High-yielding and quality banana production through plant growth-promoting rhizobacterial inoculation

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Received 9 January 2004
Accepted 14 June 2005

Fruits, 2005, vol. 60, p. 179–185

DOI: 10.1051/fruits:2005024

***RESUMEN ESPAÑOL**, p. 185*

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Abstract — Introduction. Rhizobacterial inoculation in low fertilizer-N conditions, *viz.*, 33% fertilizer-N of the total N requirement, could produce similar plant growth to the 100% N-fertilization of banana plantlets grown under hydroponic conditions. Thus, we tested PGPR inoculation in combination with fertilizer-N application to study the role played by strains of rhizobacteria in nutrient accumulation, nitrogen fixation and, consequently, improvement in yield and fruit quality of bananas. **Materials and methods**. Two PGPR strains were used in the experiments, namely, Sp_7 (*Azospirillum brasilense*) and $UPMB_{10}$ (*Bacillus sphaericus*). The design of the experiment was completely randomized with three replications. Eight treatments were applied: control without fertilizer-N application ($N_{0\%}$) and without PGPR; $N_{0\%}$ + Sp_7; $N_{0\%}$ + $UPMB_{10}$; $N_{33\%}$ without PGPR; $N_{33\%}$ + Sp_7; $N_{33\%}$ + $UPMB_{10}$; $N_{100\%}$ without PGPR and $N_{100\%}$ + $UPMB_{10}$. One tissue-cultured banana (cv. 'Berangan') plantlet was planted in a plastic pot (4 L) for 45 days and thereafter transferred to a larger polyethylene tank (1000 L) until maturity. A 100-mL broth culture of Sp_7 or $UPMB_{10}$ was added to the respective tanks after the transplanting process and repeat inoculations were performed monthly. The fruits were harvested at the maturity stage after 80–90 days of flowering. After ripening, yield and fruit quality parameters were assessed. **Results**. Inoculation with 33% fertilizer-N increased the total nutrient accumulation (N, P, K, Ca and Mg). PGPR inoculation along with 33% fertilizer-N significantly increased the bunch yield and fruit physical attributes, namely, finger weight, length and diameter, and [pulp / peel] ratio, besides inducing early flowering by 3 weeks. **Conclusion**. The results suggested that PGPR strains Sp_7 and $UPMB_{10}$ could be used as bioenhancer and biofertilizer for early, high-yielding and improved banana fruit production in 33% fertilizer-N conditions.

Malaysia / *Musa* / plant nutrition / bacteria / nitrogen fertilizers / growth / yields / fruit quality

Production de bananes de qualité, avec de forts rendements, par inoculation avec des rhizobactéries favorisant la croissance des plants (PGPR).

Résumé — Introduction. L'inoculation de rhizobactéries en cas de faibles apports d'azote, comme avec une fertilisation à 33 % de N par rapport aux besoins azotés totaux, pourrait permettre d'obtenir une croissance des bananiers équivalente à celle obtenue par fertilisation avec 100 % de N, comme cela a été constaté pour des plantules de bananes développées sous culture hydroponique. Nous avons donc étudié l'inoculation de plants avec des PGPR en combinaison avec une application d'engrais azoté ; le rôle joué par des souches de rhizobactéries sur l'accumulation des éléments nutritifs, la fixation de l'azote et, par conséquent, l'amélioration du rendement et de la qualité des bananes ont été évalués. **Matériel et méthodes**. Deux souches de PGPR ont été utilisées, à savoir Sp_7 (*Azospirillum brasilense*) et $UPMB_{10}$ (*Bacillus sphaericus*). Le dispositif expérimental a été complètement randomisé avec trois répétitions. Huit traitements ont été expérimentés : témoin sans application d'engrais azoté ($N_{0\%}$) et sans PGPR ; $N_{0\%}$ + Sp_7 ; $N_{0\%}$ + $UPMB_{10}$; $N_{33\%}$ sans PGPR ; $N_{33\%}$ + Sp_7 ; $N_{33\%}$ + $UPMB_{10}$; $N_{100\%}$ sans PGPR et $N_{100\%}$ + $UPMB_{10}$. Un seul plant issu de culture *in vitro* de bananier (cv. Berangan) a été planté par pot de plastique (4 L) pendant 45 jours, puis transféré dans un plus grand récipient de polyéthylène (1000 L) jusqu'à sa maturité. Une suspension de 100 mL de Sp_7 ou d'$UPMB_{10}$ a été appliquée dans chaque récipient après la transplantation du plant et les inoculations ont été répétées chaque mois. Les fruits ont été récoltés à maturité après 80 à 90 jours de floraison. Après maturation, le rendement et la qualité du fruit ont été évalués. **Résultats**. L'apport d'un engrais azoté à 33 % a augmenté la teneur totale en éléments minéraux (N, P, K, CA et Mg). L'inoculation de PGPR avec de l'engrais à 33 % de N a significativement augmenté le rendement en régimes et les caractères physiques du fruit, notamment le poids, la longueur et le diamètre des doigts, ainsi que le rapport [pulpe / peau] ; par ailleurs, ce traitement a induit une floraison avancée de 3 semaines. **Conclusion**. Les résultats suggèrent que les souches de PGPR – Sp_7 et $UPMB_{10}$ – pourraient être employées comme biostimulateur et bioengrais pour une production améliorée de bananes précoces et à hauts rendements, en présence d'engrais à 33 % de N.

