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Original Research Article

Dynamics of Plant Growth Promoting Rhizobacteria in Maize-Based Inter-Cropping System

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A B S T R A C T

Under present investigation 48 PGPR isolates were isolated from different rhizotic zones of maize based intercropping system by using different media from twelve different site of Bihar including diara belt. PGPR isolates are coded for *Azospirillum* spp. as AZS_1 to AZS_{12}, *Azotobacter* spp. as AZT_1 to AZT_{12}, *Pseudomonas* spp. as PSD_1 to PSD_{12} and P-solubilizing bacteria spp. as PSB_1 to PSB_{12}. These isolates were screened on the basis of seed germination, production of IAA, P-solubilization activity, antifungal activity, and nitrogenase activity for the formulation of microbial consortium. Under pot condition plant height, leaf area index, number of leaves plant^{-1}, fresh and dry weight of shoot and root, total biomass production, root volume, percentage root colonization by mycorrhiza in pot soil and microbial population of *Pseudomonas* spp. PSD_6 was maximum in treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) while microbial population of *Azotobacter* spp. AZT_4, P-solubilization bacteria PSB_4 and MPN of *Azospirillum* spp. AZS_6 were maximum in treatment T_{13} (NPK + PSB_4 + AZS_6 + AZT_4). Keeping in view of experimental findings, PGPRs of diara belt are extremely diversified and perform well in stress condition. They are also competitive in nature and efficient in nitrogen fixing, P-solubilization and producing plant growth hormones. After 60 days, growth period in maize plant N (0.78%), P (0.35%) and K (0.65%) content were significantly superior over UIC(T_1) and maximum in treatment T_{13} (NPK + PSB_4 + AZS_6 + AZT_4). Similarly N and K uptake by whole plant was found significantly influenced by co-inoculation of selected strains of PGPR and was maximum in treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) which was probably due to higher biomass production. While P-uptake was found maximum in treatment T_{13} (NPK + PSB_4 + AZS_6 + AZT_4). Microbial consortium improves crop growth and increase biomass production. Hence, it may be concluded that PGPRs (PSD_6 + AZS_6 + AZT_4) form best microbial consortium in all respect than that of others which is very significant not only in growth parameters but also in maintaining soil health for sustainable crop production.

Introduction

Major constraints in exploiting full genetic potential of the crops for achieving higher yield is the supply of adequate nutrients. The application of chemical fertilizer to fulfil the nutrient requirement of crops is advocated since the introduction of green revolution in India. However, during the period 1960-69, the response to NPK fertilization was about 12 kg of food grains per kg of nutrient. It declined to 10 kg during 1980-89 and to 9 kg during 1990-99, and the declining trend in continuing. Further, high cost of chemical fertilizer, widening gap between supply and demand and low purchasing power of small and marginal farmer contributing adversely to our Agricultural production process. The situation is further complicated during the last couple of decades due to proven negative environmental impact of chemical fertilizers and their increasing costs. In such a difficult situation, only option left is to look for other alternative sources of plant nutrient, both for augmentation of production and sustainability. Microbial consortium constitutes one of the best possibilities in this aspect, and call an organized systematic effort for the isolation of different microbial strains including PGPR strains from diverse habitats and developing an effective package of Microbial consortium.

The rhizobacteria that are beneficial to plant are called plant growth promoting rhizobacteria (PGPR). The term PGPR was first proposed by Kloepper and Schroth (1978) to describe a subset of rhizobacteria which induce increased plant growth after inoculation to seeds. Kloepper (1993) and Cattelan *et al.,* (1999) indicated that different strains of PGPR can increase crop yields, control root pathogens, increase resistance against foliar pathogens, promote legume nodulation and enhance seedling emergence. PGPR group of bacteria actively colonize plant roots and increase plant growth and yield (Wu *et al.,* 2005). It may benefit the target plant by causing plant growth promoting and also a source of biological disease control. Growth promoting activity has been reported in strains belonging to several genera, such as *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia serratia* and *Bacillus* (Kloepper 1993; Zhang *et al.,* 1996, Glick and Bashan 1997, Ramamoorty *et al.,* 2002, Bashan, *et al.,* 2004). Several mechanisms have been postulated to explain the role of PGPR as plant growth, stimulator which can be categorized as direct or indirect promotion. Direct promotion occurs due to ability of targeted strains to produce or change the concentration of phytohormons, like IAA (Mardukhova *et al*., 1991); Cytokinins (Tien *et al.,* 1979), Ethylene (Arshad and Frankenberger 1991, Glick, 1995) and N_2-fixation by some of the strain (Boddy and Dobereiner, 1995, Mrkovacki and Milic, 2001; Salantur *et al*., 2006). Indirect promotion like antagonism against phyto-Pathogenic microorganisms or deleterious bacteria (Kloepper 1993; Glick 1995; Lugtenberg *et al.,* 1991) and solubilisation of mineral phosphate and other nutrients (Cattelan *et al.,* 1999).

