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**Chaitanya S Mogal**
Department of Plant Molecular Biology and Biotechnology, NAU, Navsari, Gujarat, India

**Sanjay Jha**
ASPEE Shakilam Agricultural Biotechnology Institute, NAU, Surat, Gujarat, India

**Raj Kumar BK**
Main Cotton Research Station, NAU, Surat, Gujarat, India

**Vipul Kumar Parekh**
Department of Basic Science and Humanities, ACHF, NAU, Navsari, Gujarat, India

**DA Chauhan**
Pulses and Castor Research Stat ion, NAU, Navsari, Gujarat, India

**Nilima Karmakar**
Department of Biochemistry, NAU, Navsari, Gujarat, India

**Corresponding Author:**
**Sanjay Jha**
ASPEE Shakilam Agricultural Biotechnology Institute, NAU, Surat, Gujarat, India

# Quantification of plant hormones and synergistic effect of PGPR on yield attributing characters of mungbean (*Vigna radiata* (L.) Wilczek)

**Chaitanya S Mogal, Sanjay Jha, Raj Kumar BK, Vipul Kumar Parekh, DA Chauhan and Nilima Karmakar**

**Abstract**
The inoculation of mungbean seeds with different inoculants *(Rhizobium,* PGPR and PSB) alone and in combination, significantly increased the nodulation, pod formation and grain yield over uninoculated control. Nodulation, pod formation and grain yield were highest when seeds were inoculated with *Rhizobium* + PGPR + PSB followed by *Rhizobium* + PGPR, the two *rabi* seasons (2016 and 2017) of experimentation. In pooled analysis also, combined inoculation mungbean seeds with *Rhizobium* + PGPR + PSB gave significantly the highest number of nodules/plant (12.32), number of pods/plant (24.65) and grain yield (835. 27 kg $ha^{-1}$). It was at par with *Rhizobium* + PGPR with grain yield of 831. 60 kg $ha^{-1}$. Maximum IAA, IBA, GA3 and Salicylic acid content in root tissue was recorded in treatment Consortia 2 (T13) about 1.043, 0.036, 1.999, and 0.098 µg $g^{-1}$ FW respectively. While treatment Consortia 1 (T12) was second highest among the different treatments with compare to control.

**Keywords:** Mungbean, PGPR, synergistic effect, IAA, IBA, GA3, salicylic acid

## Introduction
Mungbean [*Vigna radiata* (L.) Wilczek], commonly known as green gram is one of the important pulse crops, which provides the best solution to alleviate protein-calorie malnutrition. Biological uptake of essential plant nutrients resulting immobilization and chemical precipitation gradually depletes the available nitrogen, phosphorus which can be replenished by using N-fixer *(Rhizobium),* Phosphate Solubilising Bacteria (PSB) and Plant Growth Promoting Rhizobacteria (PGPR) used as seed dressers in nodulating legumes. *Rhizobium* and PSB are highly beneficial in enhancing N and P content due to more N fixation by *Rhizobium* and solubilisation of native P by PSB, thus, making two essential nutrients available to plants. Plant hormone production mainly affects the plant root system and thus nutrient uptake occurs (Etesami *et al.,* 2009). It is also observed that, auxin levels in the host legume plants are necessary for nodule formation (Spaepen *et al.,* 2007) [15]. These bacteria are reported to alter the endogenous level of auxins affecting plant morphology. The most reliable approach is to inoculate the beneficial microorganism into soil as part of mixed culture, and at a sufficiently high inoculums density to maximize the probability of its adaptation to environmental and ecological conditions (Higa and Wididana 1991, Parr *et al.* 1994) [6, 11]. Inoculation of pulses with PGPR and *Rhizobium* causes growth stimulation of plant and enhances crop yield (Sharma *et al.* 1989). Various reports are available on the synergistic effect of *Azospirillum* with *Rhizobium* on legumes *viz.,* soybean (Singh and Subba Rao 1979) [14], chickpea (Kundu and Tauro 1989), pigeonpea (Kundu 1988) [9] and groundnut (Riverkar and Konde 1988) [13]. The synergism has also been reported between *Rhizobium* sp. and PSB in soybean (Dubey 1997) [3] and urdbean (Prasad *et al.* 2002) [12]. The present study was undertaken to evaluate the synergistic effect of *Rhizobium*, PSB and PGPR on nodulation and grain yield of mungbean.

