agriculture systems for perk up agricultural production with long-standing ecological balance in the agro-ecosystem.

MATERIALS AND METHODS

Inoculation: Five PGPR microorganisms *Rhizobium leguminosarum*, *Azotobacter chroococcum*, *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Bacillus spp*., acquired from the Department of Agriculture Microbiology, UAS (Bangalore). These bacteria were maintained on Yeast Extract Mannitol Agar (YEMA), Azotobacter Agar, Azospirillum Medium w/o Agar, Pikovskaya Agar, Nutrient Agar medium, respectively at 4 °C.

Screening of PGPR for multifarious plant growth promoting activities

All the PGPR bacterial isolates were screened for their growth promoting activities like Indole Acetic Acid (IAA) production, ammonia production, phosphate solubilization, HCN production, Antifungal production, Organic acid production (Bhattacharya and Pati 1999; Deshwal and Kumar 2013).

IAA Production : IAA production by individual isolates, Consortia 1 (*R. leguminosarum* + *A. brasilense* + *P. fluorescens* + *Bacillus spp.* + *Bacillus licheniformis*) and Consortia 2 (*R. leguminosarum* + *A. chroococcum* + *P. fluorescens* + *Bacillus spp.* + *Bacillus licheniformis*) were analysed. Luria Bertani broth medium (25 ml) amended with 50 µg/ml tryptophan was inoculated with the bacteria. Loop full culture was inoculated and incubated for 24 h at 28 °C on rotary shaker. Cultures were centrifuged at 10,000 g for 15 min. 2 ml of supernatant was taken and 2 to 3 drops of ortho-phosphoric acid was added. 4 ml of Salkowski reagent was added and incubated for 25 min. at room temperature and development of pink color indicates the IAA production. The amount of IAA produced was calculated from the standard graph of pure indole acetic acid. Quantification of IAA was done at 530nm. (Dey *et al*. 2004).

Production of Ammonia : Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube separately and incubated for 48-72 h at 28 ± 2 °C. Nesseler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman 1992).

Phosphate Solubilizing Activity : The plates were prepared with Pikovskaya's medium. The culture of five isolates were streaked on the plates and incubated in an incubator at 28 °C for 7 days. The plates were then examined and data were recorded (Kloepper *et al*. 1999)

HCN Production: Hydrogen cyanide (HCN) production was evaluated by streaking the bacterial isolates on Kings B agar medium amended with glycine. Whatman No.1 filter

paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each Petri plate. The plates were then sealed air-tight with parafilm and incubated at 30°C for 48 h. A colour change of the filter paper from deep yellow to reddish-brown colour was considered as an indication of HCN production (Deshwal and Kumar, 2013).

Antagonistic activities against Mungbean plant pathogenic fungi

The antagonistic effects of all 5 bacterial isolates were tested against fungal pathogens of *Rhizoctonia solani*. For this the bacterial isolates were streaked at a distance of 3.5 cm from rim of individual Petri plate containing Potato Dextrose Agar (PDA) medium. 6 mm mycelial disc from a 7-day old PDA culture of fungal pathogens were then placed on the other side of the petri dish and the plates were incubated at 28 °C for 4-7 days (Kumar *et al*. 2016)

Organic acid profiling by HPLC : The detection and quantification of organic acids was carried out on High-Performance Liquid Chromatogram (HPLC), Thermo Scientific Fisher equipped with PDA detector, autosampler, Smartline pump, Thermo Scientific Fisher inline degasser, and HyperSil Gold C-18 column 250mm × 4.6mm with 5µm particle size (Thermo Fisher, Austria). 0.02% Ortho-phosphoric acid (Merck, Germany and Hi-Media Laboratories) at flow rate of 1.0 ml/min was used as a mobile phase. Eluates were detected at 210 nm and identified by retention time and co-chromatographed by spiking the sample with the authentic organic acids. The organic acids were quantified by reference to the peak areas obtained for the authentic standards for gluconic acid, xylonic acid, formic acid, pyruvic acid and acetic acid (Merck, Germany and Hi-Media Laboratories). The values were presented as the mean of three replicates (Buch *et al*. 2005).