Numerous plant growth promoting rhizobacteria of the genus *Pseudomonas*, *Bacillus* (PSB), *Arthrobacter*, *Azospirillum*, *Klebsiella*, *Azotobacter* and *Enterobacter* have been isolated from the rhizosphere of various crops and have been evaluated for their synergistic effect on plant growth (Kloepper *et al.,* 1992). Egamberdiyeva. D. (2007) studied the rhizosphere and phyllosphare bacteria isolated from wheat and peas and examined for their plant growth promoting properties. Bacterial strains were identified as *Pseudomonas*, *Bacillus* and *Microbacterium* species. After inoculation with effective bacterial strains, the root and

shoot growth, and nodulation of peas increased. Gholami *et al.,* (2009) also reported that under *In vitro* condition seed treatment with PGPR strains improved seed germination, seedling vigour, seedling emergence and seedling stand over the control in maize.

Maize is grown almost all states of India. Bihar being one of the most important maize growing state ranked fourth in area (6.4 lakh ha) and second in production (17.20 lakh tonnes) in the country in 2006-07. In Bihar maize is grown in all three cropping seasons out of which *Rabi* maize rank first in terms of productivity. Area under winter maize is increasing at a faster rate especially under north Bihar condition. *Rabi* crop is sown in Oct.-Nov; makes little growth till mid-January, leaving enough space for inter cropping during the cropping period. It is planted in rows 60 cm apart. Legumes, Potato, turmeric, tobacco are some successful intercrops that are taken with *Rabi* maize.

Maize based cropping system is getting importance in modern agriculture. Being photoperiod insensitive and a member of C_4 group, cultivation of maize in unfertile soil of diara belt is very common. Further, with a view to earn more profit small and marginal farmers take varieties of intercrop belong to C_3 group along with maize. Such a practice favour microbial diversity. Root exudates of a specific crop either of C_3 or C_4 group determine the kind of micro-organisms to develop with in the root zone. A good crop stand is the resultant effect of dominance of an interdependence beneficial group of micro-organism with in the root zone. Therefore finding effective microbial consortium is expected in maize based cropping system only. A microbial consortium is a group of interdependent helping microbes that allow the helper to thrive with in the rhizosphere region and depriving the harmful group from essential elements. Identification of such consortium will be a boon in achieving sustainability and consistently higher yield of crop. In view the importance of PGPRs the present study has been taken up to explore the possibility of formulating microbial consortium from out of the microbial diversity in maize rhizosphere when grown either as a mono-crop or intercropped with others.

Materials and Methods

This chapter deals with the description of the material used and the methods or techniques adopted during the course of investigation.

Isolation of Plant Growth Promoting Rhizobacteria (PGPR)

Different PGPR isolates were isolated by using selective or non selective media. 10 g soil from the rhizosphere of host plant from selected site were taken and prepared 10 fold dilution series up to 10^{-6} by serial dilution method. 1 ml suspension from different dilutions (10^{-2} to 10^{-6}) was poured on plates containing respective media. The suspensions were spread on the plate by using sterilzed spreader under aseptic condition.

Medium for PGPR

Different selective and non selective media were used for isolation of Plant Growth Promoting Rhizobacteria (PGPR). Selective media used were King' B (KB) for *Pseudomonas* spp. Pikovskay'sagar (PKV) for PSB, Jensen's medium (JEN) for *Azotobacter* spp., *Pseudomonas fluorescens* agar (PFA) for *Pseudomonas* spp. and N-free malate medium (Nfb) for *Azospirillum* spp. isolates and Non-selective media used for PGPR were nutrient agar (NA), Potato dextrose agar (PDA) and soil extract agar (SEA). Seeds of Maize CV Laxmi were

obtained from All India Coordinated Maize Improvement Project (AICMIP), Department of Genetic and Plant Breeding, Tirhut College of Agriculture, Dholi, R.A.U., Pusa, Samastipur, Bihar.

Studies on survival of PGPR isolates in consortia

A modified succinate broth (MSB) was prepared to grow PGPR isolates, PSD_6, PSB_4, AZT_4 and AZS_6 together to examine their interaction in vitro condition.

Modified succinate medium broth (Prasad *et al.*, 2002)

Constituent's		**g/L**
Sodiuum glutamate	-	1.0 g
Sodium succinate	-	5.0 g
$(NH_4)_2 SO_4$	-	0.1 g
$MgSO_4. 7H_2O$	-	0.1 g
K_2HPO_4	-	0.5 g
Mannital	-	10.0 g
NaCl	-	0.1 g
Yeast Extract	-	0.5 g
Distilled water	-	1000.0 ml
pH	-	6.8

For study of survival of AZT_4 and AZS_6 with PSB_4 and PSD_6 in culture broth fresh inoculum of AZT_4 and AZS_6 were prepared by growing in Jensen's broth and N-free bromothymol blue medium respectively for 96 hrs and PSB_4 and PGPR by growing in MSB was 48 hrs.

50 ml of MSB was taken in 250 ml Erlenmeyer conical flasks, plugged with cotton and steirlized at 103.4 kilo Pascal pressure for 30 min. After cooling the broth medium was inoculated with the 1 ml inoculums of AZT_4, AZS_6, PSB_4 and PSD_6, either alone or in combination according to treatments given below. The inoculated flasks were incubated for 30 $\pm$ 1^0C for 6 days.

