

Full Length Research Paper

The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime

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Banana, an important fruit crop, requires high amounts of N-fertilizers for commercial cultivation. This, however, is costly and can be hazardous to the soil environment when used excessively. Biofertilizer is globally accepted as an alternative source of N-fertilizer and can substantially supplement the N requirement while enhancing the uptake of water and mineral nutrients of crop plants. An experiment was conducted to observe the effect of plant growth promoting rhizobacterial inoculation on growth, nutrient uptake of bananas grown under hydroponics condition. The design of the experiment was randomized complete block with five replicates. The following six treatments were imposed: T₁ (control; N₀-PGPR), T₂: (N₀+Sp7), T₃: (N₀+ UPMB10), T₄: (N_{33%}+ Sp7), T₅: (N_{33%} + UPMB10), and T₆: (N_{100%}-PGPR). The results showed that inoculation by UPMB10 with minimal fertilizer-N supply increased ($P < 0.05$) the primary root elongation and secondary root initiation and subsequently increased ($P < 0.05$) the root biomass. The same treatment also increased ($P < 0.05$) N concentration in pseudostem and leaves and Ca concentration in roots. The total accumulation of N, P, K, Ca and Mg were increased due to inoculation; a consequence of increased plant growth. Plants with this treatment produced an equivalent total dry matter as those supplied with 100% N.

Key words: Banana, rhizobacteria, root stimulation, growth, nutrient uptake.

INTRODUCTION

The banana fruit crop is widely cultivated in tropical areas where high dosages of chemical fertilizers (NPK fertilizer) are commonly applied. Commercial cultivation of banana requires a substantial amount of inorganic fertilizers namely urea. Banana plants cannot store N and such deficiency symptoms quickly develop if supply is not sufficient. Therefore, N should be supplied at short intervals during growth, which is costly and difficult to maintain (Robinson, 1996). Furthermore, excessive N, that is, beyond the critical level is not only wasteful and uneconomical but also pollutes the soil environment and water bodies through leaching. Banana suffers from a mismatch of its nitrogen demand and its nitrogen supplied

as chemical fertilizer, resulting in a 50-70% loss of the fertilizer applied. Two approaches may be used in resolving this problem; one is to regulate the timing of nitrogen application based on the banana plant nutrient requirement, thus increasing the efficiency of the plant's use of applied nitrogen. The other is to increase the efficiency of the use of available soil nitrogen and meet the additional N-demand by making banana capable of fixing its own nitrogen either directly, or via a close interaction with diazotrophic bacteria (Ladha et al., 1997).

Biofertilizer, inoculants of microorganisms, an alternative source of N-fertilizer, can be applied to increase crop growth through biological N₂ fixation, availability of soil nutrients and water uptake (Cocking, 2000) and to ensure a sustainable banana production. Over the last decades there has been a great interest in nitrogen fixing-bacteria associated to non-legumes, principally

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gramineous plants. The biological nitrogen fixation could be an alternative for this crop system, once the plants are able to establish association with *Herbaspirillum*-like and *Burkholderia* related bacteria, which are not well known yet. In N-free hydroponics condition, the plant growth promoting rhizobacteria (PGPR) inoculated plants were not able to perform normal growth due to lack of needed N which require additional N-fertilizer for a good synergy (Saad et al., 1999). Fertilizer-N has the most prominent influence on biological N₂ fixation (BNF) which efficiency increases in the presence of moderate levels of soil riched fertilizer-N but declines at high levels, reflecting an inhibitory effect of N-fertilizer on N₂ fixation (Marschner, 1995). PGPR inoculation together with 33% of the total N requirement has been shown to produce a similar biomass and yield in sweet potatoes as the plants with complete fertilizer-N (Saad et al., 1999). Significant increases in crop yields following application of PGPR have been documented under diverse field conditions (Bashan, 1998; Mia et al., 2005). They have been widely reported to fix atmospheric N₂ with grasses and cereals (Dobereiner, 1997) and enhance nutrient uptake (Kapulnik, 1991; Bashan and Holguin, 1997; Sheng, 2005; Mia et al., 2007). This demonstrates a potential saving on fertilizer cost. Based on these studies it necessitates investigating the benefits of PGPR interaction with minimal starter-N. Thus, the present study was carried out to observe the effect of PGPR inoculation with minimal fertilizer-N on nutrient uptake, root and shoot growth of banana plantlets.

MATERIALS AND METHODS

The experiment was conducted in the Shade-House of Farm 2, Universiti Putra Malaysia, under hydroponics condition. The following six treatments with five replications were imposed: T₁ (control; N₀-PGPR), T₂: (N₀+Sp7), T₃: (N₀+ UPMB10), T₄: (N_{33%}+ Sp7), T₅: (N_{33%} + UPMB10), and T₆: (N_{100%}-PGPR). Plant nutrient solution was used according to the modified Zakaria et al. (1997). Two PGPR strains were used in the experiments, namely *Azospirillum brasilense* strain Sp7, isolated from Digitaria grass and *Bacillus sphaericus* strain UPMB10 (Universiti Putra Malaysia Bacteria 10) isolated from oil palm roots. Plant growth promoting rhizobacteria strains Sp7 and UPMB10 were grown in nutrient broth (Okon et al., 1977) in 250-mL Erlenmeyer flasks containing 100 mL medium, which were agitated with a rotary shaker (125 rpm, 24 h, 30°C). One tissue-cultured banana plantlet cv. 'Berangan' was planted per plastic pot (4.0 L) containing the nutrient solution. The pots were aerated for six hours with an air pump at six-hourly intervals to ensure an uninhibited root respiration and bacterial growth.

The primary root elongation was measured with a meter scale and secondary root initiation was counted at alternate days for 15 days. The plant height, leaf number and leaf chlorophyll content were measured fortnightly and net photosynthesis was measured 30 DAI (days after inoculation). Leaf chlorophyll content of youngest fully expanded leaf (third leaf from the shoot) was indirectly measured using a chlorophyll meter (SPAD 502, Minolta Camera Ltd. Japan) (Takebe and Yoneyama, 1989). The chlorophyll meter

value was calibrated against leaf chlorophyll content determined by chemical method with acetone extraction as described by Coombs et al. (1985). The rate of net photosynthesis was measured with a Portable Photosynthesis System (LI-6200, LI-Cor, Inc., USA). The plants were terminally harvested at 45 DAI and the following parameters were recorded: plant height, leaf number, leaf area and total dry matter (TDM). Root parameters, namely root number, root length, root volume (cm³ plant⁻¹) and root dry weight were also recorded. Plant parts were separated into roots, pseudostems and leaves, dried in oven at 71°C for 48 h and weighed for TDM. Harvested plants were separated into roots, corm, pseudostem and leaves and were prepared for chemical analyses. Banana leaves were sampled following the International Reference Method (Lahav and Turner, 1983). The oven-dried samples were ground in a Willey hammer mill, passed through a 2 mm sieve, mixed thoroughly and stored in plastic vials. The ground samples were digested by Micro-Kjeldahl method (Thomas et al., 1967). The digested samples were analysed for N, P, K, Ca and Mg. The N and P were determined by Autoanalyzer (Technicon II, Technicon Ltd.) while the remaining elements were analysed by Atomic Absorption Spectrophotometer (Perkin-Elmer, 5100 pc). The collected data were analyzed statistically using the Statistical Analysis System (SAS, version 6.12, 1989). Following the analysis of variance procedure (ANOVA), differences among treatment means were determined using the Least Significant Difference (LSD) and Duncan's New Multiple Range Test (DMRT) comparison method (whenever applicable) at 5% level of significance. Correlation analysis was performed for the estimation relationships between different traits.