Pot Study : The pot experiment conducted with thirteen treatments as mentioned in Table 1 of a different combination of PGPR ($1x10^8$ ml). Seeds of Mungbean was treated by respective culture @ 10 ml/kg seed, dried in shed for 30 min before sowing. In the combination of culture treatments, the doses of individual bio-inoculants were reduced in such a manner that the total volume of the culture remained constant i.e. 10 ml/kg of seed in seed treatment. The transcriptional level study were performed in the juvenile root tissues of Mungbean on 15^{th}, 30^{th} and 45^{th} days after treatment. Total RNA was extracted from the root samples of each treatment by a modified Trizol method. For each sample, 0.1 g roots were powdered in liquid nitrogen using a sterilized pestle and mortar. The resulting powder was transferred to a 1.5 ml tight capped Eppendorf tube with 1 ml Trizol reagent and incubated at room temperature for 5 min. The double volume of chloroform (0.2 ml) was added with vigorous shaking for 15 sec and incubated at room temperature for 2 - 3 min. Tubes were centrifuged at 12000 rpm at 4 °C for 15 min. The aqueous phase was transferred in another eppendorf tube and 0.5 ml of isopropanol was added and mixed gently. Microcentrifuge tubes were kept at room temp for 10 min and again centrifuged at 12000 rpm at 4 °C for 15 min. The supernatant was removed and RNA pellet was washed with 1 ml of 75 % ethanol at 7500 rpm for 5 min at 4 °C. Pellets were air dried till ethanol evaporated and then dissolved in 25 µl Diethyl pyrocarbonate (DEPC) treated water. Tubes were kept at 60 °C on dry bath for 10 min. The extracted RNA was loaded on 1% agarose gel to check the quality of RNA (Sambrook and Russell, 2001).

Table No 1: Treatments details for field condition

No.	Treatment Details
T1	Absolute control
T2	Seed treatment with Rhizobium leguminosarum (1x108 ml) @ 10 ml/kg seeds
T3	Seed treatment with Azotobacter chroococcum (1x108/ml) @ 10 ml/kg seeds
T4	Seed treatment with Azospirillum brasilense (1x108 /ml) @ 10 ml/kg seeds
T5	Seed treatment with Pseudomonas fluorescence (1x108/ml) @ 10 ml/kg seeds
T6	Seed treatment with Bacillus spp. (1x108/ml) @ 10 ml/kg seeds
T7	Seed treatment with Azospirillum + Rhizobium (1x108/ml) @ 10 ml/kg seeds
T8	Seed treatment with Azotobacter + Rhizobium (1x108/ml) @ 10 ml/kg seeds
T9	Seed treatment with Pseudomonas + Rhizobium (1x108/ml) @ 10 ml/kg seeds
T10	Seed treatment with Bacillus spp. + Rhizobium (1x108/ml) @ 10 ml/kg seeds
T11	Seed treatment with Bacillus licheniformis (1x108/ml) @ 10 ml/kg seeds
T12	Seed treatment with Consortia 1 (T2+T3+T5+T6+T11) (1x108/ml) @ 10 ml/kg seeds
T13	Seed treatment with Consortia 2 (T2+T4+T5+T6+T11) (1x108/ml) @ 10 ml/kg seeds

Real-time PCR Analysis : Three genes (*ARF, ERF and GAI*) were initially screened with normal PCR using genomic DNA and cDNA from Mungbean root samples. PCR amplification reaction was performed in 25 µl reaction volume containing 12.5 µl Top Taq master mix (Qiagen), 0.5 µM each primer, 10.5 µl nuclease-free water and 1 µl genomic DNA/cDNA (80 ng). The PCR cycles consisted of

initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55-61 °C for 30 sec and extension at 72 °C for 1 min with a final extension at 72 °C for 10 min. The amplified products were resolved on 2.5 % agarose gel. Quantitative real time PCR (qRT-PCR) was performed on CFX96 Real Time PCR System (Biorad, USA) with three genes. The reaction was performed using DyNAmo Colorflash SYBR green qPCR kit with ROX passive reference dye (Thermo Scientific, USA). Each 20 µl reaction volume contained 80 ng cDNA, 200 nM of each primer (forward and reverse) and 2 x Master mix. Samples were initially denatured by heating at 95 °C for 3 min followed by a 40 cycles of amplification and quantification programme composing of denaturation (95 °C for 10 sec), annealing (56 °C for 10 sec) and extension (70 °C for 20 sec) in 96-well optical reaction skirted plates (Thermo Scientific, USA). A melting curve analysis was conducted to ensure amplification of a single product. PCR with no template served as control for each primer pair. The specificity of amplicons was verified by melting curve analysis (60 to 95 °C) after 40 cycles. Three biological replicates for each sample were used for real time PCR analysis and three technical replicates were analyzed for each biological replicate.

LCMS Analysis : The quantitative estimation of the plant hormones viz. IAA, IBA, GA3 and Salicylic acid, was carried out on a LC-MS system (Thermo Fisher Scientific, Surveyor). Prior to quantitation, the linearity of different phenolic acids was carried out by plotting the detector response against the concentration of the analytes by using a reversed phase C-18 (RP C-18) column. The mobile phase consisted of solvent A; water–formic acid (99.5: 0.5, v/v) and solvent B; acetonitrile. The HPLC binary pump with a flow rate of 1 mL/min was programmed to run the mobile phase as the following:

0–60 min, gradient from 0–50% B; 60– 70 min, gradient from 50–100% B; 70–73 min, isocratic at 100% B; 73–75 min, gradient rom 100–0% B; and 75–80 min, isocratic at 100% A.

Statistical Analysis : Fisher's method of analysis of variances was adopted for statistical analysis and interpretation of the data. The level of significance used in't' test was P = 0.05 (Panse and Sukhatme, 1967).

RESULTS

IAA Production : All PGPR isolates showed a significant variation in IAA production in Trp^{+} media. It was observed that range of IAA produced by these rhizobacteria between 42.10 – 83.46 µg/ml in trp- medium and 71.27 -127.84 µg/ml in the trp+ medium on 3rd day of incubation. On 5th day the maximum production of IAA by selected strains was in the range 42.15 – 83.88 µg/ml and 73.32-129.13 µg/ml in Trp- and Trp+ medium, was reported in (Figure 1).

Phosphate Solubilization : These five bacterial isolates exhibited the ability to solubilize tri-calcium phosphate. *P. fluorescens* being the most active strain showed maximum potential (5.02 mm zone of solubilization), pursued by *Bacillus spp.* (2.87 mm) after the 7th day of incubation at 28 °C on Pikovskaya agar plate.

HCN production : In the present study, the HCN production in terms of the color change from yellow to brown was considered as positive results. Out of five selected strains, *P. fluorescens* and *Bacillus spp.* were reported to show positive HCN production.

Production of Siderophore : In the following study, five rhizobacterial isolates exhibited the ability of siderophore production. All the isolates have shown potential to produce siderophores.

Antagonistic activity : The antagonistic activity was checked against the plant pathogenic fungi *Rhizoctonia solani*. Out of five selected bacteria, *A. chroococcum* and *P. fluorescens* found to inhibit the radial growth of the test fungi.

Table No 2: HPLC profiling of organic acids secreted by selected isolates

Bacteria	Gluconic/ Xylonic acids (mM)	Formic acid (mM)	Pyruvic acid(mM)	Acetic acid(mM)
R. leguminosarum ***(glucose)***	7.24	2.73	1.23	3.46
R. leguminosarum ***(xylose)***	6.19	9.41	-	2.76
A. brasilense ***(glucose)***	6.93	2.11	0.83	1.46
A. brasilense ***(xylose)***	5.81	2.32	-	1.54
A. chroococcum ***(glucose)***	10.59	1.99	0.61	2.68
A. chroococcum ***(xylose)***	9.04	3.65	-	2.72
Bacillus spp ***(glucose)***	11.46	4.78	0.52	0.79
Bacillus spp ***(xylose)***	10.87	1.08	-	0.73
P. fluorescens ***(glucose)***	12.41	4.63	0.34	1.73
P. fluorescens ***(xylose)***	10.63	7.96	-	1.28