Inoculated flasks were shaken intermittently at 30 $\pm$ 1^0C on shaker for ½ hrs 3-4 times daily.

Population of the microorganisms in their inoculums were determined after 3 and 6 days of incubation. For this one ml bacterial suspension from each flask was aseptically transferred in test tubes containing 9 ml sterilized water. Serial dilutions were prepared upto 10^{-12}. The population of AZT_4 was estimated in Jensen's medium, AZS_6 in N-free bromothymol blue medium, PSB_4 in nutrient medium, while PSD_6 in modified succinate agar medium by serial dilution and plate count method.

Germination count

Seed of maize CV laxmi were surface sterilized with 2 per cent sodium hypochlorite for 30 minute followed by washing with 0.01N HCl to avoid its any harmful effect. After proper washing with sterilized distilled water. Seeds were inoculated with different combination of selected PGPR isolates ((*Pseudomonas* spp. PSD_6, *Azotobacter* spp. AZT_4, *Azospirillum* spp. AZS_6 and *P-solubilizing* bacteria spp. PSB_4). The inoculated seeds of maize were kept for germination on moist fitter paper with the help of sterilized forcep. The inoculated seeds were allowed to germination in incubator at 28^0 $\pm$ 2^0C for 7 days according to the following treatment combination in triplicates.

After completion of incubation period the seedlings were carefully taken from the petriplates. The seedlings were then kept on the blotting paper sheet to remove any excess of media and observation regarding shoot and root length measurements were made. In control plate, the seeds treated with sterilized media were taken vigor index was

determined with following formula (Abdul Baki and Anderson, 1973).

Vigor index = (Mean root length + mean shoot length) x germination %.

A pot culture experiment was conducted at department of Microbiology, F.B.S. and H., R.A.U., Pusa during the *Rabi* season 2009.

Soil characteristics

Soil was taken from 0-15 cm depth randomly selected spots from Kitchen Garden, R.A.U., Pusa with the help of soil augar. The soil samples were mixed and make composite sample, which was subsequently stirred, dried at room temperature, powdered and finally sieved and kept in polyethylene bag for physico-chemical and microbiological analysis. The soil was found to be low in organic carbon (0.49%), medium in available Nitrogen (250.12 kg/ha), medium in available phosphorus (26.0 kg/ha) and available potash (225.00 kg/ha).

On the basis of morphological, cultural characteristics and screening, four best isolates of PGPR, (*Pseudomonas* spp. PSD_6, *Azotobacter* spp. AZT_4, *Azospirillum* spp. AZS_6 and *P-solubilizing* bacteria spp. PSB_4) were examined alone or in possible combinations with other isolates under pot experiment during Rabi season, 2009.

Pot culture experiment

The experiment was conducted at Department of Microbiology, F.B.S. & H., R.A.U., Pusa in earthen pots under controlled conditions. Soil samples were collected from Kitchen garden of RAU Pusa Campus. The soils was air dried sieved and kept in polyethylene bag. The earthen pot had capacity to 10 kg soil. 6 kg soil was filleded up in each earthen pot. All treatments arranged in 48 pots of 3 replicates with 16 pots/replications. The treatments were arranged on completely randomised design (CRD).

Preparation of seed inoculation

The maize seeds were inoculated with different possible combination of PGPR inoculation (*Pseudomonas* spp. PSD_6 (10^6 cfu ml^{-1}) Azotobacter spp. AZT_4 (10^7 cfu ml^{-1}), *Azospirillum* spp. AZS_6 (10^7 cfu ml^{-1}) and *P-solubilizing* bacteria spp. PSB_4 (10^9 cfu ml^{-1}). PGPR inoculants were prepared by growing bacteria in respective media. Mixed inoculation of different combination of these organisms were prepared by mixing equal volumes of culture suspension (10 ml) and inoculated with surface sterilized seeds. A 10 per cent solution of Pharmaceutical grade of gum Arabic served as a sticker for inoculants. Seeds were inoculated with gum Arabic as an adhesive and rolled into suspension of bacteria (10^6 to 10^9 cfu ml^{-1}) with sterilized glass rod until uniformly coated. Seeds treated with sterile water and gum Arabic served as the uninoculated control. The viable counts of bacteria per seed were found to be (10^6 to $10^{8.}$). Basal doze of fertilizer N @ 200 kg ha^{-1}, P_2O_5 80 kg ha^{-1}, and K_2O 80 kg ha^{-1} in the form of Urea, SSP and MOP were applied Nitrogen was applied in two split dozes ½ at the time of sowing and rest half at 35 days of sowing. Ten inoculated seeds were sown in each pot and thinned to one plant. The plants were irrigated when required. After 60 days of growth from the date of sowing plants were carefully uprooted from each pot, washed in acidified detergent solution followed by distilled water and dried in oven at 65^0C.