RESULTS

Root growth

Primary (1⁰) root elongation

PGPR inoculation positively influenced the 1⁰ root elongation (Figure 1). Plants inoculated without N-fertilization showed lower 1⁰ root length compared to those inoculated with minimal N-fertilization. Inoculation with 33% N showed higher root elongation; UPMB10-inoculated plants with 33% N recorded the highest root elongation. Plants inoculated with Sp7 along with 33% N-fertilization increased their root elongation from 2 DAI to 10 DAI compared to control. On the other hand, roots of UPMB10-inoculated plants with 33% fertilizer-N increased from 3 DAI until 12 DAI when compared to control. There were no significant differences ($P < 0.05$) among the controls (N₀ and N_{100%}) and both showed shorter 1⁰ root length, when compared with the inoculated plants.

Secondary (2⁰) root initiation

Secondary roots were initiated at 2 DAI. Plants inoculated with Sp7 and provided with 33% N showed positive influence on secondary root initiation (Figure 2). This treatment also showed the highest initiation of secondary roots. Plants inoculated with UPMB10 without fertilizer-N

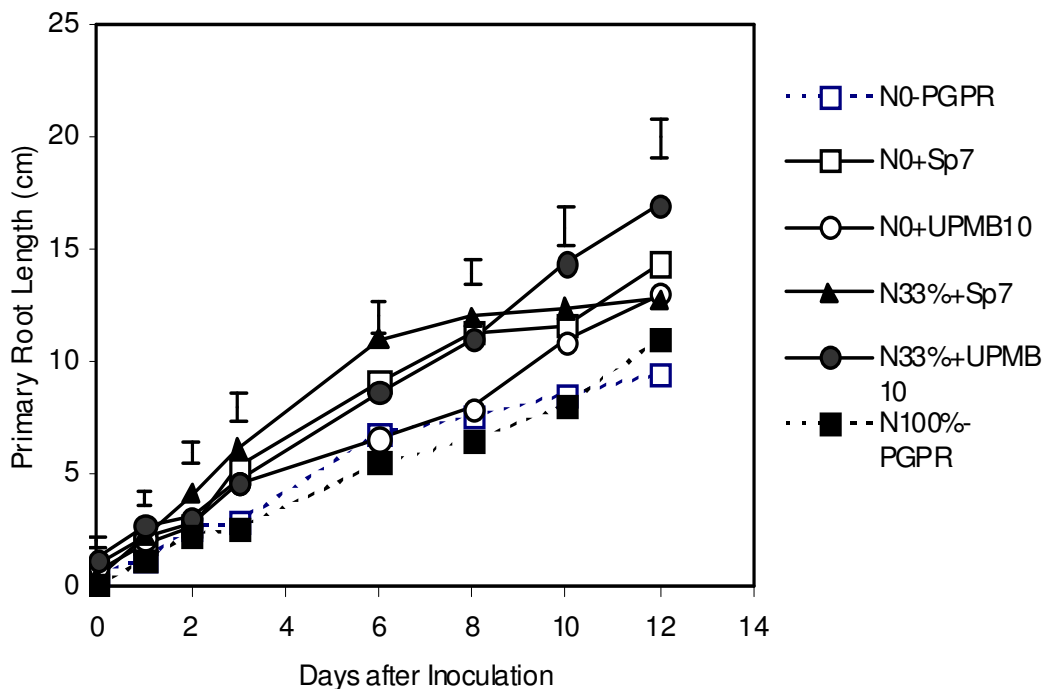


Figure 1. Primary root elongation of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 (vertical bar represents LSD at 0.05 significant level)

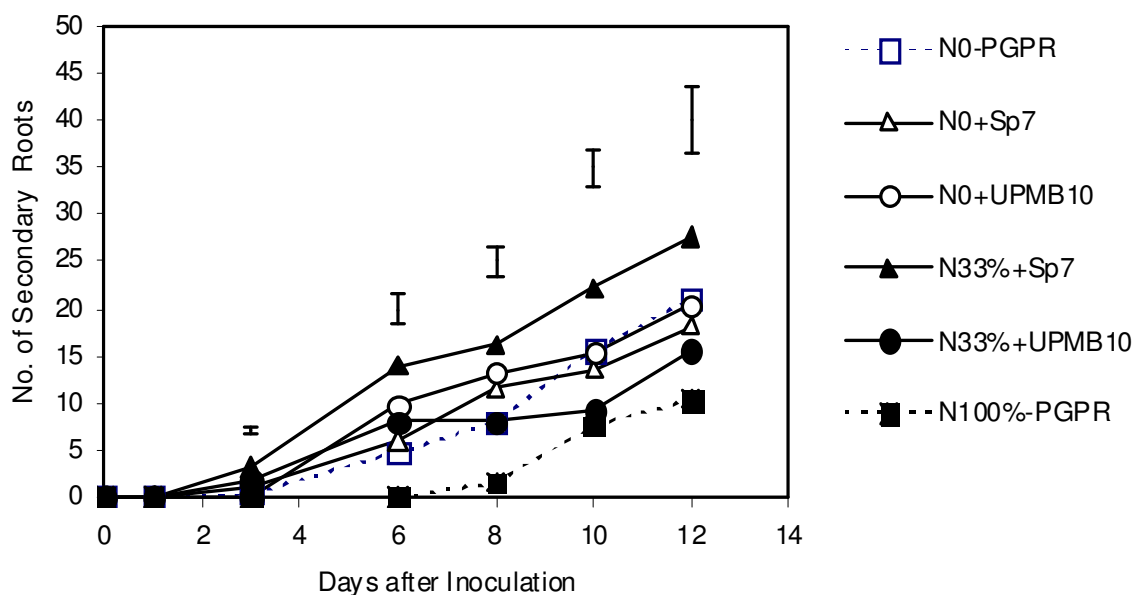


Figure 2. Secondary root initiation of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 (vertical bar represents LSD at 0.05 significant level)

showed higher 2⁰ root initiation compared to control (N₀-PGPR) until 8 DAI thereafter no differences were observed. Plants inoculated with UPMB10 and provided with 33% fertilizer-N could not increase 2⁰ root initiation compared to control. Uninoculated plants fertilized with 100% N showed the lowest initiation of secondary roots.

