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Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured *Musa* plantlets under nitrogen-free hydroponics condition

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Abstract
Banana requires large amounts of chemical fertilizers which are costly and can be hazardous to the environments when are used excessively. Biological N_2 fixation (BNF) technology can play a vital role as substitution to commercially available N-fertilizer in crop production and reduction of environmental problem to some extent. An experiment was conducted in the shade-house of University Putra Malaysia, Malaysia under hydroponics condition using nitrogen-free plant nutrient solution to evaluate the effect of PGPR (Plant Growth Promoting Rhizobacterial) inoculation on growth and N_2 fixation of tissue-cultured banana plantlets under nitrogen (N) free hydroponics condition. The experiment was a completely randomized design with six replicates. There were three treatments *viz*. T_1: (control; N_0 -PGPR), T_2: (N_0 + Sp7) and T_3: (N_0 + UPMB10). One tissue-cultured banana plantlet (ex-laboratory, about 10-11 cm height of three-leafed stage) cv. 'Berangan' (*Musa* spp. dessert type) was planted per pot (4.0 L). The results indicated that a remarkable increase in root growth, namely length (33-44%), volume (76-168%) and mass (137-141%) were recorded due to the PGPR inoculation, beside a higher shoot growth (123-202%) and N yield (94-144%). The inoculated plants showed higher formation of root hair which was visible within 7 days of inoculation. The growth attributes namely, leaf area, chlorophyll content, and consequently the total biomass were also increased due to PGPR inoculation. The overall growth performance of inoculated seedlings was higher in compare to un-inoculated control. Thus, it might be concluded that PGPR strains Sp7 and UPMB10 could be used as crop-enhancer and bio-fertilizer for vigor seedling and production of bananas.

Keywords: banana seedlings, growth, N_2 fixation, PGPR, biofertilizers, hydroponics, growth

Abbreviations: PGPR_ plant growth promoting rhizobacterial; DAI_ days after inoculation; UPMB10_The Bacillus sphaericus UPMB10; Sp7_*Azospirillum*

Introduction

Banana is always considered as a gross feeder and requires large amounts of nitrogen (N) and potassium (K) followed by phosphorus (P), calcium (Ca) and magnesium (Mg) to maintain high yields (Abdullah et al., 1999; Robinson, 1996). The physiological limitation in N-storage capacity is also a constraint for commercial cultivation of this crop. The deficiency symptoms quickly develop and extra N must be frequently applied even on fertile soil (Robinson, 1996). The excess use of chemical fertilizer is undesirable, because (1) production of chemical fertilizers is an costly process, (2) most of the energy is provided by the consumption of non-renewable fossil fuels, and (3) considerable pollution is caused through both the production and use of mineral N-fertilizers, and this is exacerbated by the relatively low efficiency of their uptake by the plants (Ladha and Reddy, 1995; Ladha et al., 1997).

Biofertilizers such as microbial inoculants, which can promote plant growth and productivity, have internationally been accepted as an alternative source of N-fertilizers. They are environmental friendly and can be used to ensure a sustainable banana production. In the biofertilizer technology, new systems are being developed to increase biological nitrogen fixation (BNF) for cereals and other non-legumes, by establishing N_2-fixing bacteria in plant roots (Cocking, 2000). Capability of Nitrogen fixation and promotion of plant

Table 1. Root growth of tissue-cultured banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown under hydroponics condition for 45 days

Treatments	Total no. of 1^0 roots (plant^{-1})	Total length of 1^0 roots (cm)	Root volume (cm^3 plant^{-1})	Root dry weight (g plant^{-1})
N_0-PGPR	6.0 a	236 b	6.2 c	0.27 b
N_0+Sp7	6.3 a	314 a	10.9 b	0.65 a
N_0+UPMB10	7.0 a	341 a	16.6 a	0.64 a

