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Original Research Article

Co-inoculation Effect of Plant Growth Promoting Rhizobacteria on Dry Matter Yield Production

A. Kumar[1], S.P.Vishwkarma[2], P. Kumar[3] and M. Kumar[3]

[1]Soil Science, IRS, Araria, BAU, Sabour, India
[2]Soil Science, IRS, Madhepura, BAU, Sabour, India
[3]KVK, Araria BAU, Sabour, India
**Corresponding author*

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. 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.

Keywords

PGPR, Rhizobacteria, Intercropping, Pseudomonas, Screening

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 (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 sterilized 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 ± 1^0C for 6 days.

Inoculated flasks were shaken intermittently at 30 ± 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 $28^0 \pm 2^0$C 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 inoculatnts 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 maize plant physiology

Plant height

Plant height of Maize at 45 and 60 DAS increased significantly over uninoculated control (UIC) Table 20. At 30 DAS PGPR consortia treatment T_{14} (NPK + PSD_6 + AZS_4 + AZS_6) gave highest Plant height (11.60 cm) followed by T_{15} (NPK + PSD_6 + AZS_6 + PSB_4) (11.57 cm). The data were non-significant. At 45 day after sowing (DAS) highest plant height was recorded under treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) 35.33 cm which was at par with T_{15} and T_{16}. The lowest plant height was recorded in UIC (T_1) 22.23 cm. Mixed inoculation or consortium T_{14} significantly superior over single and double inoculants.

At 60 day after sowing (DAS) highest plant height was recorded in T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) i.e. 65.23 cm which was significantly superior over T_{15}, T_{16} and T_{13} while lowest plant height was recorded in UIC (T_1) 31.93 cm. Treatment T_{14} significantly superior over single and double inoculants. As compared with UIC, T_{14} recorded 51.05 %, T_{15} 49.34% and T_{16} 48.14 % increased in plant height. This finding was supported by Yaseri and Patwardhan (2007) reported that application of *Azotobacter* and *Azospirillum* strains increased canola yield 21.17% number of branches 11.78 %. There are numerous report indicating beneficial effect of *Azospirillum* inoculation in crop yield. Similarly promotion in plant height, plant dry weight and grain yield of various crop plants to inoculation with PGPR were reported by other workers (Khalid *et al.*, 2004, Biswas *et al.*, 2000a; b; Shahroons *et al.*, 2006).

Leaf area index

Leaf area index at 45 and 60 DAS increased significantly over uninoculated control (UIC) Table 21. At 30 DAS leaf area index was non-significant however T_{14}, T_{15}, T_{16} recorded highest value. At 45 DAS highest leaf area index of plant was recorded in treatment T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) (0.25) which was at par with treatment T_{15} (0.24) and significantly superior over T_{16} (0.22) and T_{13} (0.22) PGPR consortia. The lowest plant leaf area index was recorded in UIC (T_1) 0.09. Mixed inoculation or consortium T_{14} significantly superior over single and double inoculants. At 60 DAS highest leaf area index of maize plant was recorded in treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) (0.46), which was at par with treatment T_{15} (0.43) and significantly superior over T_{16} (0.42) and T_{13} (0.41)PGPR consortia, while lowest plant leaf area index was recorded in UIC (T_1) 0.18.

Number of leaf plant^{-1}

Data presented in Table (22) revealed that number of leaf plant^{-1} in maize crop at 45 and 60 DAS increased significantly over the UIC (T_1). At 30 DAS number of leaf plant^{-1} was non-significant. At 45 DAS highest number of leaf plant^{-1} in maize were recorded in consortia consists of triple inoculation treatment T_{13}, T_{14}, T_{15} and T_{16} and double inoculation treatment T_8 and T_{10} (7.00), which were significantly superior over UIC (T_1) (5.0) and fertilizer treatment T_2 (5.33).
At 60 DAS highest number of leaf plant^{-1} in

