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 I), 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 Pari 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 impeding 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 Pari 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 orthophosphoric 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. Nessler’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 *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 C18 column 250mm × 4.6mm with 5um particle size (Thermo Fisher, Austria). 0.02% Orthophosphoric 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 15th, 30th and 45th 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).

**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

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**Table No 1: Treatments details for field condition**

| No. | Treatment Details |
|-----|------------------|
| T1  | Absolute control |
| T2  | Seed treatment with *Rhizobium leguminosarum* (1x10^8 ml) @ 10 ml/kg seeds |
| T3  | Seed treatment with *Azotobacter chroococcum* (1x10^8 ml) @ 10 ml/kg seeds |
| T4  | Seed treatment with *Azospirillum brasilense* (1x10^8 ml) @ 10 ml/kg seeds |
| T5  | Seed treatment with *Pseudomonas fluorescens* (1x10^8 ml) @ 10 ml/kg seeds |
| T6  | Seed treatment with *Bacillus licheniformis* (1x10^8 ml) @ 10 ml/kg seeds |
| T7  | Seed treatment with *Azospirillum ⋅ Rhizobium* (1x10^8 ml) @ 10 ml/kg seeds |
| T8  | Seed treatment with *Azotobacter ⋅ Rhizobium* (1x10^8 ml) @ 10 ml/kg seeds |
| T9  | Seed treatment with *Pseudomonas ⋅ Rhizobium* (1x10^8 ml) @ 10 ml/kg seeds |
| T10 | Seed treatment with *Bacillus spp. ⋅ Rhizobium* (1x10^8 ml) @ 10 ml/kg seeds |
| T11 | Seed treatment with *Bacillus licheniformis* (1x10^8 ml) @ 10 ml/kg seeds |
| T12 | Seed treatment with Consortia 1 (T2+T3+T5+T6+T11) (1x10^8 ml) @ 10 ml/kg seeds |
| T13 | Seed treatment with Consortia 2 (T2+T4+T5+T6+T11) (1x10^8 ml) @ 10 ml/kg seeds |
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 from 100%–B; and 75–80 min, isocratic at 100% A.

| Bacteria              | Gluconic/ Xyloonic 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            |

**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

*Plantae Scientia, July 2020; Volume 03 Issue 04:20-29*
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 (Gibberellin 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 T15 while second highest expression (5.93 folds) of ARF gene was reported in treatment T12 on 30th days after treatment as compared to control (Figure 3).

2. Ethylene response factor (ERFI): 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 sowing. T15 and T12 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 (1.60, 1.58 and 1.59 g), number of nodules (12.85, 11.79 and 12.32), and number of pods (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, GA3, and Salicylic acid content in root tissue was recorded in treatment T13 Consortia 2 about 1.043, 0.036, 1.999, and 0.098 µg g⁻¹ FW respectively. While treatment T12 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 D-Glucaric acid, and producing 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.

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   | 30.30   | 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                      | 23.61    | 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\(_2\)H\(_4\)/h \(^{1}\) culture \(^{1}\) followed by A. chroococcum to the amount of 645.48 n moles of C\(_2\)H\(_4\)/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 was 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.

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 \(\beta\)-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\(_3\), Consortia 2 about 1.043, 0.396, 1.999, and 0.098 \(\mu g\) 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 15th, 30th and 45th 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\(_3\) (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.

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Table 5: Concentrations of Plant Growth Hormones

| No. | Treatments                     | IAA Conc. (µg g\(^{-1}\) FW) | IBA Conc. (µg g\(^{-1}\) FW) | GA\(_3\) Conc. (µg g\(^{-1}\) FW) | Salicylic acid Conc. (µg g\(^{-1}\) FW) |
|-----|--------------------------------|-----------------------------|-------------------------------|----------------------------------|--------------------------------------|
| T\(_1\) | Absolute control               | 0.003                       | 0.003                         | 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 \(\mu g\) ml\(^{-1}\). 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.
While the slight increase in GAI receptor expression was observed in root tissues of 45 days about 6.45 folds and 4.54 in T11 and T12 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 nodule1 (1.60, 1.58 and 1.59 g), number of nodules1 (12.85, 11.79 and 12.32), and number of pods1 (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.

ACKNOWLEDGMENTS

The author conveys heartfelt thanks to Dean PG Studies, Navsari Agricultural University, Navsari for providing funding for the present research work.
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