Treatment

T_1	-	Uninoculated Control (UIC)
T_2	-	NPK
T_3	-	PSD_6

$T_{4`}$ - AZT_4
T_5 - AZS_6
T_6 - PSB_4
T_7 - $PSB_4 + AZT_4$
T_8 - $PSB_4 + AZS_4$
T_9 - $PSD_6 + PSB_4$
T_{10} - $AZT_4 + AZS_6$
T_{11} - $PSD_6 + AZT_4$
T_{12} - $PSD_6 + AZS_6$
T_{13} - $PSB_4 + AZT_4 + AZS_6$
T_{14} - $PSD_6 + AZS_6 + AZT_4$
T_{15} - $PSD_6 + AZS_6 + PSB_4$
T_{16} - $PSD_6 + AZT_4 + PSB_4$

Results and Discussion

This study was conducted to asses the effect of either single or in different possible combinations of selected PGPR isolates i.e. diazotrop a N_2-fixers *Azospirillum* spp. AZS_6, *Azotobacter* spp. (AZT_4), biocontrol agent *Pseudomonas* spp. (PSD_6) and Phosphate solubilizing bacteria PSB_4 on maize plants under pot experiment. Total biomass production, nutrients uptake, microbial population dynamics were also assessed by these PGPR consortia on post harvested soil. There is little information available on the possible synergistic effect of these microbes on maize under pot conditions. PGPR isolates selected for each functions were combined together and their effect evaluated in pot experiment.

Co-inoculation effect of PGPR on root volume and root colonization per cent (%) of maize plant at 60 day growth period

Root volume

The data on root volume in maize plant at 60 days growth period as influence by different consortium of PGPR have been present in Table 25 and Fig. 6. Higher value of root volume was recorded in treatments received three mixed inoculant (T_{13}, T_{14}, T_{15} and T_{16}) as compared with PGPR inoculant containing two inoculant (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}). Similarly higher value of root volume was recorded in PGPR consortium consists of two inoculant as compared to single inoculants.

Treatment T_{14} consists of mixed isolate of diazotrophs *Azospirillum* isolate (AZS_6), *Azotobater* (AZT_4) and *Pseudomonas* isolates (PDS_6). These isolates have a potential to fix atmospheric nitrogen and synthesize considerable quantities of biologically active substances in the rhizosphere. There biological substance is phyto hormones (IAA and G.A.) which increase the root volume. Similar result had also been reported by Dewan and Subba Rao (1979). Significantly increased in root volume over control was also reported by Bhawmik and Singh (2004) in microbial preparation of PGPR with A.M. fungi

Percentage of root colonization by mycorrhiza

The data on root colonization by mycorrhiza in maize plant at 60 days growth period as influence by different consortium of PGPR have been presented in Table 25. Highest percentage root colonization was recorded under treatment T_{14} (NPK + PSD_6 + AZS_6 + PSB_4) (90 %) followed by treatment T_{15} (83%). Higher value of root colonization percentage by mycorrhiza was recorded in treatment receiving three mixed inoculant (T_{13}, T_{14}, T_{15} and T_{16}) (77 to 90%) as compared with PGPR inoculant containing two inoculants (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}) (40 to 68%) Similarly higher value of root colonization percentage was recorded in PGPR consortium consists of two inoculant as compared to Single inoculants, except *Azospirillum* spp. (AZS_6) T_5.

PGPR (PSD_6, AZS_6 and PSB_4) rhizobacteria interaction enhancing the root colonization

percentage particularly PSD_6. PGPR should act as the biological control of plant pathogens and improve plant growth. They enhance root development either directly by producing phytohormonas, or indirectly by inhibiting pathogens through the synthesis of different compounds. The colonization of roots by inoculated bacteria is an important step in the interaction between beneficial bacteria and host plant. Kloepper and Schroth (1978) describe soil bacteria that colonize the roots of plants following inoculation on to seed and that enhance plant growth. Colonization process include, of ability to survive inoculation on seed, to multiply in the spermospher (region surrounding the seed) in response to seed exudates, to attach to the root surface, and to colonize the developing root system. Benizri *et al.,* (2001) also reported inoculation with PGPR enhancing the root colonization percentage. Similar report was also observed by Kumar and Yadav (2005).

Effect of Microbial Consortium of PGPR on Microbiological count and Physico-chemical properties at 60 days growth period of experimental soil

Most probable number (MPN) of *Azospirillum* isolates AZS_6, and bacterial population of *Pseudomonas*, (PSD_6) P-solubilizing bacteria (PSB_4) and *Azotobacter* (AZT_4) in the post harvested soils under different treatment has been presented in Figure 1.

MPN of *Azospirillum* isolates

Higher value of MPN of *Azospirillum* spp. (AZS_6) was recorded in treatment receiving three mixed inoculant treatment T_{14} and T_{15} (0.30×10^5 and 0.38×10^5) s compared with PGPR inoculant containing two inoculant (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}) (0.11×10^5 to 0.20×10^5). Similarly higher value of MPN of *Azospirillum* spp. was recorded in PGPR consortium consists of two inoculant as compared to single inoculants.