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

growth are important criteria for rhizobacteria to be used as an effective biofertilizer. Inoculation of associative and free-living N_2-fixing bacteria have been shown to produce beneficial effects on plant growth, thus they are termed plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980; Bashan and Holguin, 1998). Significant increases in crop yields have been reported following the application of PGPR under diverse field conditions (Bashan, 1998). They have been widely used to fix atmospheric N_2 on grasses and cereals (Dobereiner, 1997) and enhance uptake of nutrients (Bashan and Holguin, 1997). Application of PGPR strains, especially *Azospirillum* spp. was reported to fix N_2 in oil palm (*Elaeis guineensis*) and sweet potato (*Ipomoea batatas*). *Bacillus sphaericus* UPMB 10 was observed to produce beneficial effects on oil palm (Amir et al., 2001). In sweet potato, PGPR inoculation along with 33% N of the total fertilizer–N requirement produced a similar plant biomass and yield like the fully fertilized plants, consequently presenting a 67% saving of fertilizer-N (Saad et al., 1999). Halimi et al. (2000) found that PGPR can supplement the nutrient requirement of tomato on soilless culture media under protected environment. *Azospirillum* inoculation process has reportedly increased the N_2 fixation, mineral nutrient content (P, K, Ca and Mg) and growth of maize (Rai and Hunt, 1993).

The plant growth promoting effects of PGPR are mainly derived from morphological and physiological changes of the inoculated plant roots and their functions, and the enhancement of water and mineral uptake (Sarig et al., 1988). Root surface area and length were increased due to *Azospirillum* inoculation (Okon and Kapulnik, 1986). PGPR inoculation has promoted cell divisions of wheat's root (Levanony and Bashan, 1989) increased the diameter and length of lateral roots of maize (Hartmann et al., 1983), and enhanced the development of root hair and cortex (Kapulnik and Okon, 1983). In banana, improved root system is an important criterion for better anchorage, faster growth and higher fruit yield (Blomme, 2000).

PGPR inoculants have been shown to be effective as bioenhancer and biofertilizer for the growth of young oil palm seedlings (Amir et al., 2001). Although, there are many beneficial effects for PGPR to the host plant, their survival and effectiveness as N_2 fixer and bioenhancer associated to various factors. Only limited information is available about the PGPR inoculation process of banana for N_2 fixation, roots and shoots growth. Therefore, we conducted this experiment to evaluate the effect of PGPR inoculation on root stimulation, shoot growth and N incorporation in tissue-cultured banana plantlets, particularly for vigor seedling production.

Materials and methods

The experiment was conducted in the shade-house, University Putra Malaysia, Malaysia under hydroponics condition using N-free plant nutrient solution, with some modification from Cooper (1979), composition as N-150, P-45, K-250, Ca-127, Mg-36, S-48, Fe-9, Mn-1.5, B-0.225, Cu-0.075, Zn-0.075, Mo-0.200 mg L^{-1}. pH of the nutrient solution was adjusted to 6.0. The experiment was set up as a completely randomized design with six replications, one plantlet in each replicates. There were three treatments *viz*. T_1: (control; N_0 -PGPR), T_2: (N_0 + Sp7) and T_3: (N_0 + UPMB10).

One tissue-cultured banana plantlet (ex-laboratory, about 10-11 cm height of three-leafed stage) cv. 'Berangan' (*Musa* spp. dessert type) was planted per pot (4.0 L). Before transplanting, the plantlets were acclimatized with sterile water for 3 days and the existing roots around the corm of the plantlets were removed gently with a sterile blade.

A 40 mL broth cultures (OD_{600}, 1.0) of the PGPR was applied to each respective pot prior to transplanting. The same volume of sterile media (without inocula) was applied to the control pots. The pots were wrapped with aluminum foil to prevent light effect from inhibiting root growth and aerated with an air pump for six hours at six-hourly intervals to ensure an uninhibited root respiration and bacterial growth.

Leaf chlorophyll content of youngest fully expanded leaf (third leaf from the shoot) of each plant was indirectly measured by a chlorophyll meter (SPAD 502, MINOLTA^{TM} Camera Ltd. Japan) at harvest, 45 DAI (days after inoculation). Measurements of morphological parameters, namely, plant height, leaf number, leaf area, number of roots, total primary root length, and fresh weight of roots and shoot were also taken. The separated plant parts oven dried at 71^0C for 48 h and dry matter were weighted. Harvested plants were separated into roots, corm, pseudo-stem, 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 well and stored in plastic vials. The ground samples were digested by micro-Kjeldahl method (Thomas et al., 1967). The digested samples were analysed for total N and were determined by Auto-analyzer (Technicon II, Technicon Ltd.).