maize were recorded in consortia consists of triple inoculation treatment T_{13}, T_{14}, T_{15} and T_{16} (11.00) which were significantly superior over UIC (T_1) (7.33) and fertilizer treatment T_2 (7.67). Present studies revealed that maize seed inoculated with all PGPR consortia resulted in an increased plant height, leaf area index and number of leaf plant^{-1} (Table 20, 21 & 22). The enhancing effect of seeds inoculation with rhizobacteria applied either double or triple as compared to single inoculant on plant height, leaf area index and number of leaf plant^{-1} might be attributed to nitrogen fixing and phosphate solubilizing ability of these micro organisms to produced growth promoting substances. Similar increases in plant height, leaf area, were observed in different crops inoculated with *Pseudomonas, Azospirillum* and *Azotobacter* strain (Martinez-Toledo *et al.,* 1988; Shaukat *et al.,* 2006a, b, Siddiqui and Shaukat, 2002, Burd *et al.,* 2000). In comparison to *Azotobacter* isolates (AZT_4). *Azospirillum* isolates (AZS_6) was found superior, this was because of sandy clay nature of soil, which suited more to *Azospirillum* than *Azotobacter.* Srinivasan (1989) has suggested that *Azospirillum* was more effective in loam to clay soils with high moisture levels, while *Azotobacter* was more effective in semi dry loam. Mixed inoculation of *Azotobacter* and *Azospirillum* were alway superior at all N-levels compared to individual application in sugarcane as reported by Navale *et al.,* (1985) in extensive trials for three year. Mixed co-inoculation of diazotrophic isolates *Azotobacter* and *Azospirillum* (AZT_4 + AZS_6) and bio control agent isolates *Pseudomonas* PDS_6 proved to enhance root and foliage growth and to produce significantly plant height, leaf area index and number of leaf plant^{-1} than the UIC. Mixed culture of PGPR consortia i.e. AZS_6 + PSD_6 + AZT_4 produced significant increase in the uptake of mineral nutrients over UIC. This increase may be related to the production of hormones by *Azospirillum* isolates (Tie *et al.,* 1979) such as auxin, gibberelline and cytokinins. *Pseudomonas* (PSD_6) PGPR act as biological control of plant pathogens and improve plant growth by inhibiting pathogens through the synthesis of different compounds (Hill *et al.,* 1994). Badaway *et al.,* (2003) reported that mixed co-inoculation of diazotrophic strains; such as *Azotobacter chroococcum* or *Azospirillum lipoferum* with a phosphate solubilizing strains as *B. Polymyxa*, proved to enhance root and foliage growth, than the Uninoculated control. Afzal and Asghari (2008) reported that dual inoculation of PGPR significantly increased plant height, spike length, root and shoot weight of the test wheat crop. The plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phyto-hormones, increasing the availability of nutrients, facilitating the uptake of nutrients (Burd *et al.,* 2000).

Inoculation of seedlings of both fir and spruce with *Pseudomonas* isolates increased the plant height, number of leaf, girth of stem and weight of plants significantly over the control has been reported by Zargar *et al.,* (2005). PGPR consortia which produced high amount of IAA also significantly promoted plant height.

Co-inoculation effect of PGPR on Dry matter yield production of Maize plant at 60 days growth period

Fresh weight of shoot and root

Fresh weight of shoot (g plant^{-1}) and root (g plant^{-1}) at 60 days growth period varied from 30.73 to 75.80 g plant^{-1} and 21.67 to 65.10 g plant^{-1} respectively, as affected by different treatments (Table 23 and Fig. 4). At 60 days growth period maximum fresh weight of shoot and root was recorded in treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) 75.80 g plant^{-}

^{1} and 65.10 g plant^{-1} respectively, which was at par with T_{15}, T_{16} and T_{13}. The minimum fresh weight of shoot and root was recorded in uninoculated control (UIC) 30.73 g plant^{-1} and 21.67 g plant^{-1} respectively. Treatment T_{14} significantly superior over single inoculant treatment T_3, T_4, T_5 and T_6 and double inoculants treatments T_7, T_8, T_9, T_{10}, T_{11} and T_{12}.

Dry weight of shoot and root (g plant^{-1})

Dry weight of shoot (g plant^{-1}) and root (g plant^{-1}) at 60 days growth period varied from 3.65 to 7.58 g plant^{-1} and 2.60 to 7.47 g plant^{-1} respectively, as affected different by treatments Table 23. Maximum dry weight of shoot and root were recorded in treatment T_{14} (NPK + PSD_6 + AZS_6 + AZT_4) 7.58 g plant^{-1} and 7.47 g plant^{-1} respectively, which was at par with T_{15}, T_{16} and T_{13}. The minimum dry weight of shoot and root was recorded in UIC 3.65 g plant^{-1} and 2.60 g plant^{-1} respectively. The seeds inoculated with *Azospirillum* isolate (AZS_6) or *Azotobacter* (AZT_4) or *Pseudomonas* isolate (PSD_6) or either single or in combination resulted higher dry weight of shoot and root (g plant^{-1}) with the UIC. Treatment T_{14} 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}.