Determination of Organic acid : Production of gluconic acid, pyruvic acid, formic acid and xylonic acid was analysed by HPLC method. Selected strains were attributed to their ability to produce high concentrations of gluconic acid as seen by secretion of 12.41 mM and 11.46 mM of gluconic acid in the medium supernatant of *P. fluorescens* and *B.spp.*, respectively. The obtained results are mentioned in Table 2.

Relative gene expression analysis of target genes

The relative expression analysis of three genes *i.e. ARF* (Auxin response factors), *ERF1* (ethylene-responsive factor 1) and *GAI* (Gibberellic-Acid Insensitive), at transcriptional level were performed in the juvenile root tissues of Mungbean on 15th, 30th and 45th days after treatment, using qRT-PCR technique.

1. ***Auxin response factor* (*ARF*) :** As compared to 15 days old mungbean root tissue, 7.46 folds increase in *ARF* gene expression is reported in treatment T_{13} while second highest expression (5.93 folds) of *ARF* gene was reported in treatment T_{12} on 30th days after treatment as compared to control (Figure 3).

2. ***Ethylene response factor* (*ERF1*) :** As compared to 15, 30 and 45 days old mungbean root tissue, the expression of *ERF1* gene was found normal (Figure 4).

3. ***Gibberellic acid Insensitive* (*GAI*) receptor :** In case of GAI receptor expression, all treatments showed increase in gene expression after 30 days of sawing. T_{13} and T_{12} showed 6.45 and 4.54 fold increase in GAI expression as compare to control, after 45 days after treatment (Figure 5).

Synergistic effect of consortium in Mungbean

Growth and symbiotic traits : The response of mungbean for single as well as combination of PGPR inoculants under field conditions at vegetative and flowering stage revealed improvement in growth (plant height, weight of nodule, number of nodules as well as number of pods) over the un-inoculated control treatment. It was found higher plant height (36.74, 38.08 and 37.41 cm), weight of nodule^{-1} (1.60, 1.58 and 1.59 g), number of nodules^{-1} (12.85, 11.79 and 12.32), and number of pods^{-1} (25.61, 23.70 and 24.65) at harvest during both the year as well as in pooled analysis, respectively. Similar drift was noticed for all growth parameters at flowering stage over the un-inoculated control treatment (Table 3 and Table 4).

Total nitrogen (N), phosphorous (P) and potassium (K) content of Mungbean : Total N, P and K content of Mungbean with single and combination inoculation treatments was found non-significant at harvesting stage. Total N content of Mungbean enhanced in response to bacterial treatments. It was found in consortia N% (3.57, 3.35 and 3.46), P% (0.46, 0.49 and 0.48), and K% (0.41, 0.44 and 0.43) during both the year as well as in pooled analysis, respectively.

LC-MS Analysis: All phenolic acids were found to be linear in the range of 0.5 –5.0 µg.g^{-1}. Correlation Co-efficient (R2) for concentration (µg) and detector response (mAU) was in the range of 0.9812 to 0.9998 for all the phenolic acids under the study.

Among the different treatments tested, maximum IAA, IBA, GA_3 and Salicylic acid content in root tissue was recorded in treatment T_{13} Consortia 2 about 1.043, 0.036, 1.999, and 0.098 µg g^{-1} FW respectively. While treatment T_{12} Consortia 1 was second highest among the different treatments with compare to absolute control (Table 4).