Population of Pseudomonas isolates

Highest count of *Pseudomonas* isolates were recorded in treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) (8.42×10^5 CFU) followed by T_{15} (NPK + PSD_6 + AZS_6 + PSB_4) (8.22×10^5 CFU). Higher count of *Pseudomonas* spp. isolate (PSD_6) was recorded in treatment receiving three mixed inoculant T_{13} and T_{16} (7.05×10^5 CFU and 7.98×10^5 CFU) as compared with PGPR inoculant containing two inoculant (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}) (4.75×10^5 CFU to 6.33×10^5 CFU). Similarly higher count of *Pseudomonas* spp. was also recorded in PGPR consortium consists of two inoculants as compared to single inoculants.

Population of P-solubilizing bacteria isolates (PSB_4)

Similar trends also found in population of P-solubilizing bacteria spp. Maximum population of P-solubilizing bacteria spp. was recorded in treatment T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) (24.30×10^4 CFU) followed by T_{15} (NPK + PSD_6 + AZS_6 + PSB_4) (23.20×10^4 CFU). Higher value of P-solubilizing bacteria was recorded in treatment receiving three mixed inoculant T_{14} and T_{16} (20.00×10^4 CFU and 22.40×10^4 CFU) as compared with PGPR inoculant containing two inoculant (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}) (12.50×10^4 CFU to 14.20×10^4 CFU). Similarly higher count of P-solubilizing bacteria spp. (PSB_4) was also recorded in PGPR consortium consists of two inoculant as compared to single inoculants.

Population of *Azotobacter* isolate

Similar trends of microbial population has

also been found in *Azotobacter* spp. (AZT_4) Maximum population of *Azotobacter* spp. was recorded in treatment T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) (20 x 10^4 CFU) followed by T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) (16.00 x 10^4 CFU). Higher count of *Azotobacter* spp. was recorded in treatment receiving three mixed inoculant T_{15} and T_{16} (15.00 x 10^4 CFU and 12.00 x 10^4 CFU) as compared with PGPR inoculant containing two inoculant (T_7, T_8, T_9, T_{10}, T_{11} and T_{12}) (7.40 x 10^4 CFU to 20.50 x 10^4 CFU), except T_7 (NPK + PSB_4 + AZT_4) Similarly higher count of *Azotobacter* spp. (AZT_4) was also recorded in PGPR consortium consists of two inoculant as compared to single inoculants, except T_4 (NPK + AZT_4).

The data summarised in table 26 demonstrate that N_2-fixing and P-solubilizing bacteria were present in the natural soil (UIC), but that their populations were quite low. Seed inoculation with effective isolates of bacteria might be significantly increased the bacteria population. Data indicated that inoculation of the *Azotobacter* spp. (AZT_4) and *Azospirillum* spp. (AZT_6), *Pseudomonas* spp. (PSD_6) and P-solubilizing bacteria spp. (PSB_4) helped in maintaining a higher number of total bacteria. The beneficial effect of inoculation on microbial population may have due to an increase in the supply of available P and N directly and indirectly through changing the growth rate and metabolic activities of the crop, which resulted in more root exudates and there by created a favourable habitat for the growth and development of microbes (Ramzan *et al.*, 2007). Besides these two factors might be accounted for the relative change in bacterial population in different treatments. First soil organic matter serves as the main source of energy and nutrition as for the most of micro organisms. Second pH plays a crucial role in it as neutral or slightly alkaline pH which generally favours the bacterial population (Yadav, 1984). The fertility status of soil coupled with temp are some of the ecological factors responsible for including variability in P-dissolving bacteria Kundu and Gaur 1980, Anu and Kundu 2005, Singh *et al.*, 2002)).

Co-inoculation effect of PGPR on nutrients concentration (NPK) and uptake in maize plant at 60 days growth period

N-concentration in maize plant

The data on N-content in maize plant at 60 days growth period as influence by different consortium of PGPR has been presented in Table 24 & Fig. 5. The maximum N-content (0.78 %) was recorded in Treatment T_{13} (NPK + AZT_4 + AZS_6 + PSB_4) while lowest (0.31 %) in UIC (T_1). Treatment T_{14} was at par with T_{15} and significantly superior over T_{13} and $T_{16.}$ Single inoculant treatment T_3, T_4, T_5 and T_6 and double inoculants T_7, T_8, T_9, T_{10}, T_{11} and $T_{12.}$ The variation of N-content might be due to fixation of nitrogen by effective PGPR isolates AZT_4 + AZS_6, resulted in the increment of N-content in maize plant. Such an increment of N-uptake in maize plant might be attributed to N_2-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganism to produced growth promoting substances.

Azospirillum which lives on or in the roots may be responsible for the production of hormones affecting the plant growth by stimulating root growth proliferation of maize. Increased root surface area might have resulted in enhanced mineral uptake by inoculated plants. These observations supported the findings of Lin *et al.,* (1983) that *A. brasilense* inoculation can improve N uptake of Plant and improve the availability and efficiency of use of applied mineral nutrients.