Significant increase in shoot and root dry weight were obtained by inoculation with any single isolates, mixed inoculants with two or three isolates as compared to UIC. Mixed inoculants of PGPR consortium consisted of (PSD_6 + AZS_6 + AZT_4) was superior in plant dry matter (yield) performance. Hussain and Vancura, 1970 reported that inoculation of maize seeds with strains *Pseudomonas fluorescence* and *Chromobacterium violaccum* significantly increased the dry matter yield of plants. Diazotrophs (AZT_4 and AZS_6) being an efficient N_2-fixer fixed atmospheric nitrogen to the plant which helps in enhancing photosynthetic efficiency and therefore, there was more accumulation of dry matter. Similar results of increment in dry matter yield of maize due to inoculation of diazotrophs were reported (Rai and Hunt, 1993, Shahroons *et al.,* 2006). Rhizobacteria (PSD_6) influence the plant dry matter which probably due to production of plant growth hormones and colonization of beneficial microbes (Goel, 1999; Sindhu *et al.,* 1999; Singh and Pareek, 2003).

Total biomass production

A perusal of data presented in table 23 indicated a significant increase in total Biomass production at 60 days growth period which varied from 6.25 to 15.05 g plant^{-1} as affected by different treatments. The maximum total biomass production was recorded in the treatment (T_{14}) ((NPK + PSD_6 + AZS_6 + AZT_4) 15.05 g plant^{-1}, while lowest in the UIC (T_1) 6.25 g plant^{-1}. Treatment T_{14} was at par with 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}.

The highest biomass production in PGPR consortium T_{14} ((NPK + PSD_6 + AZS_6 + AZT_4) may be due to higher leaf area index and better root and shoot and nutrients from the deeper zone of the soil combined with the better nitrogen fixation capacity of *Azospirillum* (AZS_6) and *Azotobacter* (AZT_4) isolates and root colonizer by *Pseudomonas* isolates (PDS_6). Similar result was reported by Kennedy and Tehan (1992).

The plant growth promoting rhizobacteria play an active role in soil through their natural ability to provide important but scarce nutrients to the plants. The inoculation effect of PGPR including N_2-fixer AZS_6 and AZT_4

and Root colonizer *Pseudomonas* isolate (PSD_6) in processed calcareous soils produced a significant increase in the uptake of mineral nutrients over uninoculated control (UIC). This increase may be related to the production of hormones such as auxin (Mordukhova *et al.,* 1991) gibberellins (Mahmaud *et al.,* 1984) and cytokinins (Tien *et al.,* 1979). *Azospirillum* and *Pseudomonas* 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 might have resulted in enhanced mineral uptake (Okon, 1985). These observations support the findings of Lin *et al.,* 91983) that *A. brasilense* inoculation can improve N uptake of plants and improve the availability and efficiency of applied mineral nutrients. Use of diazotrophs as microbial inoculants has resulted in 20, 15 and 60-80 % increase in yield of paddy, wheat and legume respectively and saving of 5-100 % chemical fertilizers (Haffeez *et al.,* 2002). Increased in plant height and root and shoot biomass of wheat was reported earlier from twelve different isolates of PGPR (De Freitas and Germida 1990, 1992). Several possible mechanism of plant growth promotion has been reported. Among these, the production of biologically active metabolites, particularly the plant growth regulators by rhizosphere microbiota affect plant growth directly after being taken up by the plant or indirectly by modifying the rhizosphere environment (Arshad and Frankenberger, 1998).