Root number

PGPR inoculation positively influenced the number of primary roots especially the inoculated plants with 33% N fertilization (Table 1). Plants inoculated with Sp7 and UPMB10 and supplied with 33% fertilizer-N produced

Table 1. Root growth of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under low fertilizer-N regimes

Treatments	No. of 1 ⁰ roots plant ⁻¹	Total 1 ⁰ root lengths (cm)	Base diameter of 1 ⁰ root (mm)		Root volume (cm ³ plant ⁻¹)	Root dry wt. (g plant ⁻¹)
			Feeder	Pioneer		
N ₀ -PGPR	6.0 b	236 b	0.13 b	0.25 c	11.3 d	0.27 c
N ₀ +Sp 7	8.3 ab	314 ab	0.17 ab	0.27 bc	18.0 c	0.65 b
N ₀ +UPMB 10	7.0 ab	341 a	0.23 a	0.35 abc	27.3 b	0.61 b
N _{33%} +Sp 7	9.6 a	308 ab	0.21 ab	0.38 b	55.0 a	1.02 a
N _{33%} +UPMB 10	9.6 a	355 a	0.19 ab	0.42 a	59.0 a	0.99 ab
N _{100%} -PGPR	8.6 ab	372 a	0.15 ab	0.42 a	50.0 a	0.69 b

Means having same letter (s) in a column do not differ significantly at 0.05 level by DMRT

Feeder root: thin 1⁰ root with more 2⁰ root formation

Pioneer root: thick 1⁰ root with less 2⁰ root formation

equivalent 1⁰ root number as the 100% fertilizer-N control. Control plants with 100% fertilizer-N, however, did not show any significant difference compared to control with N₀.

Total 1⁰ root length

Total 1⁰ root lengths were significantly increased due to inoculation with PGPR (Table 1). Plants inoculated with UPMB10 without fertilizer-N application showed 44% longer primary roots compared to control (N₀-PGPR) whereas Sp7 did not show any significant increment. PGPR inoculated plants together with 33% fertilizer-N produced similar root length as 100% N control plants but could not increase root length compared to inoculated plants with N₀. Uninoculated plants with 100% fertilizer-N showed longer 1⁰ roots (372 cm plant⁻¹) compared to uninoculated plants without fertilizer-N (236 cm plant⁻¹).

Root base diameter

Root base diameter, indication of the thickness of a root and vigour of a plant, is positively influenced by PGPR inoculation. Plants inoculated with UPMB10 and without fertilizer-N showed larger (77% more) base diameter in feeder root compared to control (N₀-PGPR). Plant inoculated with UPMB10 and provided with 33% N-fertilization showed an equal base diameter in pioneer roots (0.42 mm) as the 100% N applied control plant (0.42 mm) whereas Sp7 did not produce a similar effect. Plant with 100% fertilizer-N increased their pioneer root base diameter compared to N₀ while the similar trend was not found in feeder root. However, inoculated plant provided with 33% fertilizer-N could not show any increment in root base diameter neither in feeder nor in pioneer when compared to inoculated plant without fertilizer-N.

Root volume

Remarkable increment in root volume was observed in inoculated plants with and without N-fertilization (Table 1 and Plate 1). Inoculated plants with Sp7 and UPMB10 without N-fertilization produced more root volume compared to the control (N₀-PGPR) by 59 and 142%, respectively. Similarly, these inoculated plants with 33% fertilizer-N also increased root volume by 10 and 18% respectively, an equivalent root volume as control plant with 100% fertilizer-N. Root volume increased with the application of fertilizer-N. The plant supplied with 100% fertilizer-N produced significantly more root volume (342%) compared to those without fertilizer-N.

Root dry weight

Root dry weight significantly increased due to inoculation with PGPR (Table 1). Plants inoculated with Sp7 and UPMB10 without fertilizer-N produced more root dry weight compared to the control (N₀-PGPR) by 141 and 126%, respectively. Similarly plants inoculated with Sp7 and provided with 33% fertilizer-N produced significantly greater root-dry weight (1.02 g plant⁻¹) compared to 100% fertilizer-N applied control plants. It is interesting to note that plants inoculated without N-fertilization produced similar amounts of root dry weight as the 100% N-fertilized plants. Application of fertilizer-N, especially uninoculated plants, increased their root dry weight compared to plants without fertilizer-N.

Shoot growth

Plant height

In general, there were no differences among the treatments

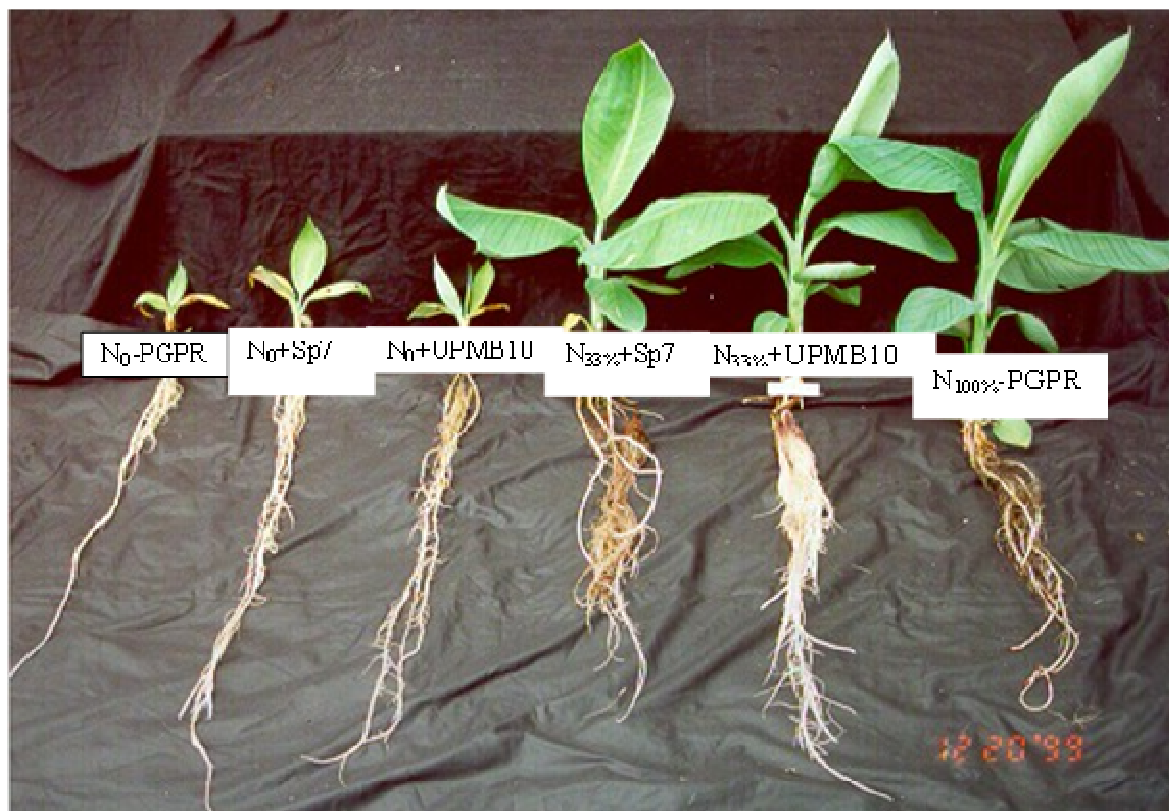


Plate 1. Root and shoot growth of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition at low fertilizer-N regimes for 45 days.