Table 2. Shoot growth of hydroponically-grown tissue-cultured banana plantlets inoculated with PGPR strains Sp7 and UPMB10 for 45 days

Treatments	Plant height (cm)	Leaf no. (plant^{-1})	Leaf area (cm^2 plant^{-1})	Leaf chlorophyll content (mg cm^{-2})	Total dry matter (g plant^{-1})
N_0-PGPR	19.6 b	5.7 a	99 b	0.57 b	0.70 b
N_0+Sp7	27.9 a	7.3 a	226 a	0.76 a	1.61 a
N_0+UPMB10	29.4 a	6.3 a	232 a	0.71 a	1.94 a

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

Statistical Analyses

The collected data were analyzed statistically using the Statistical Analysis System (SAS, version 9.0, 2004). 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.

Results

Root Growth

PGPR inoculation stimulated the root hair formation (Fig 1). The plants inoculated with PGPR, initiated more root hairs compare to control treatments. Inoculation greatly increased the production of primary, secondary and tertiary roots (Fig 2) and total primary root length (33-44%) (Table1). However, PGPR inoculation did not increase the total number of primary roots. Root volume was also significantly increased and effect of UPMB10 produced a greater volume than Sp7, an increase of 52% (Table 1). PGPR inoculation also increased root dry weight (137-141%) but with no significant differences between the two strains used.

Shoot Growth

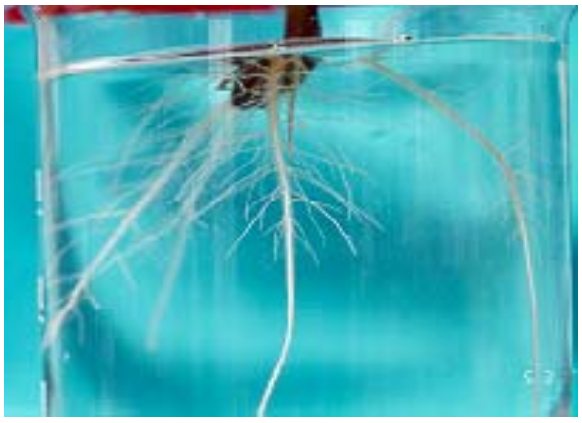
The effect of PGPR inoculation resulted in more shoot growth compared to un-inoculated control plants. Plant height (42-50%), leaf area (128-134%) as well as total dry matter (TDM) content were significantly increased in inoculated plants (Table 2). The increase in TDM due to inoculation with Sp7 and UPMB10 were 129% and 176%, respectively. Plants inoculated with Sp7 and UPMB10 produced more leaf area (128 to 134 % increases) and higher total chlorophyll content (25 to 33 % increases) compared to control treatment but produced no significant effect due to the different inoculum strains. The number of leaves was not influenced by PGPR inoculation.

Nitrogen Accumulation

PGPR inoculation greatly increased the N concentrations in roots and leaves (Table 3) but not in the stem. The highest N concentration (0.84-1.10 %) was recorded in the leaf followed by root (0.65-0.91%) and stem (0.53-0.7 4%). The total N accumulation was heavily influenced by PGPR inoculation where plants

N_0-PGPR

N_0+Sp7



N_0+UPMB10

Fig 1. Roots of tissue-cultured banana plantlets 7 days after inoculation with different treatments of PGPR strains Sp7 and UPMB10 grown under hydroponics condition

Table 3. Concentration and total N yield of hydroponically grown tissue-cultured banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days

Treatments	Root	Stem	Leaf	Total N accumulation
	Concentration (% N)			(mg plant^{-1})
N_0-PGPR	0.65 b	0.53 a	0.84 b	5.96 b
N_0+Sp7	0.83 a	0.60 a	1.11 a	11.59 a
N_0+UPMB10	0.91 a	0.74 a	1.10 a	14.55 a