## Materials and Methods
The field study was conducted in *rabi* 2016 and 2017 at Main Pulses and Castor Research Station, NAU, Navsari, to evaluate the synergistic effect of *Rhizobium*, PSB and PGPR on nodulation and grain yield of mungbean. The soil of pH 6.39 having organic carbon 0.62% and available N, P and K respectively, 282, 18 and 312 kg/ha was used.

**Inoculation**

For present study, five inoculants were used. The following five PGPR microorganisms *Rhizobium leguminosarum, Azotobacter chroococcum, Azospirillum brasilense, Pseudomonas aeruginosa, Bacillus spp.* were maintained on Yeast Extract Mannitol Agar (YEMA), Azotobacter Agar, Azospirillum Medium w/o Agar, Pikovasky Agar, and Nutrient Agar medium respectively, at 4+l°C and multiplied whenever required.

**Preparation of carrier-based inoculants**

Carrier based inoculants of *Rhizobium,* PSB and PGPR were prepared by growing each of these microorganism on respective media upto stationary phase.

**Inoculation before sowing**

Mungbean seeds *var*. Co4 were treated with carrier based *above* PGPR microorganisms each at the rate of 10 ml/kg per kg seeds and mixed well to ensure the inoculums to stick onto the surface of the seeds. For the rest of the combinations 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 and treated seeds were dried in shade for an hour and used for sowing. An uninoculated check was also maintained. The same strains of *Rhizobium,* PSB and PGPR were for both the seasons of experimentation (*rabi* 2016 and 2017).

The experiment conducted with 13 treatments of a different combination of PGPR *viz*., $T_1$: Control, $T_2$: *Rhizobium*, $T_3$: *Azospirillum*, $T_4$: *Azotobacter*, $T_5$: *Pseudomonas*, $T_6$: *Bacillus spp*., $T_7$: *Azospirillum* + *Rhizobium*, $T_8$: *Azotobacter* + *Rhizobium*, $T_9$: *Pseudomonas* + *Rhizobium*, $T_{10}$: *Bacillus spp*. + *Rhizobium*, $T_{11}$: *Bacillus licheniformis*, $T_{12}$: Consortia 1 (*Rhizobium* + *Azospirillum* + *Pseudomonas* + *Bacillus spp*. + *Bacillus licheniformis*), $T_{13}$: Consortia 2 (*Rhizobium* + *Azotobacter* + *Pseudomonas* + *Bacillus spp*. + *Bacillus licheniformis*).The experiment was replicated thrice in Randomized Block Design (RBD) manner with plot size of 19.8 m X 10 m = 198 $m^2$. All the package practices prescbied for the mungbean were followed to raise the good crop. Number of nodules were counted at 30 days after sowing (DAS) and yield recorded after harvest.

**LCMS Parameter**

The chromatographic separation of the methanol extract was carried out by High Performance Liquid Chromatography (HPLC) 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.

**Results and Discussion**

All the treatments under study showed significantly higher nodulation status and grain yield over uninoculated control (Table 1). Among 13 treatments, presowing inoculation of mungbean seeds with Consortia 2 ($T_{13}$: *Rhizobium* + *Azotobacter* + *Pseudomonas* + *Bacillus spp*. + *Bacillus licheniformis*) showed maximum nodulation status in terms of a number of nodulation per plant and highest grain yield in all the two seasons *(rabi* 2016 & 2017). It was followed by combined inoculation Consortia 1 ($T_{12}$: *Rhizobium* + *Azospirillum* + *Pseudomonas* + *Bacillus spp*. + *Bacillus licheniformis*) (Table 1). Inoculations with PSB or PGPR alone were least effective with minimum number of nodules/plant, and grain yield in all the two seasons of experimentation. Bhatnagar *et al.* (1979) [1] have also reported that use of *Bacillus megatherium* in case of *Vigna radiata* and *Glycine max* gave significantly higher yield than that obtained by the use of *Rhizobium* alone as inoculant. *Rhizobium* and PSMs *(Aspergillus awamorii* and *Pseudomonas striata)* as dual inoculants reported to increase the grain yield of chickpea under field conditions (Dudeja *et al.* 1981).