Table.1 Combined effect of plant growth promoting rhizobacteria on electro-chemical properties of harvested soil after 60 days growth period of maize crop at different DAS

Treatment	Post harvest soil		
	PH	EC (dSm^{-1})	Organic carbon ($g.kg^{1}$)
T1-Control	7.88	0.320	5.08
T2-NPK	7.84	0.321	5.11
T3-NPK+PSD_6	7.81	0.320	5.30
T4-NPK+AZT_4	7.77	0.321	5.50
T5-NPK+AZS_6	7.79	0.322	5.55
T6-NPK+PSB_4	7.80	0.322	5.35
T7-NPK+PSB_4+AZT_4	7.66	0.319	5.69
T8-NPK+PSB_4+AZS_6	7.61	0.320	5.67
T9-NPK+PSD_6+PSB_6	7.69	0.322	5.65
T10-NPK+AZT_4+AZS_6	7.57	0.320	5.71
T11-NPK+PSD_6+AZT_4	7.54	0.321	5.74
T12-NPK+PSD_6+AZS_6	7.50	0.321	5.80
T13-NPK+PSB_4+AZT_4+AZS_6	7.39	0.322	5.88
T14-NPK+PSD_6+AZS_6+AZT_4	7.48	0.325	5.96
T15-NPK+PSD_6+AZS_6+PSB_4	7.42	0.324	5.92
T16-NPK+PSD_6+AZT_4+PSB_4	7.44	0.323	5.90
S.Em +_	0.22	0.01	0.17
CD (P=0.05)	0.61	NS	0.46

Fig.1 Combined effect of plant growth promoting rhizobacteria on dry matter production of maize crop at 60 days growth period

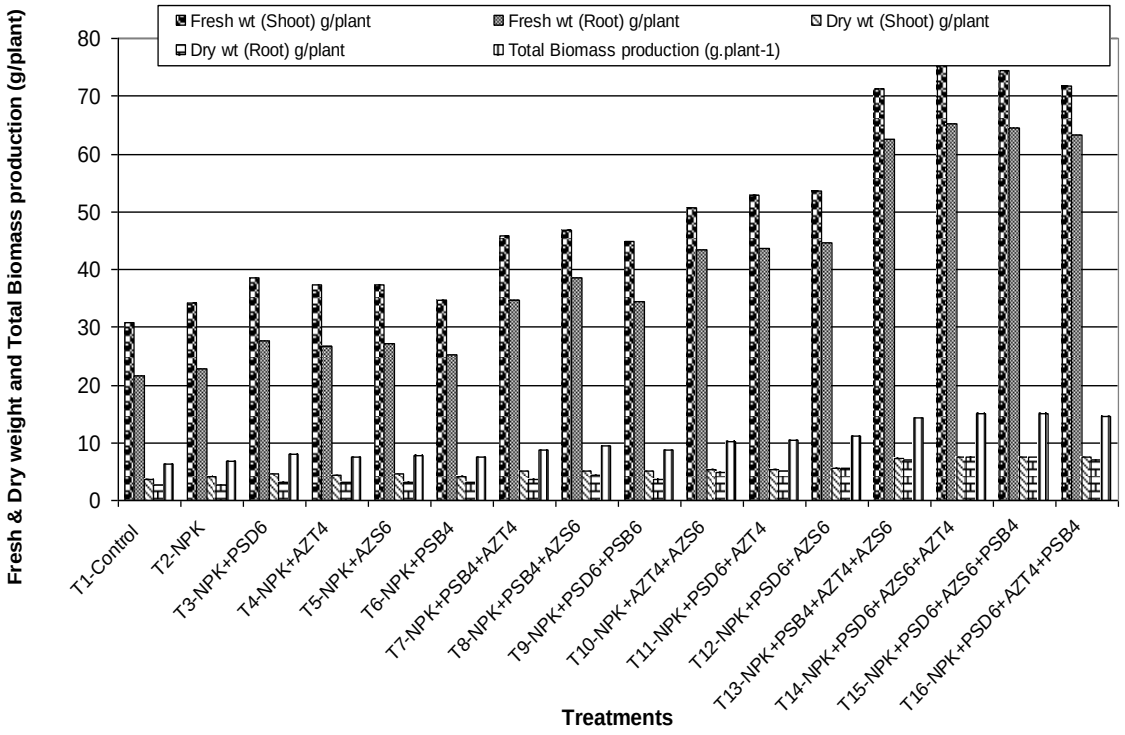
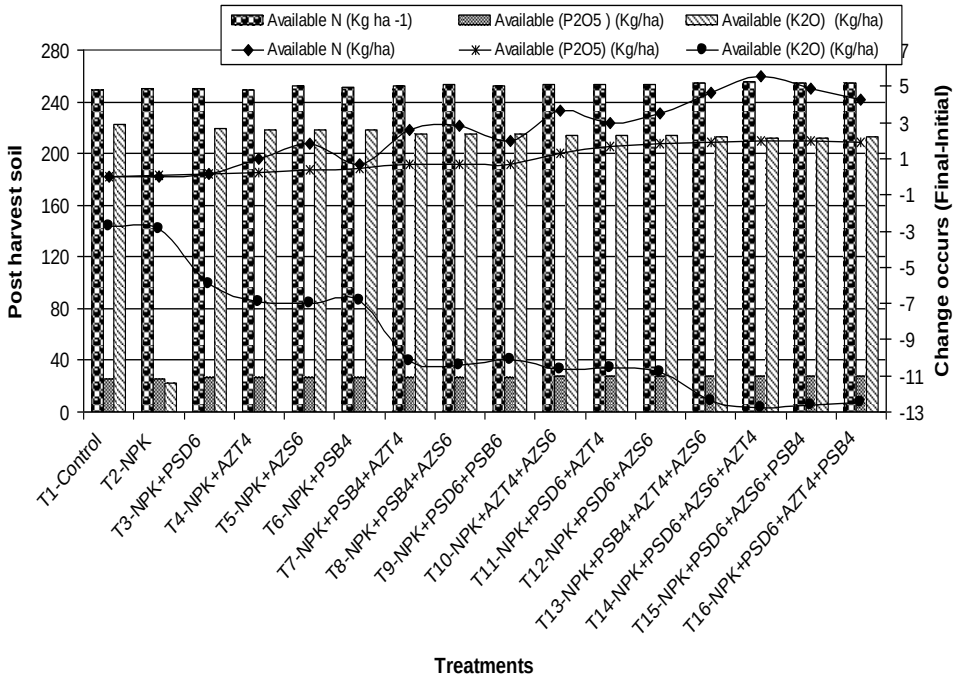