DISCUSSION

In modern cultivation process indiscriminate use of fertilizers, particularly the nitrogenous and phosphorus, has led to substantial pollution of soil, air and water. Excessive use of these chemicals exerts deleterious effects on soil microorganism, affects the fertility status of soil and also pollutes environment. Plant growth-promoting rhizobacteria (PGPR) consortia are a group of free-living bacteria that colonize the rhizosphere and benefit the plant growth (Vessey, J. K. (2003). Five potential strains were used and studied for multifarious PGP traits *viz.* Indole-3-acetic acid (IAA), phosphate (P) solubilization, siderophore production, ammonia production, production of 1-Aminocyclopropane-1- carboxylate deaminase (ACC), phytohormone production and exhibiting antifungal activity.

It was scrutinized that range of maximum IAA produced by these rhizobacteria between 42.15 – 83.88 µg/ml and 73.32-129.13 µg/ml in Trp- and Trp+ medium, respectively. Tryptophan is an amino acid commonly found in root exudates, has been identified as main precursor molecule for biosynthesis of IAA in bacteria.

A wide range in the production of gibberellins by the selected rhizobacteria was observed (83.3-110.3 µg/ml). In our study all the isolates were able to produce the Gibberellic acid which can be helpful for root elongation and development, flowering and fruit setting of plants (Itoh *et al.* 2002).

Table No 3: Effect of combined inoculation of PGPR in Mungbean during *rabi* 2016 and 2017.

No	Treatments	Number of pods plant^{-1}			Plant height (cm) at harvest		
		2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T_1	Control	19.61	18.86	19.23	28.24	29.47	28.8
T_2	*Rhizobium*	21.61	20.59	21.10	32.07	33.36	32.7
T_3	*Azospirillum*	20.61	19.42	20.02	30.00	31.26	30.6
T_4	*Azotobacter*	20.94	19.78	20.36	30.50	31.76	31.1
T_5	*Pseudomonas*	21.27	19.95	20.61	31.83	33.11	32.4
T_6	*Bacillus spp.*	20.61	19.20	19.90	29.53	30.79	30.1
T_7	*Azospirillum + Rhizobium*	21.94	20.98	21.46	33.43	34.73	34.0
T_8	*Azotobacter + Rhizobium*	22.61	21.37	21.99	34.42	35.73	35.0
T_9	*Pseudomonas + Rhizobium*	22.90	20.59	21.75	35.22	36.54	35.8
T_{10}	*Bacillus spp. + Rhizobium*	21.94	20.98	21.46	32.53	33.81	33.1
T_{11}	*Bacillus licheniformis*	20.61	19.18	19.89	28.66	29.90	29.2
T_{12}	Consortia 1	24.61	22.54	23.57	35.42	36.74	36.0
T_{13}	Consortia 2	25.61	23.70	24.65	36.74	38.08	37.4
	C. D. (P=0.05)	2.96	2.55	1.93	4.59	4.50	4.55
	C.V. %	8.2	7.5	5.5	8.7	8.2	8.4

Table No 4: Effect of combined inoculation of PGPR in Mungbean during *rabi* 2016 and 2017

No	Treatments	Number of nodules plant^{-1}			Weight of nodules plant^{-1}		
		2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T_1	Control	8.06	8.00	8.00	0.58	0.55	0.57
T_2	*Rhizobium*	9.98	9.62	9.62	0.91	0.86	0.88
T_3	*Azospirillum*	9.16	9.10	9.10	0.71	0.68	0.69
T_4	*Azotobacter*	9.41	9.35	9.35	0.73	0.70	0.72
T_5	*Pseudomonas*	9.81	9.52	9.52	0.71	0.74	0.73
T_6	*Bacillus spp.*	8.66	9.04	9.04	0.68	0.69	0.69
T_7	*Azospirillum + Rhizobium*	10.40	9.92	9.92	0.94	0.89	0.91
T_8	*Azotobacter + Rhizobium*	10.47	10.42	10.42	1.10	1.06	1.08
T_9	*Pseudomonas + Rhizobium*	10.68	11.45	11.45	1.16	1.12	1.14
T_{10}	*Bacillus spp. + Rhizobium*	10.10	9.68	9.68	0.89	0.86	0.87
T_{11}	*Bacillus licheniformis*	8.58	8.60	8.60	0.68	0.70	0.69
T_{12}	Consortia 1	11.18	11.45	12.15	1.51	1.49	1.50
T_{13}	Consortia 2	12.85	11.79	12.32	1.60	1.58	1.59
	C. D. (P=0.05)	1.53	1.64	1.40	0.71	0.48	0.44
	C.V. %	9.4	10.2	8.6	1.53	1.64	1.40