Pooled analysis of two seasons (Table 1) showed significantly higher number of nodules /plant (12.32), number of pods/plant (24.65) and grain yield (835. 27 kg $ha^{-1}$) when inoculation was done with Consortia 2 over other treatments and uninoculated control (627.77 kg $ha^{-1}$). While the combined inoculation with Consortia 1 was at par for grain yield. The single inoculation with *Rhizobium,* PSB or PGPR was less effective as compared to combined inoculations. The increased yield of mungbean in combined inoculation may be attributed to the growth stimulation of the plant. Besides, PGPRs are also known to secrete a variety of secondary metabolites and contribute considerably in plant protection and production. PGPRs are also known to enhance levels of flavonoid like compounds in roots of legumes, which on seed bacterization might be an additional factor in nodule promotion (Sharma *et al.* 2007). Earlier studies also showed that inoculation of legumes with root colonizing bacteria (PGPR) and *Rhizobium* affect symbiotic nitrogen fixation by enhancing root nodule number or mass (Nishijima *et al.* 1988) [10]. Chanway *et al.* (1989) [2] also reported that inoculation of lentil with one or more of the rhizobacterial strains significantly increases emergence, vigour and nodulation. PGPR and PSB are known to improve Biological Nitrogen Fixation (BNF) by enhancing nodulation through colonizing root system and suppressing growth of deleterious micro-organisms.

The quantitative estimation of the plant hormones *viz*., IAA, IBA, GA3 and salicylic acid, was carried out on a Liquid Chromatography Mass Spectrometry (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. 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.

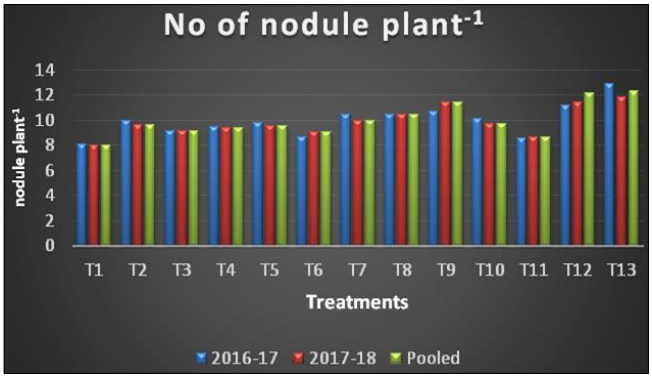
Among the different treatments tested, maximum IAA, IBA, $GA_3$ and salicylic acid content in root tissue was recorded in treatment Consortia 2 ($T_{13}$) about 1.043, 0.036, 1.999, and 0.098 µg $g^{-1}$ FW respectively. While treatment Consortia 1 ($T_{12}$) was second highest among the different treatments as compare to absolute control. Auxins principally affect plant roots (Salisbury, 1994). Plant hormone released by rhizobacteria mainly affects the root system, increasing its size, weight, branching number, and the surface area in contact with soil. All these changes lead to an increase in its ability to probe the soil for nutrient exchange, thereby improving plant nutrition and growth capacity (Gutierrez Manero *et al*., 1996) [5]. Thus, it can be concluded that presowing combined inoculation of mungbean seeds with *Rhizobium* + PGPR + PSB can increase grain yield to about 25-30 per cent with a significant increase in nodulation in terms of number of nodules, number per pod and seed yield.