N-uptake by plant

The data present in Table 24 showed the response of PGPR consortium on N-uptake by maize plant of 60 days growth period. The maximum N-uptake was recorded in treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_{14}) (115.89 mg $plant^{-1}$) which was at par with T_{15}, T_{16} and T_{13} and superior over the single inoculant treatment T_3, T_4, T_5 and T_6 and double inoculants, treatment T_7, T_8, T_9, T_{10}, T_{11} and T_{12} probably due to higher biomass production. The minimum N-uptake was recorded in UIC (T_1) (19.38 mg. $Plant^{-1}$). The result was in conformity with the result of Chaykavskaya *et al.*, (2002), Kapulnik *et al.*, (1981, 1982), Zambre *et al.*, (1984), Boddey *et al.*, 1985.

P-concentration in maize plant

Variation in the p-content in maize plant at 60 days growth period due to consortium of PGPR isolates have been presented in table (24). PGPR consortium caused the significantly variation in P-content in maize plant. P-content varied from 0.09 to 0.35 per cent as affected by different treatment. The maximum P-content in plant was recorded in T_{13} (NPK + PSB_6 + AZT_4 + AZS_6) (0.35%) while lowest in UIC (T_1) (0.09%). Treatment T_{14} was at par with treatment T_{15}, T_{16} and T_{13} and significantly superior over single inoculant treatment T_3, T_4, T_5 and T_6 and double inoculants, treatment T_7, T_8, T_9, T_{10}, T_{11} and T_{12}.

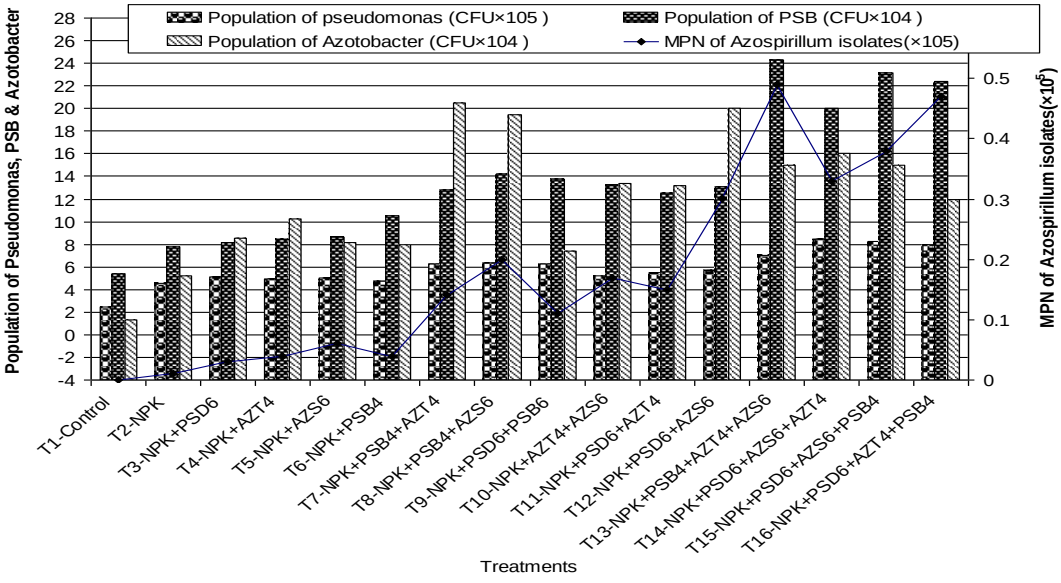
Table.1 Combined effect of plant growth promoting rhizobacteria on Root Volume and Root colonization (%) by mycorrhiza in maize crop at 60 days growth period

Treatment	Root volume(cc)	Root colonization (%) (Mycorrhiza)
T1-Control	23.23	19.00
T2-NPK	24.20	20.00
T3-NPK+PSD_6	28.30	34.00
T4-NPK+AZT_4	27.63	32.00
T5-NPK+AZS_6	27.73	42.00
T6-NPK+PSB_4	25.13	38.00
T7-NPK+PSB_4+AZT_4	40.57	40.00
T8-NPK+PSB_4+AZS_6	40.73	45.00
T9-NPK+PSD_6+PSB_6	39.50	53.00
T10-NPK+AZT_4+AZS_6	43.090	58.00
T11-NPK+PSD_6+AZT_4	44.17	62.00
T12-NPK+PSD_6+AZS_6	49.47	68.00
T13-NPK+PSB_4+AZT_4+AZS_6	56.57	77.00
T14-NPK+PSD_6+AZS_6+AZT_4	62.93	90.00
T15-NPK+PSD_6+AZS_6+PSB_4	59.70	83.00
T16-NPK+PSD_6+AZT_4+PSB_4	57.50	80.00
S.Em +_	0.97	2.23
CD (P=0.05)	2.78	6.42

Table.2 Combined effect of plant growth promoting rhizobacteria on nutrient concentration (NPK) and uptake in maize crop at 60 days growth period