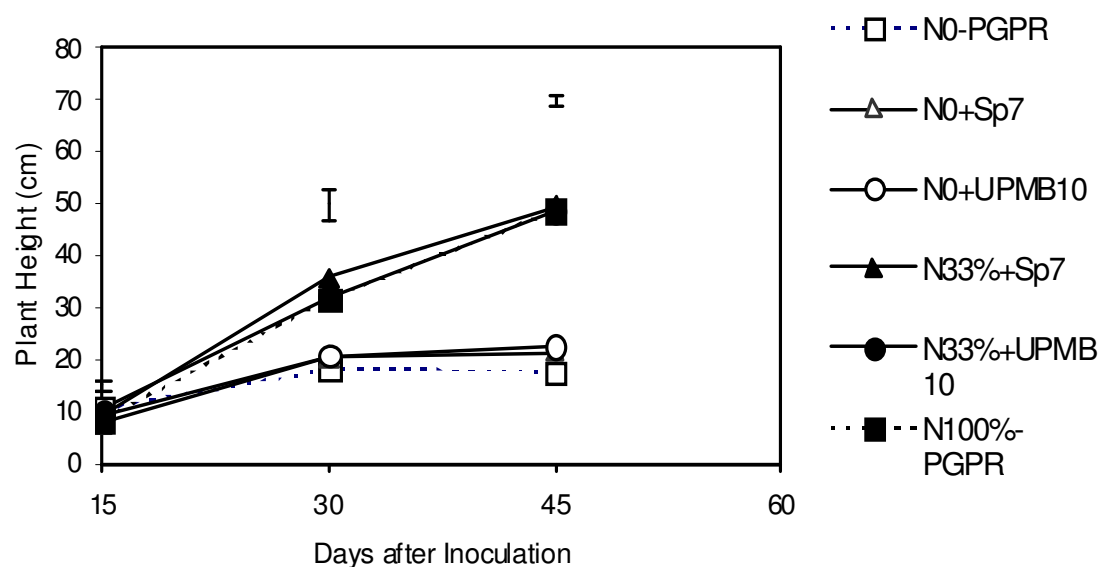


Figure 3. Plant height of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days (vertical bars represent LSD at 0.05 significant level)

at D₁₅ (Figure 3). Thereafter, plants with fertilizer-N showed taller plants compared to plants without fertilizer-N. Plants given 100% fertilizer-N were significantly taller

compared to uninoculated plants without fertilizer-N. Similarly inoculated plants with 33% fertilizer-N showed taller height compared to inoculated plants without

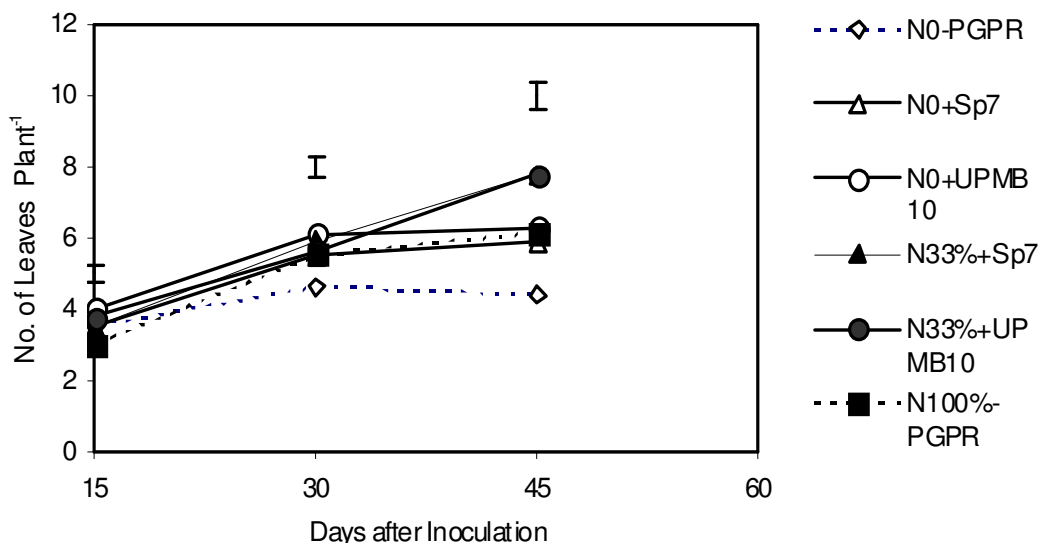


Figure 4. Leaf number of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days (vertical bars represent LSD at 0.05 significant level)

fertilizer-N. However, inoculation with PGPR showed positive effect on plant height. Inoculated plants with 33% fertilizer-N showed an equivalent plant height (48.8 to 49.8 cm) as the control plant with 100% fertilizer-N (49.0 cm). Similarly inoculated plants without fertilizer-N showed taller plant compared to control (N₀-PGPR) at 45 DAI.

Leaf development

Leaf development, measured by leaf number and area, was positively influenced by PGPR inoculation (Figure 4 and 5). Initially at D₁₅ there was no difference of leaf number among the treatments with and without inoculation. Uninoculated plants without fertilizer-N showed the lowest number of leaves from 30 to 45 DAI unlike those supplied with 100% fertilizer-N. Inoculated plant with 33% fertilizer-N resulted in similar leaf number as the 100% N-applied plants at 30 DAI but higher at 45 DAI. Inoculated plant without fertilizer-N produced similar leaf number as the uninoculated plant with fertilizer-N (100% N). However, inoculated plant with 33% fertilizer-N produced significantly more leaf number compared to plants inoculated without fertilizer-N at 45 DAI. Inoculated plants without fertilizer-N produced more leaf area compared to the control (N₀-PGPR) (Figure 5). Similarly inoculated plants provided with 33% fertilizer-N produced an equivalent leaf area as the 100% N applied control plant. Plants with fertilizer-N showed higher leaf area when compared with the plants without fertilizer-N under both inoculated and uninoculated conditions.

Leaf chlorophyll content

Leaf chlorophyll content significantly increased by PGPR

inoculation especially plants without N-fertilization (30 - 45 DAI) (Figure 6). There was a decreasing trend of chlorophyll content with the plant age among the treatments of N₀ with inoculated or uninoculated conditions. However, inoculated plants together with 33% N-fertilization showed an equivalent chlorophyll content as the 100% N-fertilized plants. Plants with 100% fertilizer-N showed higher chlorophyll content compared to plants without fertilizer-N (30 to 45 DAI). Similarly, inoculated plants with 33% fertilizer-N showed higher chlorophyll content compared to inoculated plants without fertilizer-N (30 - 45 DAI).

Net leaf photosynthesis (Pn)

There were no significant differences of net leaf photosynthesis by the inoculation process without fertilizer-N (Figure 5). But inoculation with PGPR (Sp7 and UPMB10) using 33% fertilizer-N resulted in similar photosynthetic rate (11.9 to 14.1 $\mu\text{mole CO}_2\text{ m}^{-2}\text{ sec}^{-1}$) as the 100% N applied control (13.1 $\mu\text{mole CO}_2\text{ m}^{-2}\text{ sec}^{-1}$). Plants with 100% fertilizer-N showed higher Pn when compared with the plants without fertilizer-N. Similarly plants inoculated with 33% fertilizer-N showed significantly higher Pn compared to inoculated plants without fertilizer-N.