In our study three Rhizobacteria showed appreciable amount of nitrogenase activity. *R. leguminosarum* showed Acetylene Reductase Assay of maximum 679.26 n moles of C_2H_4/h^{-1} culture^{-1} followed by *A. chroococcum* to the amount of 645.48 n moles of C_2H_4/h^{-1} culture^{-1}. Biological nitrogen fixation could have important agronomic implications and enable the use of N-fertilizers to be reduced. Symbiotic nitrogen fixation is a mutualistic relationship between a microbe and the plant. Nitrogenase is an enzyme responsible for catalyzing nitrogen fixation, which is the reduction of nitrogen (N_2) to ammonia (NH_3).

P. fluorescens was found to be most phosphate solubilizing strain which showed maximum potential (5.02 mm zone of solubilization), followed by *Bacillus spp.* (2.87 mm). However, maximum phosphate solubilized in glucose as well as in xylose at pH 5.0 was observed in *Pseudomonas fluorescens i.e.* 686.32 (µM) in glucose and 435.12 (µM) in xylose at pH 5.0 (µM). Initially the phosphate solubilization were less but as soon as the incubation period increased it started solubilizing RP with decreased in the pH. *Pseudomonas fluorescens* solubilize unavailable forms of phosphorus and in turn also help in making phosphorus available for plants to absorb by producing

organic acids *i.e* (gluconic acid, xylonic acid, pyruvic acid, acetic acid).

Selected strains were attributed to their ability to produce high concentrations of gluconic acid as seen by secretion of 12.41 mM and 7.46 mM in the medium supernatant of *Pseudomonas fluorescens* and *Bacillus spp.*, respectively. This might have led to acidification of microbial cells and surrounding environment with ultimate release of P-ions by H^+ substitution for the cation bound to phosphates.

Table 5 : Concentrations of Plant Growth Hormones

No.	Treatments	IAA	IBA	GA_3	Salicylic acid
		Conc ($\mu g\ g^{-1}$ FW)	Conc ($\mu g\ g^{-1}$ FW)	Conc ($\mu g\ g^{-1}$ FW)	Conc ($\mu g\ g^{-1}$ FW)
T_1	Absolute control	0.003	0.005	0.786	0.008
T_2	*Rhizobium*	0.005	0.007	0.922	0.016
T_3	*Azospirillum*	0.027	0.006	1.040	0.043
T_4	*Azotobacter*	0.033	0.004	1.546	0.047
T_5	*Pseudomonas*	0.038	0.008	1.644	0.053
T_6	*Bacillus spp.*	0.049	0.018	1.749	0.055
T_7	*Azospirillum + Rhizobium*	0.052	0.018	1.793	0.060
T_8	*Azotobacter + Rhizobium*	0.323	0.019	1.872	0.064
T_9	*Pseudomonas + Rhizobium*	0.836	0.033	1.902	0.078
T_{10}	*Bacillus spp. + Rhizobium*	0.386	0.032	1.887	0.068
T_{11}	*Bacillus licheniformis*	0.413	0.028	1.953	0.079
T_{12}	Consortia 1	0.979	0.033	1.980	0.085
T_{13}	Consortia 2	1.043	0.036	1.999	0.098

The amount of K released from muscovite mica in the broth by the isolates were studied at 20 days after incubation (DAI) in lab condition. The maximum solubilization was observed in case of *Bacillus spp* i.e. 33.75 µg/ml. It is well known that the application of KSB can be a promising technique to solubilize the K reserves from soil and make it available to the plants, resulting in promotion of plant growth and minimizing the application of K-fertilizers. Earlier, researches well explained that by excreting organic acids KSB were capable to release K from various insoluble sources of K-minerals.