Treatment	N %	N uptake (mg. plant^{-1})	(P) %	P uptake (mg.plant^{-1})	(K) %	K uptake (mg. plant^{-1})
T1-Control	0.31	19.38	0.09	5.63	0.24	21.25
T2-NPK	0.33	22.41	0.12	8.15	0.38	25.80
T3-PSD_6	0.38	30.17	0.15	11.91	0.42	31.08
T4-AZT_4	0.46	34.68	0.14	10.56	0.44	33.18
T5-AZS_6	0.48	37.20	0.15	11.63	0.45	34.88
T6-PSB_4	0.49	36.60	0.16	11.95	0.48	35.88
T7-PSB_4+AZT_4	0.58	51.16	0.25	20.05	0.52	45.86
T8-PSB_4+AZS_6	0.62	58.34	0.28	26.35	0.56	52.70
T9-PSD_6+PSB_6	0.48	41.71	0.22	19.12	0.53	46.06
T10-AZT_4+AZS_6	0.54	54.76	0.21	21.30	0.51	51.71
T11-PSD_6+AZT_4	0.52	54.50	0.18	19.91	0.49	51.35
T12-PSD_6+AZS_6	0.53	58.57	0.19	21.00	0.50	55.25
T13-PSB_4+AZT_4+AZS_6	0.78	111.85	0.35	50.19	0.65	93.21
T14-PSD_6+AZS_6+AZT_4	0.77	115.89	0.30	45.15	0.63	94.81
T15-PSD_6+AZS_6+PSB_4	0.75	112.13	0.29	43.35	0.60	94.19
T16-PSD_6+AZT_4+PSB_4	0.70	111.34	0.27	39.04	0.62	89.66
S. Em. +_	0.03	1.61	0.006	0.65	0.015	1.28
CD (P=0.05)	0.07	4.45	0.02	1.79	0.04	3.56

Fig.1 Combined effect of pl ant growth promoting rhizobacteria on Microbiological count in maize corp at 60 days growth



PGPR including phosphate solubilizing bacterial isolate (PSB_4) is able to solubilize the unavailable forms of P in soil by acidification, chelation, and exchange reaction in the soil environment (Ponmnugan and Gopi, 2006). Zaidi *et al.,* 2004 reported dual inoculation of asymbiotic N_2 fixer *A. chroococcum* and A

M fungi, *G. fasciculatum* resulted in enhanced root infection, which stimulated plant growth, and increased N and P uptake in green gram.

P-uptake by plant

A perusal of data presented in Table 24 indicated a significant increase in P-uptake at 60 days growth period, which varied from 5.63 mg plant^{-1} to 50.19 mg plant^{-1} as affected by different treatments. The maximum P-uptake was recorded in treatment T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) (50.19 mg Plant^{-1}) is probably due to higher P concentration, followed by T_{14}, T_{15} and T_{16} and significantly superior over single inoculant treatment T_3, T_4, T_5 and T_6 and double inoculants, treatment T_7, T_8, T_9, T_{10}, T_{11} and T_{12} while minimum P-uptake was recorded in UIC (T_1) 5.63 mg plant^{-1},. De Freitas *et al.,* 1997 reported that PGPR solubilise the fixed P and increase the P-content of plant. PGPR significantly influenced the P-uptake by plant as reported by Kapulnik *et al.,* 1981, 1982, Zambre *et al.,* 1984 and Boddy *et al.,* 1985. Khan and Zaidi (2007) also reported that Co-inoculation of *A. chroococcum*, *Bacillus* sp. and *G. fasciculatum* increased the P of roots, shoots and whole plant by 0.9, 0.7 and 0.4 fold respectively, over the control.

K-concentration in maize plant

The data on K-content in maize plant at 60 days growth period as influence by different consortium of PGPR has been presented in Table 24.

The K-concentration in maize plants varied from 1.50 to 1.98 per cent as influenced by different treatment combinations. The higher K-concentration (1.98 %) was recorded in T_{14} (NPK + PSD_6 + PSB_4 + AZT_4 which was at par with T_{15}, T_{16} and T_{13} and significantly superior over the single inoculant treatment T_3, T_4, T_5 and T_6 and double inoculants, treatment T_7, T_8, T_9, T_{10}, T_{11} and T_{12}. The lowest K-concentration was recorded in UIC T_1 (1.50%). One of the most common ways that PGPR improve nutrient uptake for plant is by altering plant hormone levels. This changes roots growth and shape by increasing root branching, root mass, root volume, root length, and/or the amount of root hairs. This leads to greater root surface area which in turn, helps it to absorb more nutrients.

K-uptake by plant

The uptake of K by maize plant as affected by different treatments combination of PGPR consortium has been presented in table 24. The maximum K-uptake of maize plant at 60 days growth period was recorded in treatment T_{14} (NPK+ PSD_6 + AZS_6 + AZT_4) (94.82 mg. Plant^{-1}) is probably due to higher biomass production (T_{14}) while minimum K-uptake was recorded in UIC (T_1) (21.25 mg plant^{-1}). Treatment T_{14} at par with T_{13}, and T_{15} and significantly superior over single inoculant treatment T_3, T_4, T_5 and T_6 and double inoculants, treatment T_7, T_8, T_9, T_{10}, T_{11} and T_{12}.