Shoot dry weight

Shoot dry weight greatly increased by PGPR inoculation. Plants inoculated with Sp7 and UPMB10 without N-fertilization showed more shoot dry weight compared to the control (N₀-PGPR) by 93 and 100%, respectively

□ N0-PGPR ▨ N0+Sp7 ▩ N0+UPMB10
 ▤ N33%+UPMB10 ▦ N33+Sp7 ▧ N100-PGPR

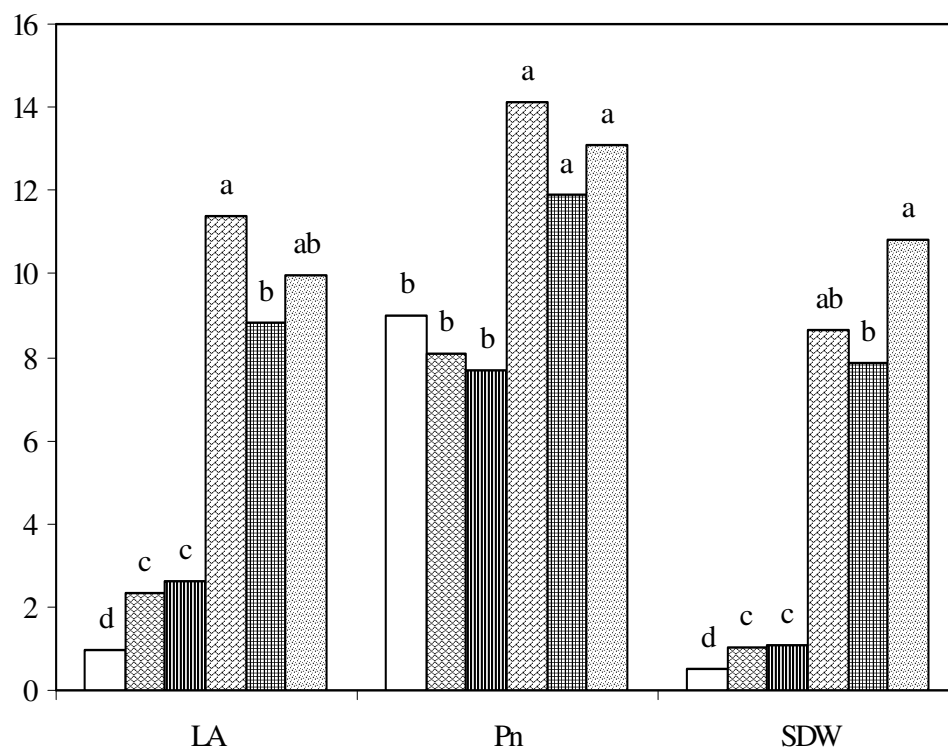


Figure 5. Leaf area (LA; dm² plant⁻¹), photosynthetic rate (Pn; μmole CO₂ m⁻² sec⁻¹) and shoot dry weight (SDW; g plant⁻¹) of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days (values having same letter (s) in a column do not differ significantly at 0.05 level by DMRT)

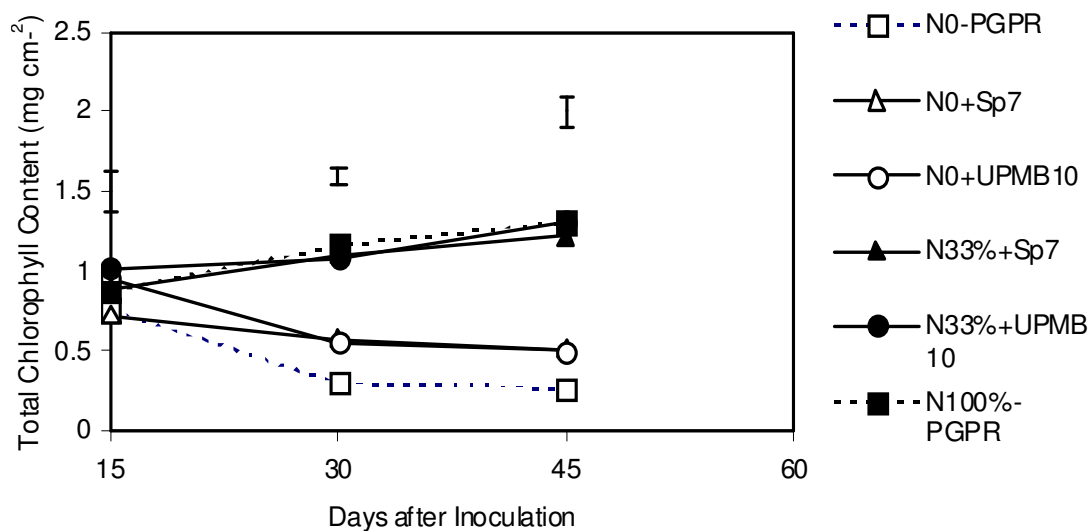


Figure 6. Leaf chlorophyll content of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days (vertical bars represent LSD at 0.05 significant level)

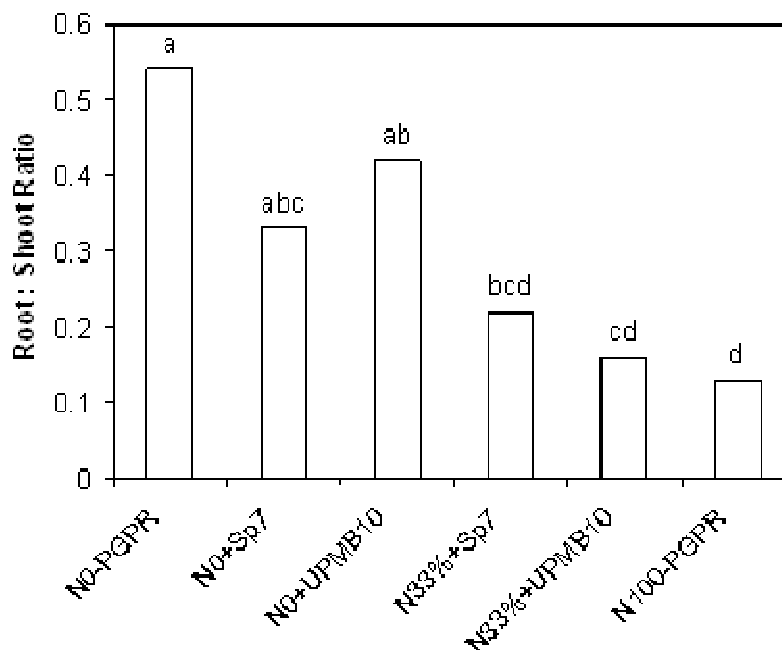


Figure 7. Root: shoot ratio of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition at low fertilizer-N regimes for 45 days (values having same letter (s) in a column do not differ significantly at 0.05 level by DMRT)

(Figure 5). On the other hand, inoculated plants with UPMB10 and provided with 33% fertilizer-N produced an equivalent amount of shoot dry weight as the 100% N-fertilized control plants while Sp7 could not show the similar effect. There were significant differences of shoot dry weight between the treatment of N₁₀₀ and N₀. Similarly inoculated plants with 33% fertilizer-N showed significantly higher shoot dry weight compared to inoculated plants without fertilizer-N.

Root to Shoot Ratio (R/S)

Application of fertilizer-N resulted in lower R/S value (0.13 to 0.22) indicating better shoot growth and without N-fertilization showed higher R/S value (0.33 - 0.54) reflecting less shoot growth (Figure 7). Inoculated plants without fertilizer-N could not show any difference of R/S value compared to the control (N₀-PGPR). However, inoculated plants with 33% fertilizer-N showed an equivalent R/S values as the 100% N-applied control plants. Plants using 100% fertilizer-N significantly showed lower R/S values compared to plants without fertilizer-N. Similarly plants inoculated with UPMB10 and given with 33% fertilizer-N showed lower R/S value when compared with inoculated without fertilizer-N. However, Sp7 could not

show similar effect.