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

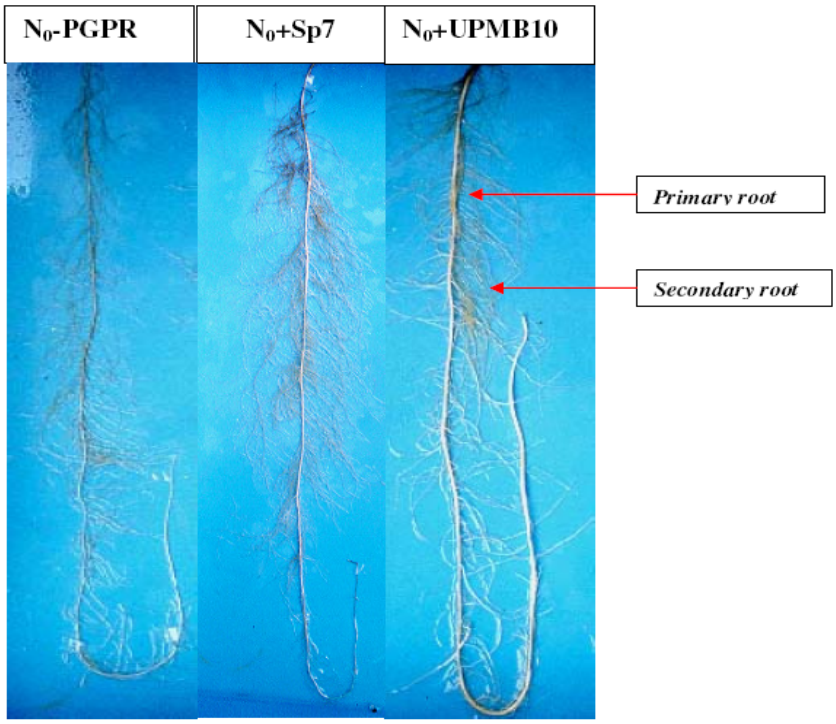


Fig 2. Primary, secondary and tertiary roots of tissue-cultured banana plantlets inoculated with PGPR strains Sp7 and UPMB10 at 45 days after inoculation grown under hydroponics condition

inoculated with Sp7 and UPMB10 resulted in 94 and 144% more N yield, respectively, compared to control (Table 3).

Discussion

PGPR inoculation substantially increased root growth and development of tissue-cultured banana plantlets, where the initial response was observed during root hair formation. The initiation of more root hairs might be due to the results of bacterial interactions with the root surface of the host plant. This interaction resulted in more root hair formation. Similar results were found in different cereal crops and tomato seedlings where PGPR inoculation enhanced the appearance of root hairs (Okon, 1985; Hadas and Okon, 1987). The PGPR inoculation in wheat has been shown to result in enhanced cell division in the root tips (Levanony and Bashan, 1989) while in maize it increased diameter and length of lateral roots (Hartmann et al., 1983), and promoted root hair development and branching which caused alteration in arrangement of root cortex cells (Kapulnik and Okon, 1983). Similarly, inoculation also increased the root growth of tomato seedlings (Hadas and Okon, 1987). Correspondingly, Lin et al. (1983) and Kapulnik et al. (1985) found changes in the external layers of root cortex in maize and wheat seedlings. *Azospirillum* (Sp7) has the potential to synthesize plant hormone which can replace indole acetic acid (IAA) to stimulate root growth in vegetable soybean (Molla et al., 2001). Dobbelarere et al. (1999) suggested that secretions of plant growth promoting substances such as auxins, gibberellins and cytokinins by the bacteria seem to be responsible for these effects. Sarig et al. (1988) further suggested that growth promoting effects of PGPR inoculation are mainly derived from morphological and physiological changes in inoculated sorghum roots and enhancement in water and plant nutrient uptake.

In our study, PGPR inoculation, increased (28–40%) the N concentration in roots and leaves, which conseq-uently resulted in greater accumulation of N as

evidenced by more (129-176%) total dry matter production. This increase in N concentration consequently increased N accumulation which certainly due to the N_2 fixation by the PGPR, since no fertilizer-N was applied to the treatments. The strains Sp7 and UPMB10 efficiently fixed a substantial amount of N_2 with the association of banana roots. Similarly, Malik et al. (1997) found that *Azospirillum* and other rhizobacterial inoculation could contribute about 70% of the total N requirement of the host plant. In addition, PGPR inoculation on oil palm plantlets grown under *in vitro* has been shown to contribute up to 89% of the total N requirement (Shamsuddin, 1994). In bananas, inoculation can contribute a considerable amount of N via BNF system. Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with PGPR. This was mainly because of an increase in number of nitrogenous compound and nitrogenase activity in inoculated plants (Bashan and Holguin, 1997).