Summary and conclusions are as follows:

The present investigation was under taken to isolate plant growth promoting rhizobacteria (PGPR) from twelve different diversified area of Bihar including, diara belt (Koshi and Ganga), where microbial diversity was maximum under maize based inter cropping

system. Efforts were also made to screen the PGPRs for their plant growth promoting attributes like seed germination, production of I.A.A., P-solubilizing activity, antifungal activity and nitrogenase activity. The insertion of isolates for the formulation of microbial consortium and its effect on plant physiological parameters, total biomass production, nutrients uptake, microbial population on post harvest soil were under taken. Selected four best isolates of PGPR *Pseudomonas* (PSD_6), P-solubilizer (PSB_4), *Azospirillum* (AZS_6) and *Azotobacter* (AZT_4) were examined alone or in the possible combinations with other isolates. The pot experiment was conducted at the Department of Microbiology, F.B.S. & H., R.A.U., Pusa in completely randomised design (CRD) with three replications during rabi 2008-09.

A brief summary of the result on each aspect has been presented given here in following paragraphs.

MPN count of *Azospirillum* isolate AZS_6 varies due to influence of different treatments combination. Maximum MPN count of *Azospirillum* (AZS_6) was recorded in treatment T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) 0.49 x 10^5 followed by T_{16} (NPK + PSD_6 + AZT_4 + PSB_4) 0.47 x 10^5.

Highest population of *Pseudomonas* isolate PSD_6 was recorded in T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) 2.43 x 10^5 CFU and minimum in UIC (T_1) 2.43 x 10^5 CFU.

Maximum population of P-solubilizing bacteria (PSB_4) and nitrogen fixer microbes diazotroph *Azotobacter* (AZT_4) was recorded in T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) 24.30 x 10^4 and 20.00 x 10^4 CFU respectively while minimum in UIC (T_1) 5.40 x 10^4 and 1.30 x 10^4 CFU, respectively.

Root volume of maize at 60 day growth period was significantly increased due to influence of different treatments combination. The highest root volume was recorded in the treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) (62.93 cc plant^{-1}) followed by T_{15} (59.70 cm plant^{-1}) and minimum (23.23 cc plant^{-1}) in UIC (T_1)

Percentage of root colonization by soil mycorrhiza increased significantly over uninoculated control (UIC). The maximum percentage root colonization was recorded under treatment T_{14} (NPK + PSD_6 + AZS_6 + PSB_4) (90.00 %) followed by T_{15} (83.00%) and minimum (19.00 %) in UIC (T_1).

Nitrogen, Phosphorus and Potash contain in maize plant after 60 days growth period increased significantly over UIC which were maximum in PGPR consortia T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) and followed by T_{15} and minimum in UIC, respectively.

N and K uptake in maize plant after 60 days growth period increased significantly over UIC, which were maximum in PGPR consortia T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) (115.89 mg plant^{-1} and 94.81 mg plant^{-1}) followed by T_{15} (112.13 mg plant^{-1} and 94.19 mg plant^{-1}) and minimum in UIC (19.38 mg plant^{-1} and 21.25 mg plant^{-1}).In case of P maximum uptake was found in T_{13} (NPK + PSB_4 + AZT_4 + AZS_6) (50.19 mg plant^{-1}) followed by T_{14} (45.15 mg plant^{-1}) and T_{15} (43.35 mg plant^{-1}) and minimum in UIC (5.63 mg plant^{-1}) respectively.

In the light of summarised result following conclusion emerged.

Under this investigation it was confirmed that maize based cropping system in diara belt was very specific in respect of microbial diversity. This might be due to intercropping with various C_3 species including legumes, C_4 nature and photo insensitiveness of maize crops low fertility status of soil. It was also

found that microbial isolates like *Pseudomonas* spp. (PSD_6), *Azotobacter* spp. (AZT_4), *Azospirillum* spp. (AZS_6) and P-solubilizing bacteria spp. (PSB_4) of these areas are very efficient in biomass production by synthesizing growth hormones in maize based cropping system. On the basis of screening test, with regard to seed germination test, production of IAA, P-solubilization test, antifungal activity and nitrogenase activity, four most efficient native PGPR isolates of *Pseudomonas* spp. (PSD_6), *Azotobacter* spp. (AZT_4), *Azospirillum* spp. (AZS_6) and P-solubilizing bacteria spp. (PSB_4) were selected, out of 48 isolates, which were collected from different sites of Bihar. In pot culture experiment maize seeds were inoculated with selected isolates of PGPR alone and different possible consortium and observed enhancement in maize plant physiological attributes, total biomass production and nutrient uptake which were significantly superior over the uninoculated control. The present studies revealed that microbial consortium (*Pseudomonas* PSD_6 + *Azotobacter* AZT_4 + *Azospirillum* AZS_6) was the best in all respect than that of others and very significant not only in growth parameters but also in maintaining soil health for sustainable crop production. From these result we conclude that the native isolates of PGPR activity isolates from Bihar soil can play an important role in helping plant to establish and growing calcareous condition. It is advisable that isolates may be used in field trial to ascertain whether this combination of these isolates enhancement growth and yield of maize would be feasible.

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