Nutrient uptake

The PGPR inoculation and fertilizer-N application significantly increased the N concentration and accumulation in banana plantlets grown under hydroponics condition for 45 days (Tables 2 and 3). Application of fertilizer-N resulted in higher N concentration in root, pseudostem and leaf. Plant using 100% fertilizer-N showed significantly higher N concentration compared to plant without fertilizer-N. Similarly, inoculated plants with 33% fertilizer-N resulted in higher N concentration in pseudostem and leaf while no significant difference was found in root.

Inoculated plants without fertilizer-N could not increase the N concentration in root and leaf while Sp7 showed higher N in pseudostem. Inoculated plants with UPMB10 and provided with 33% fertilizer-N increased their N concentration in pseudostem and leaf which resulted in an equivalent concentration as the 100% fertilizer-N applied plant. However, inoculated plants with Sp7 and supplied with 33% fertilizer-N showed similar increment in leaf only. Plants inoculated with UPMB10 and provided with 33% fertilizer-N showed significantly higher P concentration in root compared to inoculated plants with UPMB

Table 2. The N, P, K, Ca and Mg concentrations in root, pseudostem and leaves of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition under low fertilizer-N regimes for 45 days

Plant part	Treatments	Concentration (%)				
		N	P	K	Ca	Mg
Root	N ₀ -PGPR	1.67 c	1.09 ab	4.08 b	0.58 c	0.51 a
	N ₀ +Sp7	1.63 c	1.14 ab	4.72 ab	0.58 c	0.44 ab
	N ₀ +UPMB10	2.28 bc	0.98 b	5.27 a	0.49 c	0.33 c
	N _{33%} +Sp7	2.41 bc	1.14 ab	4.98 ab	1.06 a	0.43 ab
	N _{33%} +UPMB10	2.98 b	1.33 a	4.76 ab	1.06 a	0.34 bc
	N _{100%} -PGPR	4.16 a	1.27 ab	4.50 ab	0.93 b	0.33 c
Pseudostem	N ₀ -PGPR	1.30 d	0.73 a	6.91 a	0.47 a	0.24 ab
	N ₀ +Sp7	2.12 c	0.65 a	6.24 a	0.46 a	0.28 a
	N ₀ +UPMB10	1.25 d	0.78 a	5.39 a	0.42 ab	0.25 ab
	N _{33%} +Sp7	3.08 b	0.66 a	5.52 a	0.30 bc	0.21 ab
	N _{33%} +UPMB10	4.65 a	0.67 a	5.31 a	0.26 c	0.26 a
	N _{100%} -PGPR	4.72 a	0.66 a	5.41 a	0.28 bc	0.16 b
Leaf	N ₀ -PGPR	1.58 b	1.21 a	4.21 a	0.43 a	0.33 a
	N ₀ +Sp7	1.72 b	0.97 b	4.71 a	0.34 ab	0.32 a
	N ₀ +UPMB10	1.33 b	0.81 bc	4.26 a	0.34 ab	0.39 a
	N _{33%} +Sp7	3.63 a	0.63 cd	4.26 a	0.30 b	0.35 a
	N _{33%} +UPMB10	3.76 a	0.58 d	4.31 a	0.28 b	0.40 a
	N _{100%} -PGPR	3.98 a	0.75 cd	3.80 a	0.32 b	0.34 a

Means having same letter (s) in a column of a parameter do not differ significantly at 0.05 level by DMRT

Table 3. Total accumulation of N, P, K, Ca and Mg in banana plantlets inoculated with PGPR strains Sp7 and UPMB10 at low fertilizer-N regimes for 45 days

Treatments	Total nutrient accumulation (mg plant ⁻¹)				
	N	P	K	Ca	Mg
N ₀ -PGPR	6.0 d	8.5 d	23.4 c	4.0 d	2.9 d
N ₀ +Sp7	12.6 c	15.5 c	62.4 b	10.5 c	6.5 c
N ₀ +UPMB10	14.8 c	18.0 c	67.4 b	9.0 c	7.1 c
N _{33%} +Sp7	173.9 b	35.4 b	148.9 a	17.3 b	19.0 b
N _{33%} +UPMB10	185.9 b	53.6 a	173.4 a	36.2 a	29.2 a
N _{100%} -PGPR	243.6 a	44.8 ab	135.5 a	6.2 c	16.7 b

Means having same letter (s) in a column do not differ significantly at 0.05 level by DMRT

10 without fertilizer-N. Other treatments could not show any significant differences in P concentration. In pseudostem, P concentration was unaffected by inoculation with PGPR. However, in leaf, application of fertilizer-N decreased the P concentration. Plants supplied with 100% fertilizer-N resulted in significantly lower P concentration compared to plants without fertilizer-N. Similarly inoculated plants with 33% fertilizer-N also showed lower P concentration in leaf compared to inoculated plant without fertilizer-N. PGPR inoculation could not increase K concentration except in root in the treatment of UPMB10 without fertilizer-N. PGPR inoculation greatly increased Ca concentration in root

especially in plants inoculated and supplied with 33% fertilizer-N (14% more over 100%-N control). However, inoculation without fertilizer-N could not show any increment. Application of fertilizer-N decreased Ca concentration in pseudostem and leaf. Using 100% fertilizer-N showed significantly lower Ca concentration in pseudostem compared to the control (N₀-PGPR). Similarly, inoculated plants provided with 33% fertilizer-N also showed lower concentration when compared to inoculated plants without fertilizer-N. However, in leaf, application of 100% fertilizer-N showed lower Ca concentration compared to the control (N₀-PGPR) while other treatments were unaffected.

Plants inoculated with Sp7 and supplied with 33% fertilizer-N recorded higher (30% more over 100% N control) Mg concentration in root compared to uninoculated plants fertilized with 100% N. On the other hand inoculated plants with UPMB10 and given with 33% fertilizer-N showed higher Mg concentration in pseudostem when compared to plants fertilized with 100% N. Magnesium concentration in leaf was not influenced by PGPR inoculation. The total accumulation of N, P, K, Ca and Mg were significantly increased with PGPR inoculation. Inoculated plants without fertilizer-N showed significantly higher accumulation compared to the control (N_0 -PGPR). Inoculated plants provided with 33% fertilizer-N recorded an equivalent amount of P and K as the 100% N applied control plant. Application of 100% fertilizer-N showed significantly higher accumulation of those nutrients (N, P, K and Ca) compared to the control (N_0 -PGPR). Similarly inoculated plants with 33% fertilizer-N also showed significantly higher accumulation of nutrient compared to inoculated plants without fertilizer-N. Moreover, plants inoculated with UPMB10 and supplied with 33% fertilizer-N accumulated significantly higher amounts of Ca and Mg compared to 100% N-fertilizer applied plants while Sp7 showed higher Ca but similar Mg.