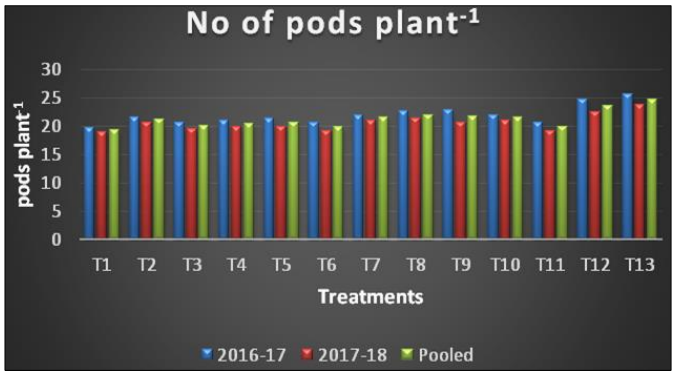
**Table 1:** Effect of combined inoculation of *Rhizobium*, PSB and PGPR in mungbean during *rabi* 2016 and 2017

| No | Treatments | Yield attributing characters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of nodules plant$^{-1}$ | | | Number of pods plant$^{-1}$ | | | Seed yield (kg ha$^{-1}$) | | |
| | | 2016-17 | 2017-18 | Pooled | 2016-17 | 2017-18 | Pooled | 2016-17 | 2017-18 | Pooled |
| $T_1$ | Control | 8.06 | 8.00 | 8.00 | 19.61 | 18.86 | 19.23 | 603.52 | 652.01 | 627.77 |
| $T_2$ | *Rhizobium* | 9.98 | 9.62 | 9.62 | 21.61 | 20.59 | 21.10 | 698.52 | 748.49 | 723.51 |
| $T_3$ | *Azospirillum* | 9.16 | 9.10 | 9.10 | 20.61 | 19.42 | 20.02 | 684.86 | 698.74 | 691.80 |
| $T_4$ | *Azotobacter* | 9.41 | 9.35 | 9.35 | 20.94 | 19.78 | 20.36 | 692.19 | 711.01 | 701.60 |
| $T_5$ | *Pseudomonas* | 9.81 | 9.52 | 9.52 | 21.27 | 19.95 | 20.61 | 693.52 | 734.68 | 714.10 |
| $T_6$ | *Bacillus spp.* | 8.66 | 9.04 | 9.04 | 20.61 | 19.20 | 19.90 | 683.52 | 695.68 | 689.60 |
| $T_7$ | *Azospirillum* + *Rhizobium* | 10.40 | 9.92 | 9.92 | 21.94 | 20.98 | 21.46 | 809.86 | 789.34 | 799.60 |
| $T_8$ | *Azotobacter* + *Rhizobium* | 10.47 | 10.42 | 10.42 | 22.61 | 21.37 | 21.99 | 811.86 | 808.68 | 810.27 |
| $T_9$ | *Pseudomonas* + *Rhizobium* | 10.68 | 11.45 | 11.45 | 22.90 | 20.59 | 21.75 | 827.52 | 825.68 | 826.60 |
| $T_{10}$ | *Bacillus spp.* + *Rhizobium* | 10.10 | 9.68 | 9.68 | 21.94 | 20.98 | 21.46 | 724.86 | 783.21 | 754.03 |
| $T_{11}$ | *Bacillus licheniformis* | 8.58 | 8.60 | 8.60 | 20.61 | 19.18 | 19.89 | 624.52 | 681.57 | 653.05 |
| $T_{12}$ | Consortia 1 ($T_2$ + $T_3$+ $T_5$+ $T_7$ + $T_8$ + $T_9$ + $T_{10}$ + $T_{11}$) | 11.18 | 11.45 | 12.15 | 24.61 | 22.54 | 23.57 | 837.52 | 825.68 | 831.60 |
| $T_{13}$ | Consortia 2 ($T_2$ + $T_4$+ $T_6$ + $T_7$ + $T_8$ + $T_9$ + $T_{10}$ + $T_{11}$) | 12.85 | 11.79 | 12.32 | 25.61 | 23.70 | 24.65 | 838.19 | 832.34 | 835.27 |
| | C. D. (P=0.05) | 1.53 | 1.64 | 1.40 | 2.96 | 2.55 | 1.93 | 85.36 | 76.54 | 58.71 |
| | C.V. % | 9.4 | 10.2 | 8.6 | 8.2 | 7.5 | 5.5 | 7.1 | 6.2 | 4.8 |