Fig.2 Combined effect of plant growth promoting rhizobacteria on available nutrients N, P and K of harvested soil after 60 days growth period of maize crop



Physico-chemical properties of experimental soil

Data presented in table 27 revealed that pH value in soil decreased due to influence of different consortium of PGPR treatments. The lowest pH was recorded in treatment T_{14} (NPK + AZS_6 + PSB_4 + AZT_4) (7.39) while maximum was recorded in the UIC (T_1) (7.88). Treatment T_{14} was at par with T_{15}, T_{16} and T_{13} and significantly superior over single and double inoculants. This might be P-solubilizing isolate (PSB_4) exude higher amount of organics acid in the rhizospher (Groleau-Renud *et al.*, 1998) on young (16 days old) maize crops. This organics acid might be decreased the pH of the soil. However, in EC value in soil was non-significant.

Similar trends also follows in organic carbon. Organic carbon present in soil increased due to influence of different treatments combination. The maximum organic carbon was recorded in the treatment T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) 5.96 g kg^{-1} while minimum was recorded in the UIC (T_1) 5.08 g kg^{-1}. T_{14} was at par with T_{15}, T_{16} and T_{13} and superior over single and double inoculants. This might be due to higher microbial population (Diazotoph and P-solubilizing), increment of soil biomass, deposition of fallen leaf, senescence of roots, dead microbial cells, and deposition of microbially synthesized compound.

Effect of PGPR consortium on available nutrients (N, P, and K) in experimental soil after harvest of 60 days growth period of maize plant

The data on available N, P_2O_5 and K_2O of pre sown and post harvest soil have been presented in Table 28 and Fig. 8. N-content of post harvest soil was non significant. However PGPR consortia T_{14} recorded high value of available N in soil followed by T_{15} and T_{16}. The gain in available N as recorded in the different treatments was as follow:
$T_{14} > T_{15} > T_{13} > T_{16} > T_{12} > T_{10} > T_{11} > T_8 > T_7 > T_9 > T_5 > T_4 > T_6 > T_3 > T_2 > T_1$.

The increment in soil available N in post harvest soil sample might be due to nitrogen fixation by N-fixing organisms. The variation in the value of soil available N might be due to amount of biological nitrogen fixed by N-fixing bacterial isolates.