DISCUSSION

The results strongly indicated that PGPR strains Sp7 and UPMB10 inoculation influenced the plant growth of banana plantlets grown for 45 days. Highly positive responses were observed in root stimulation. Primary root elongation and 2^o root initiation of inoculated plant increased due to PGPR inoculation. The strain UPMB10 with 33% fertilizer-N improved 1^o root elongations while Sp7 with 33% fertilizer-N promoted 2^o root initiations. Uninoculated plants with N_0 and N_{100} showed lowest response in root elongation and initiation. This indicated that N supply is not critical in root stimulation. The results showed that PGPR inoculation with low fertilizer-N could produce a synergistic effect on root growth parameters. Higher initiation of 2^o roots of Sp7 inoculated plants may be due to the presence of more bacterial cells and their beneficial interaction in this root zone. Root colonization study supported this results that more bacterial cells of Sp7 were colonized in the root hair proliferation zone. The findings are in agreement with the results of Bilal et al. (1993) who found that the area around the point of emergence of lateral roots usually shows more bacterial colonization in grasses.

The study indicated that PGPR inoculation increased the root function and when supplemented with fertilizer-N could accelerate root growth. Using 33% of the total fertilizer-N contributed a similar or even higher root growth ($1.02 \text{ g plant}^{-1}$) compared to the 100% N-applied

plants ($0.69 \text{ g plant}^{-1}$). Inoculated plant with UPMB10 and provided with 33% fertilizer-N significantly increased root volume (18%), root mass (43%) and root diameter. Inoculation alone increased the thickness of the root while fertilizer-N could not produce any synergistic effect. PGPR inoculation showed positive effect on root volume and this increment is enhanced with the application of fertilizer-N indicating a synergistic effect with both strains. Similarly root dry weight increased due to inoculation and fertilizer-N application; strain Sp7 showed a highly synergistic effect (48% increased) with 33% fertilizer-N whereas UPMB10 could not produce the same effect. This might be due to production of more 2^o roots rather than length in the Sp7 inoculated plants which increased the root mass. Application of fertilizer-N alone did not increase root number whereas inoculated plants together with 33% fertilizer-N had a highly synergistic effect on 1^o root emergence. Plant inoculated with UPMB10 increased 1^o root lengths but could not show the synergy with fertilizer-N. Thus, the effect may be due to bacterial interaction alone which is sufficient to increase 1^o root length. However, a highly synergistic effect was observed in root mass and volume.

The findings concerning increased root growth are supported by Morgenstern and Okon (1987) who found that inoculation with *Azospirillum* spp affected the root function by stimulating the appearance of lateral roots and enhancement in the number of adventitious roots in wheat and sorghum. Tien et al. (1979) also found that inoculation of PGPR in *Setaria* increased the number of lateral roots and root hairs. *Azospirillum* inoculation also increased the diameter and length of the lateral roots in maize seedlings (Hartmann et al., 1983).

The study revealed that PGPR inoculation could increase N, P and Ca concentrations in root, stem and leaves. Plants inoculated with UPMB10 especially those supplemented with 33% fertilizer-N resulted in an equivalent N concentration in stem and leaves as the 100% fertilizer-N treated plants. On the other hand, Sp7 could not produce a similar trend but showed higher N concentration in leaves only. The latter strain also showed higher N concentration in pseudostem under N_0 condition. The higher N uptake in fertilizer-N treated plant diluted the P concentration in leaf, similarly Ca concentration in pseudostem and leaf. The dilution capacity of inoculated plants with 33% fertilizer-N is similar as 100% fertilizer-N applied plant. Moreover, plants inoculated with UPMB10 with 33% fertilizer-N showed higher dilution of P and Ca which are indication of more N accumulation. The increased N concentration of stem and leaves may be due to N_2 fixation and enhanced uptake of NO_3^- from the nutrient solution. Similarly, Kapulnik et al. (1985) concluded that beneficial effects of host plants by PGPR inoculation might be due to increase uptake of combined-N from the soil. Inoculation of *Azospirillum* spp. increases

N content and consequently increases the plant growth in carrots (Govedarica et al., 1994). PGPR can contribute about 31% of the total N requirement in maize through BNF process (El Kommy et al., 1998). Similarly, Malik et al. (2000) found increased plant biomass by PGPR inoculation through N_2 fixation in wheat and rice.

The higher level of fertilizer-N decreased the ionic ratio of P/N (diluted the P concentration) in the tissue of leaf whereas increased in root. In leaf, N concentration of N-fertilized plant is higher which may have restricted the luxuriant accumulation of P. In N_0 condition, P was not diluted due to the absence of NO_3^- . The lower P concentration in leaf indicated an increased uptake of NO_3^- from the nutrient solution.

A higher Ca concentration was observed in roots of inoculated plants with 33% fertilizer-N application which might be due to minimum mobility of this element from root to shoot. Application of fertilizer-N (33 and 100%) resulted in low Ca concentration in pseudostem and leaves. This may be due to higher dry matter production which diluted the Ca concentration. However, total accumulation of Ca in inoculated plant was greater, an effect accelerated by the application of fertilizer-N which led to a good synergistic effect between PGPR strain and fertilizer-N. Lahav and Turner (1983) concluded that the uptake of Ca during the course of plant growth follows dry matter accumulation until bunch emergence. The higher Ca uptake by the inoculated plants in this experiment might not only be due to higher plant growth but also higher uptake capacity which is induced by bacterial interaction through the acceleration of proton efflux, which results in the acidification of the rhizosphere. It is one of the important mechanism in cation uptake by the roots which is the consequence of stimulation of root membrane ATP-ase activity. PGPR inoculation could increase this enzyme activity through bacteria-root interaction as both the strain successfully colonized banana roots. Higher activity of this enzyme resulted in higher Ca concentration in roots (Marschner, 1995). Similarly, Bashan (1990) concluded that *Azospirillum* could increase the proton efflux by stimulating ATP-ase activity in the root of wheat seedlings.

PGPR might improve the efficiency of absorbing applied mineral nutrients by helping the plant scavenge limiting nutrients. The enhanced root growth could absorb more nutrients and consequently more total nutrient accumulation. Inoculation process could increase root volume *vis-a-vis* more root surface area which assisted plants to absorb more nutrients. Similarly Bashan (1990) concluded that *A. brasilense* is capable of increasing the mineral nutrient content in maize plants. Enhancement in uptake of NO_3^- , NH_4^+ , HPO_4^{2-} , K^+ , Rb^+ and Fe^{2+} by *Azospirillum* was suggested as the cause for an increase in accumulation of minerals in stems and leaves (Kapulnik et al., 1985; Morgenstern and Okon, 1987; Sarig et al., 1988).

PGPR inoculation stimulated shoot growth namely plant height, leaf number, leaf area and shoot dry weight. These growth attributes increased with plant age and fertilizer-N application. Positive responses of inoculation on those growth attributes were clearly marked within 30 - 45 DAI. This increased shoot growth can be attributed to the observed improvement in root growth which consequently enhanced nutrient uptake. The better root development also helped to colonize more bacterial cells which increased N_2 fixation. The findings are supported by Saad et al. (1999) who found out that *Azospirillum* inoculation increased the grain yield and total N content in wheat and sweet potato. Photosynthetic activity was increased due to PGPR inoculation especially to plants supplied with 33% fertilizer-N resulted in an equivalent Pn as the 100% fertilizer-N applied control plants. This indicated that minimal fertilizer-N along with PGPR inoculation produced a synergistic effect on Pn activity. Several researchers have shown that photosynthetic capacity of N_2 -fixing plants were higher compared to non-fixing plants, since the former need more photosynthate to meet the demand of microorganisms for BNF process (Quilici and Medina, 1998). Similarly Amir et al. (2001) found higher photosynthetic capacity in oil palm seedlings inoculated with PGPR.