**Table 2:** Area and concentrations of plant growth hormone

| No. | Treatments | *IAA* | | IBA | | $GA_3$ | | Salicylic acid | |
|---|---|---|---|---|---|---|---|---|---|
| | | Area | Conc (µg g$^{-1}$ FW ) | Area | Conc (µg g$^{-1}$ FW ) | Area | Conc (µg g$^{-1}$ FW ) | Area | Conc (µg g$^{-1}$ FW ) |
| $T_1$ | Absolute control | 116016 | 0.003 | 101523 | 0.005 | 80362 | 0.786 | 4931 | 0.008 |
| $T_2$ | *Rhizobium* | 165152 | 0.005 | 156423 | 0.007 | 366373 | 0.922 | 7112 | 0.016 |
| $T_3$ | *Azospirillum* | 243496 | 0.027 | 185631 | 0.006 | 413790 | 1.040 | 14654 | 0.043 |
| $T_4$ | *Azotobacter* | 12545356 | 0.033 | 203695 | 0.004 | 614974 | 1.546 | 15806 | 0.047 |
| $T_5$ | *Pseudomonas* | 1178064 | 0.038 | 213936 | 0.008 | 653796 | 1.644 | 17470 | 0.053 |
| $T_6$ | *Bacillus spp.* | 1516679 | 0.049 | 448732 | 0.018 | 695697 | 1.749 | 17958 | 0.055 |
| $T_7$ | *Azospirillum* + *Rhizobium* | 1586136 | 0.052 | 451403 | 0.018 | 713358 | 1.793 | 19281 | 0.060 |
| $T_8$ | *Azotobacter* + *Rhizobium* | 12373067 | 0.323 | 476893 | 0.019 | 744154 | 1.872 | 20318 | 0.064 |
| $T_9$ | *Pseudomonas* + *Rhizobium* | 9811317 | 0.836 | 754927 | 0.033 | 756350 | 1.902 | 24172 | 0.078 |
| $T_{10}$ | *Bacillus spp.* + *Rhizobium* | 1431802 | 0.386 | 723646 | 0.032 | 750685 | 1.887 | 21640 | 0.068 |
| $T_{11}$ | *Bacillus lichenoformis* | 916226 | 0.413 | 738298 | 0.028 | 776353 | 1.953 | 24698 | 0.079 |
| $T_{12}$ | Consortia 1 | 29718756 | 0.979 | 783848 | 0.033 | 787706 | 1.980 | 26200 | 0.085 |
| $T_{13}$ | Consortia 2 | 31663710 | 1.043 | 814927 | 0.036 | 794637 | 1.999 | 29904 | 0.098 |