In case of soil available P_2O_5, non-significant change in available P_2O_5 was recorded soil.

$T_{13} > T_{15} > T_{16} > T_{14} > T_{12} > T_{11} > T_{10} > T_7 > T_8 > T_6 > T_5 > T_4 > T > T_2 > T_1$.

The change in available P_2O_5 in post harvest soil sample might be due to combined role of P-solubilizing isolates PSD_6 and PSB_4 and also due to P_2O_5 Utilization by crop during crop growth.

In case of available K_2O in post harvest soil samples variations were non-significant. The decrement of available K_2O in post harvest soils were as follow:

$T_{14} > T_{15} > T_{13} > T_{16} > T_{13} > T_{11} > T_{10} > T_8 > T_7 > T_9 > T_5 > T_4 > T_3 > T_2 > T_1$.

The decrement of available K_2O in post harvest soil as compared to pre-sowing soil might be due to plant utilization of potassium during growth period.

Summary and conclusions of the present study 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.

1 Under pot culture condition plant height of maize at 45 and 60 day after sowing (DAS) was significantly increased by over uninoculated control (UIC). At 45 and 60 DAS, maximum plant height was recorded in PGPR consortia T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) (35.33 cm and 65.23 cm), followed by T_{15} (34.33 and 63.03 cm) and minimum in UIC (T_1) (22.23 cm and 31.93 cm), respectively.

2. Leaf area index of maize plant at 45 and 60 day after sowing (DAS) was significantly increased over uninoculated control (UIC). At 45 and 60 DAS, maximum leaf area index was recorded in PGPR consortia T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) (0.25 and 0.46), followed by T_{15} (0.24 and 0.43) and minimum (0.09 and 0.18) in UIC (T_1), respectively.

3. Average number of leaf plant^{-1} at 45 and 60 day after sowing (DAS) was significantly increased over uninoculated control (UIC). At 45 and 60 DAS, maximum average number of leaf plant^{-1} was recorded in T_{14}, T_{15}, T_{16} and T_{13} and minimum in UIC (T_1) respectively.

4. Fresh weight (g plant^{-1}) of shoot and root after 60 days growth period was significantly increased over UIC. Maximum Fresh Weight of shoot and root was recorded in PGPR consortia T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) 75.80 g plant^{-1} and 65.10 g plant^{-1}), followed by T_{15} (74.33 g plant^{-1} and 64.57 g plant^{-1}), T_{16} (71.67 g plant^{-1} and 63.27 g plant^{-1}) and T_{13} (71.27 g plant^{-1} and 62.47 g plant^{-1}) and minimum in UIC (T_1) (30.73 g plant^{-1} and 21.67 g plant^{-1}) respectively.

5. Dry weight (g plant^{-1}) of shoot and root after 60 days growth period was significantly increased over UIC. Maximum dry weight of shoot and root was recorded in PGPR consortia T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) (7.58 g plant^{-1} and 7.47 g plant^{-1}), followed by T_{15} (7.43 g plant^{-1} and 7.52 g plant^{-1}), T_{16} (7.41 g plant^{-1} and 7.05 g plant^{-1}) and T_{13} (7.34 g plant^{-1} and 7.00 g plant^{-1}) and minimum in UIC (T_1) (3.65 g plant^{-1} and 2.60 g plant^{-1}), respectively.

6. Total biomass production after 60 day growth period was significantly increased over UIC, which was maximum in PGPR consortia T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) (15.05 g plant^{-1}), followed by T_{15} (14.95 g plant^{-1}), T_{16} (14.46 g plant^{-1}) and T_{13} (14.34 g plant^{-1}) and minimum in UIC (T_1) (6.25 g plant^{-1}).

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 weather this combination of these isolates enhancement growth and yield of maize would be feasible.

7. Organic carbon content in post harvest oil was influenced by different combination of PGPR. The maximum organic carbon was recorded in the treatment T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) 5.96 g kg^{-1} followed by T15 (5.92 g kg^{-1}) and minimum 5.08 g kg^{-1} in UIC (T_1).

8. The available N, P_2O_5 and K_2O at post harvest soil were non-significantly affected by different consortia of PGPR. Treatment T_{14} (NPK + PSD_6 + AZT_4 + AZS_6) recorded maximum available N (6.58 kg ha^{-1}).

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