Conclusion

The results of this study concluded that PGPR inoculation increased the root growth, namely length, thickness, volume and root mass. Plant inoculated with UPMB10 and provided with 33% fertilizer-N could increase the primary root elongation while Sp7 increased 2^o root initiations. Root volume and root mass were also increased due to synergy with PGPR and minimal fertilizer-N. Plants inoculated with UPMB10 with 33% fertilizer-N produced an equivalent shoot growth as those with the 100% fertilizer-N treatment. PGPR inoculation could increase the nutrient concentration in different plant parts. Plants inoculated with both PGPR strains with 33% fertilizer-N increased the Ca concentration in root. Those inoculated with Sp7 and supplied with 33% fertilizer-N recorded higher Mg concentration in root, while the same with UPMB10 treatment showed higher concentration only in the pseudostem. The total accumulation of plant nutrients (N, P, K, Ca and Mg) were increased due to PGPR inoculation. The study suggested that PGPR strains Sp7 and UPMB10 could be used together with minimal fertilizer-N as bioenhancer for improved root and shoot growth and more nutrient accumulation of banana plantlets at seedling stage.

REFERENCES

Amir HG, Shamsuddin ZH, Halimi MS, Ramlan MF, Marziah M (2001).

- Effects of *Azospirillum* inoculation on N₂ fixation and growth of oil palm plantlets at nursery stage. J. Oil Palm Res. 13(1): 42-49.
- Bashan Y (1990). Short exposé to *A. brasilense* Cd inoculation enhanced proton efflux of intact wheat root. Can. J. Microbiol. 36: 419-425.
- Bashan Y (1998). Inoculants of plant growth promoting rhizobacteria for use in agriculture. Biotechnol. Adv. 16: 729-770.
- Bashan Y, Holguin G (1997). *Azospirillum*/plant relationship: environmental and physiological advances (1990-1996). Can. J. Microbiol. 43: 103-121.
- Bilal R, Rasul G, Arshad M, Malik KA (1993). Attachment, colonization and proliferation of *Azospirillum brasilense* and *Enterobacter* spp. on root surface of grasses. World J. Microbiol. Technol. 9: 63-69.
- Cocking EC (2000). Helping plants get more nitrogen from air. European Review, 8(2): 193-200.
- Coombs J, Hall DO, Long SP, Scurlock JMO (1985). Techniques in Bioproductivity and Photosynthesis (2nd edition). pp. 223-234. Pergamon International Library, Oxford.
- Dobereiner J (1997). Biological nitrogen fixation in the tropics: social and economic contributions. Soil Biol. Biochem. 29(5/6): 771-774.
- El-Kommy HMA, Moharram TMM, Safwat MSA (1998). Effects of *Azospirillum* inoculation on growth and N₂ fixation of maize subjected to different levels of FYM using ¹⁵N-dilution method. In *Nitrogen Fixation with Non-legumes*, ed: Malik KA pp. 49-59. Kluwer Academic Publishers, London.
- Govedarica M, Milosevic N, Jarak M (1994). Efficient application of associative nitrogen fixing bacteria in vegetable production. Savrem. Poljor, 42: 303-308.
- Hartmann A, Singh M, Klingmüller W (1983). Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. Can. J. Microbiol. 29: 916-923.
- Kapulnik Y (1991). Non-symbiotic nitrogen fixing microorganisms. In: Plant Roots: The Hidden Half ed: Waisel, Y., Eshel, A. and Kafkafi, U. pp. 703-716. Marcel Dekker, New York.
- Kapulnik Y, Gafny R, Okon Y (1985). Effect of *Azospirillum* spp. inoculation on root development and NO₃⁻ uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponics systems. Can. J. Bot. 63: 627-631.
- Ladha JK, De Bruijn FJ, Malik KA (1997). Assessing opportunities for nitrogen fixation in rice: a frontier project. Plant Soil, 194: 1-10.
- Lahav E, Turner DW (1983). Banana Nutrition. International Potash Institute Bulletin, No.7 pp. 17-22, Bern.
- Malik KA, Sajjad M, Mehnaz S, Rasul G (2000). The role of plant-associated beneficial bacteria in rice-wheat cropping system. Abstract 8th International Symposium on Nitrogen Fixation with Non-legumes p. 46 The University of Sydney, NSW, Australia.
- Marschner H (1995). Functions of Mineral Nutrients: Macronutrients In Mineral Nutrition of Higher Plants. p. 889. Academic Press, London.
- Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2005). High-yielding and quality banana production through plant growth promoting rhizobacterial inoculation. Fruits, 60: 179-185.
- Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2007). Associative nitrogen fixation by *Azospirillum* and *Bacillus* spp. in bananas. Infomusa, 16(1&2): 11-15.
- Morgenstern E, Okon Y (1987). Promotion of plant growth and NO₃⁻ and Rb⁺ uptake in *Sorghum bicolor* x *Sorghum sudanense* inoculated with *A. brasilense*-Cd. Arid Soil Res. Rehab. 1: 211-217.
- Okon Y, Albrecht SL, Burris RH (1977). Methods for growing *Spirillum lipoferum* and for counting it in pure culture and different solution in association with plants. Appl. Environ. Microbiol. 33: 85-88.
- Quilici A, Medina E (1998). Photosynthesis-nitrogen relationships in pioneer plants of disturbed tropical montane forest sites. Photosynthetica, 35(4): 525-534.
- Robinson JC (1996). Bananas and Plantains. CAB International, Wallingford, Oxon, UK. pp. 172-174.
- Saad MS, Shabuddin ASA, Yunus AG, Shamsuddin ZH (1999). Effects of *Azospirillum* inoculation on sweet potato grown on sandy tin-tailing soil. Commun. Soil Sci. Plant Analysis, 30(11&12): 1583-1592.
- Sarig S, Blum A, Okon Y (1988). Improvement of water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*. J. Agric. Sci. 110: p. 271.
- SAS Version 6.12. (1989). SAS/STAT. Guide to Personal Computers, SAS.
- Sheng XF (2005). Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biol. Biochem. 37: 1918-1922.
- Takebe M, Yoneyama T (1989). Measurement on leaf color scores and its implication to nitrogen nutrition of rice plants. Jpn. Agric. Res. Quart. (JARQ) 23(2): 86-93.
- Thomas RL, Sheard RW, Moyer JR (1967). Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. Agron. J. 59: 240-243.
- Tien TM, Diem HG, Gaskins MH, Hubbel DH (1979). Plant growth substances produced by *A. brasilense* and their effects on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. 37: 1016-1024.
- Yanni YG, Rizk RY, Corich V, Squatini A, Ninke K, Philip-Hollingsworth S, Orgambide G, De Bruijn FD, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997). Natural endophytic associations between *Rhizobium leguminosarum* bv. *trifoli* and rice roots and assessment of its potential to promote rice growth. Plant Soil, 194: 99-114.
- Zakaria W, Mahmud TMM, Razak AR (1997). Hydroponics banana: a potential tool in banana R&D. Agro-Search. Res. Bull. Fac. Agric. Malaysia, 4(2): 1-3.