The shoot growth, namely plant height (42-50%), leaf area (128–134%), leaf chlorophyll content (25–33%) and total dry matter (TDM) (129–176%) increased significantly due to PGPR inoculation. This increased growth of inoculated plants might be due to the higher N accumulation by bacterial N_2 fixation and better root growth, which promoted the greater uptake of water and nutrients. The higher N incorporation has apparently increased the formation of protein and enzyme for better physiological activities. The higher N also contributed to the formation of chlorophyll, which consequently, increased the photosynthetic activity. PGPR inoculation increased the physiological properties of the host plants namely, photosynthetic rate (Mia et al., 2005). Photosynthetic capacity of N_2-fixing bacteria was higher, compare to N user, since the former needed more photosynthate to meet the higher demand by diazotrophs during the N_2-fixing process (Quilici and Medina, 1998). Similarly, *Azospirillum* inoculation has been shown to increase plant growth in maize through N_2-fixation and enhanced uptake of mineral nutrient P, K, Ca and Mg (Rai and Hunt, 1993; Shengh. 2005). The improved plant growth due to PGPR inoculation on different fruit crops has also been reported by several investigators (Bashan and Holguin, 1997; Ghai and Thomas, 1989; George, 1990). PGPR inoculated sorghum plants has been shown to improve leaf water potential under field condition (Fallik et al., 1994). Strong sink strength of inoculated roots has been shown to induce an increase in source leaf photosynthesis of soybean plants (De Veau et al., 1990).

Conclusions

The results of our experiment indicated that PGPR inoculation significantly increased the root properties (length, volume, mass), shoot growth, the plant height (42–50%), leaf area (128-134%), chlorophyll content (25-33%) and total dry matter of banana plantlets grown under hydroponics condition. A substantial increase in chlorophyll content and N yield (144%) was also observed in banana plantlets inoculated with PGPR. This study suggested that PGPR strains, Sp7 and UPMB10, can be used as crop-enhancer and bio-fertilizer to enhance better seedling production of bananas.

References

Abdullah MY, Hassan NM, Mahmood Z, Talib Z (1999) Trend in foliar nutrient concentrations and contents and its implication on leaf area index development and yield in banana cultivar 'berangan'. *Proc.1st National Banana Seminar* eds: Zakaria et al. pp 95-105. Genting, Malaysia

Amir HG, Shamsuddin ZH, Halimi MS, Ramlan MF and Marziah M (2001) Effects of *Azospirillum* inoculation on N_2 fixation and growth of oil palm plantlets at nursery stage. J Oil Palm Res 13: (1) 42-49

Bashan Y (1998) Inoculants of plant growth promoting rhizobacteria for use in agriculture. Biotechnol Adv 16:729-770

Bashan Y and Holguin G (1997) *Azospireillum*/plant relationship: environmental and physiological advances (1990-1996). Can J Microbiol 43:103-121

Bashan Y, Holguin G (1998) Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. Soil Biol Biochem 30: (8, 9): 1225-1228.

Blomme G (2000) Interdependence of root and shoot development in banana (*Musa* spp.) under field conditions and the influence of different biophysical factors on this relationship. Ph.D. Thesis. Catholic University, Leuven, Belgium

Cocking EC (2000) Helping plants get more nitrogen from air. Euro Review 8(2):193-200

Cooper A (1979) The ABC of NFT. Grower Books. London, 181 pp

De Veau EJ, Robinson JM, Warmbrodt RD, van Berkum P (1990) Photosynthesis and photosynthate partitioning in N_2-fixing soybeans. Plant Physiol 94: 259-267

Dobereiner J (1997) Biological nitrogen fixation in the tropics: social and economic contributions. Soil Biol Biochem 29 (5/6): 771-774

Dobbelarere S, Cronnenborgh A, Thys A, Vande Broek A, Vanderleyden J (1999) Phytostimulatory effects of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. Plant Soil 212: 155-164