Out of five selected strains, *P. fluorescens* and *Bacillus spp.* were positive in HCN production; also all rhizobacteria able to produce siderophore. Hydrogen cyanide is formed during the early stationary growth phase. It does not take part in growth, energy storage or primary metabolism, but is generally considered to be a secondary metabolite that has confers a selective advantage on the producer strains. Production of HCN by PGPR was originally thought to promote plant growth by suppressing pathogens, however, HCN indirectly increases P availability by metal chelation and sequestration. Iron is not readily assimilated by either bacteria or plants because ferric ion or Fe^{+3}. *P. aeruginosa* and *Bacillus spp.* have evolved specialized mechanisms for the assimilation of iron, including the production of low molecular weight iron-chelating compounds known as siderophores, which transport this element into their cells.

Mechanisms responsible for antagonistic activity include inhibition of the pathogen by antibiotics, toxins and surface-active compounds (biosurfactants) and a mechanism that develops production of extracellular cell wall degrading enzymes such as chitinase and β-1,3-glucanase. In our results *P. fluorescens* found to inhibit the radial growth of the test fungi *Rhizoctonia solani* due to partial secretion of siderophores and HCN.

Among the different treatments tested, maximum IAA, IBA, GA_3 and Salicylic acid content in root tissue was recorded in treatment T_{13} Consortia 2 about 1.043, 0.036, 1.999, and 0.098 µg g^{-1} FW respectively. Phytohormones such as auxins (e.g., IAA, IBA), GAs, ET and SA plays vital role in growth and development of plants. In our results *Rhizobium leguminosarum*, *Azotobacter chroococcum*, *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Bacillus spp.* are also claimed to be most potent IAA as well as GA_3 producers. The presence of phytohormones affects the endogenous mechanism of plants and stimulates plant growth. The major effects of these phytohormones include stimulation of lateral root and root hair formations which facilitate nutrient acquisition as well as water absorption. However, microbially produced phytohormones are more effective due to the reason that the threshold between inhibitory and stimulatory levels of chemically produced hormones is low, while microbial hormones are more effective by virtue of their continuous slow release.

The transcriptional level study were performed in the juvenile root tissues of Mungbean on 15^{th}, 30^{th} and 45^{th} days after treatment, using qRT-PCR technique. As compared to 15 days old mungbean root tissue, 7.46 folds increase in *ARF* gene expression is reported in treatment T_{13} (Consortia 2). *Auxin response factors* (*ARF*) are transcription factors known to be rapidly induced by active auxins. Due to this might be the *ARF* gene was up regulated in the root at the time of intervals.

The expression of *ERF1* gene was found repressed. *ERF1* gene expression was repressed in root due to 1-aminocyclopropane-1-carboxylate (ACC) deaminase synthesized by PGPR and reduce the ethylene production.

While the slight increase in *GAI* receptor expression was observed in root tissues of 45 days about 6.45 folds and 4.54 in T_{13} and T_{12} respectively. Ethylene is a key phytohormone has a wide range of biological activities can affect plant growth and development in a large number of different ways including promoting root initiation, inhibiting root elongation, promoting fruit ripening, promoting lower wilting, stimulating seed germination, promoting leaf abscission, activating the synthesis of other plant hormones. The high concentration of ethylene induces defoliation and other cellular processes that may lead to reduced crop performance. The enzyme 1-aminocyclopropane-1 carboxylic acid (ACC) is a pre-requisite for ethylene production, catalyzed by ACC oxidase. The biosynthesis and degradation of gibberellic acid (GA) play distinct roles in SNF in addition to signaling mediated by DELLA receptors. Auxin positively regulates rhizobial infection while gibberellic acid appears to be a negative regulator of this process.