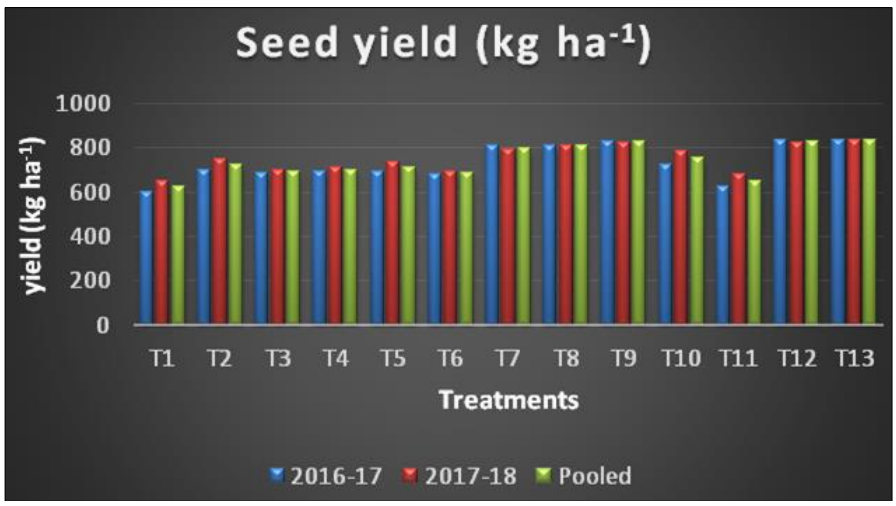




**Fig 1:** Effect of combined inoculation of *Rhizobium*, PSB and PGPR in mungbean during *rabi* 2016 and 2017

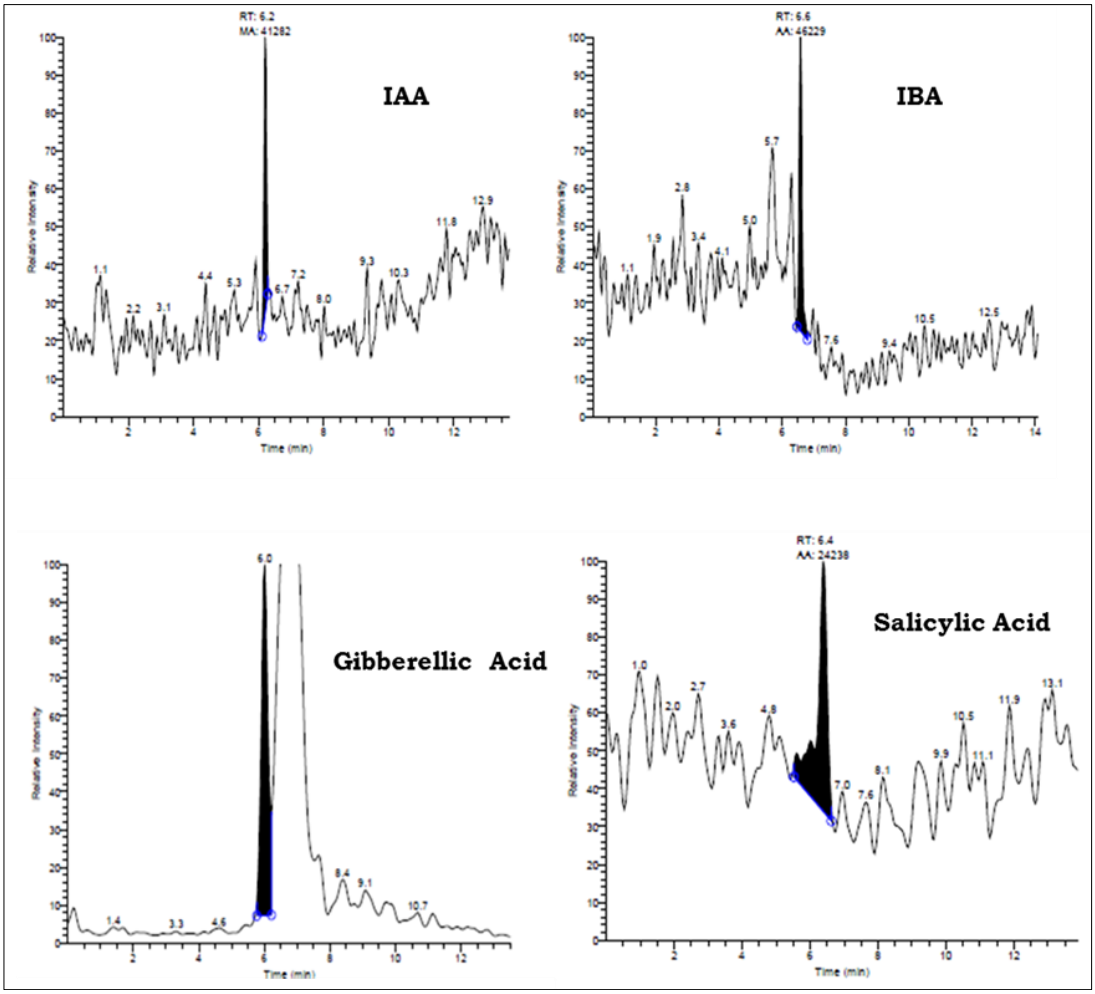



**Fig 2:** Retention time of plant hormones

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