Fallik E, Sarig S, Okon Y (1994) Morphology and physiology of plant roots associated with *Azospirillum*. In: *Azospirillum* / *Plant Associations*. ed: Okon, Y. pp.77-85, CRC Press, Florida

George M (1990) *Azospirillum* for nitrogen fixation in coconut. Philippine J Coconut Studies 15 (2):1-3

Ghai SK, Thomas GV (1989) Occurrence of *Azospirillum* spp. in coconut-based farming systems. Plant Soil 114:235-241

Hadas R, Okon Y (1987) Effect of *A. brasilense* on root morphology and respiration in tomato seedlings. Biol Fertil Soils 5:241-247

Halimi MS, Raffidah Z, Ismail, MR (2000) Growth responses of tomatoes (*Lycopersicon esculentum* Mill) under protected environment to rhizobacterial inoculation. Trans Malaysian Soc Plant Physiol 9:235-239

Hartmann A, Singh M, Klingmuller W (1983) Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. *Can J Microbiol* 29: 916-923

Kapulnik Y, Okon Y (1983) Benefits of *Azospirillum* inoculation on wheat: effects on root development, mineral uptake, nitrogen fixation and crop yield. *In: Azospirillum* II. ed.: Klingmuller, W. pp 163-170. Brikhauser Verlag. Stuttgart.

Kapulnik Y, Okon Y, Henis Y (1985) Changes in root morphology of wheat caused by *Azospirillum* inoculation. Can J Microbiol 31:881-887

Kloepper JW, Leong J, Teintze M, Schorth MN (1980) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286:885-886

Ladha JK, de Bruijin FJ, Malik KA (1997) Assessing opportunities for nitrogen fixation in rice: a frontier project. Plant Soil 194: 1-10

Ladha JK, Reddy PM (1995) Extension of nitrogen fixation to rice: necessity and possibilities . GeoJournal 35:363-372

Lahav E, Turner DW (1983) *Banana Nutrition*. International Potash Institute Bulletin No.7 pp17-22. Bern

Levanony H, Bashan Y (1989) Enhancement of cell division in wheat root tips and growth of root elongation zone induced by *Azospirillum brasilense* Cd. Can J Bot 67:2213-2216

Lin W, Okon Y, Hardy RW (1983) Enhanced mineral uptake by *Zea mays* and *Sorghum bicolor* roots inoculated with *Azospirillum brasilense.* Appl Environ Microbiol 45:1775-1779

Malik KA, Rakhshanda B, Mehnaz S, Rasul G, Mirza MS, Ali S (1997) Association of nitrogen-fixing plant-growth promoting rhizobacteria (PGPR) with kallar grass and rice. Plant Soil 194:37-44

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2005) High-yielding and quality banana production through plant growth promoting rhizobacterial inoculation. Fruits 60: p.179-185

Molla AH, Shamsuddin ZH, Halimi MS (2001) Mechanism of root growth and promotion of nodulation in vegetable soybean by *Azospirillum brasilense.* Commun.Soil Sci Plant Anal 32: (13&14) 2177-2187.

Okon Y (1985) *Azospirillum* as a potential inoculant for agriculture. Trends Biotechnol 3:223-228

Okon Y and Kapulnik Y (1986) Development and function of *Azospirillum* inoculated roots. Plant Soil 90: 3-16

Quilici A, Medina E (1998) Photosynthesis-nitrogen relationships in pioneer plants of disturbed tropical montane forest sites. *Photosynthetica* 35(4): 525-534

Rai R, Hunt P G (1993) Inoculation of maize varieties with salt tolerant mutants of *Azospirillum brasilense* and VAM fungi in saline calcareous soil. Microbiol. Release 1:243-251

Robinson JC (1996) Bananas and Plantains. CAB International, Wallinford, Oxon, UK. pp172-174

SAS Version 9.0 (2004) SAS/STAT Guide to Personal Computers. SAS Institute Inc., Cary, North Carolina

Saad MS, Shabuddin ASA, Yunus AG, Shamsuddin Z H (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 110:271

Shamsuddin ZH (1994) Application of microbial inoculant for crop production. In FFTC/RDA International Seminar on ‘ The Use of Microbial and Organic Fertilizers in Agricultural Production’ pp 1-13. Suweon Korea

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

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