The response of mungbean for single as well as combination of PGPR inoculants under field conditions at vegetative and flowering stage revealed improvement in growth (plant height, number of nodules as well as number of pods) over the un-inoculated control treatment. It was found higher plant height (36.74, 38.08 and 37.41 cm), weight of nodule^{-1} (1.60, 1.58 and 1.59 g), number of nodules^{-1} (12.85, 11.79 and 12.32), and number of pods^{-1} (25.61, 23.70 and 24.65) at harvest during 2016-17, 2017-18 year and as well as in pooled analysis, respectively. Similar drift was noticed for all growth parameters at flowering stage over the un-inoculated control treatment. Dual bio-inoculants, screened in single medium revealed enhanced multifarious PGP traits over the single inoculants.

Co-inoculation of *Rhizobium leguminosarum*, *Azotobacter chroococcum*, *Azospirillum brasilense*, *Pseudomonas fluorescens and Bacillus spp.* enhances their population synergistically in soil which may leads to development in root nodules. In addition they also increase available nutrients (N, P, K, and Fe) to plants and help in the uptake of nutrients by plants and promote growth of the plant.

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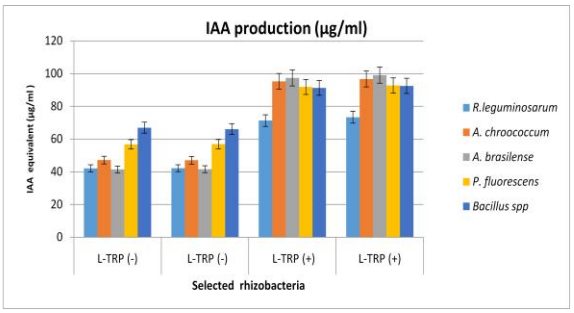

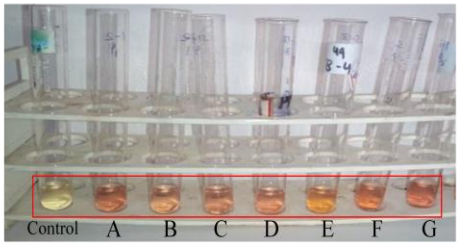

A. *R. Leguminosarum* **B.** *A. chroococcum* **C.** *A. brasilense* **D.** *P. fluorescens* **E.** *Bacillus. Spp* **F.** Consortia 1 **G.** Consortia 2

Fig.1: IAA production by the selected isolates

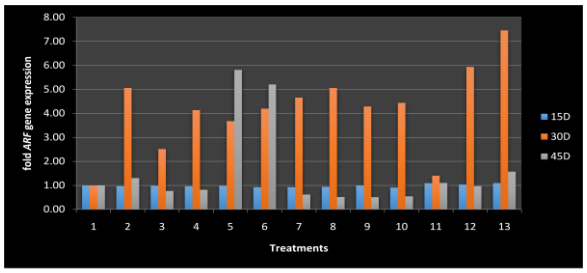

Fig. no 2: Relative fold expression of *ARF* gene

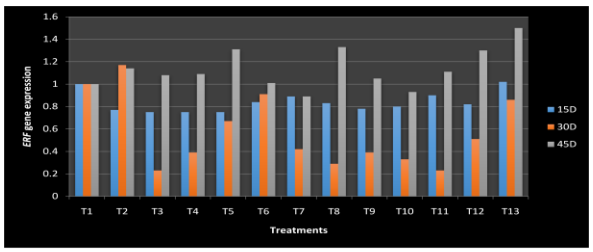

Fig. no 3: Relative fold expression of *ERF* gene

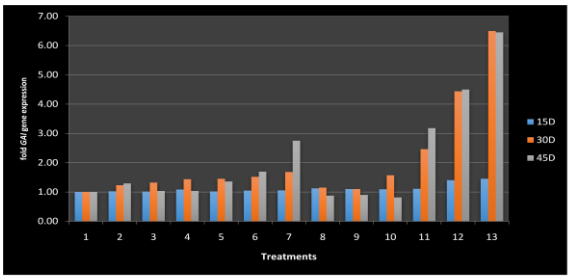

Fig. no 4: Relative fold expression of *GAI* receptor gene

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