Malaisie / *Musa* / nutrition des plantes / bacteria / engrais azoté / croissance / rendement / qualité du fruit

1. Introduction

Recently, biofertilizers are gaining prominence because they play an important role in the maintenance of soil fertility. Rhizobacteria are able to promote growth and yield of agriculturally important crops grown under different soil and climatic conditions [1]. Rhizobacteria could effectively form colonies on the root surface of banana plantlets and their inoculation significantly increased the root growth and nutrient accumulation, which consequently enhanced the shoot growth [2]. Rhizobacterial inoculation in low fertilizer-N conditions, *viz*. 33% fertilizer-N of the total N requirement, could produce similar plant growth to the 100% N-fertilization of banana plantlets grown under hydroponic conditions in smaller pots for 45 days where the beneficial effects are exerted on plant growth [3]. Wange and Patil [4] found increased plant growth of banana by Plant Growth-Promoting Rhizobacteria (PGPR) inoculation together with fertilizer-N, but could not show root development and yield improvement. Application of PGPR can save up to 67% of the total requirement of N in sweet potato [5] and 48% in oil palm seedlings [6]. Thus, we tested PGPR inoculation in combination with fertilizer-N application to study the role played by strains of rhizobacteria in nutrient accumulation, nitrogen fixation and, consequently, improvement in yield and fruit quality of bananas.

2. Materials and methods

Our experiment was conducted in an experimental field of the University Putra Malaysia Farm under hydroponic conditions. Plant nutrient solution was used according to the modification of Clarkson *et al*. [7]. Two PGPR strains were used in the experiments, namely, Sp_7 (*Azospirillum brasilense Azospirillum*, a Gram-negative diazotropohic bacteria, which fixes atmospheric nitrogen as free-living or in association with roots of grasses and cereals) and $UPMB_{10}$ (*Bacillus sphaericus,* anaerobic, rod-shaped, endospore-forming, Gram-positive bacteria with a nif-gene). Both strains were obtained from cultures maintained at the Soil Microbiology Laboratory, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia. The strain Sp_7 was originally provided by Dr. J. Dobereiner, EMBRAPA, Brazil, while strain $UPMB_{10}$ was isolated from oil palm roots in Malaysia.

The hydroponic assembly was built in an open field and the plants were supported by wooden frames. The design of the experiment was completely randomized with three replications. The following eight treatments were imposed for the experiment: T_1, control, $N_{0\%}$ – PGPR; T_2, $N_{0\%}$ + Sp_7; T_3, $N_{0\%}$ + $UPMB_{10}$; T_4, control + $N_{33\%}$ – PGPR; T_5, + $N_{33\%}$ + Sp_7; T_6, + $N_{33\%}$ + $UPMB_{10}$; T_7, control + $N_{100\%}$ – PGPR and T_8, + $N_{100\%}$ + $UPMB_{10}$. One tissue-cultured banana plantlet cv. 'Berangan' was planted in a plastic pot (4 L) for 45 days and thereafter transferred to a larger polyethylene tank (1000 L) until maturity.

A 100-mL broth culture (OD_{600} 1.0) of PGPR strains Sp_7 or $UPMB_{10}$ was added to the respective tanks after the transplanting process and repeated inoculations were performed monthly. The tanks were aerated with air pumps at 6-hourly intervals for 6 h to ensure an uninhibited root respiration and bacterial growth. Nutrient solution in every tank was monitored on alternate days for pH and electrical conductivity (EC). Bunches were covered by perforated blue polyethylene bags after completion of flowering to protect the fruits from leaf scarring, dust and light according to Robinson [8]. The fruits were harvested at the maturity stage after 80–90 days of flowering. At harvest, bunch weight was recorded and fruits were treated with calcium carbide (1 $g \cdot kg^{-1}$ green banana) for 18–20 h to hasten ripening.

Finger length was measured by meter scale and finger diameter by slide caliper according to Stover and Simmonds [9]. Finger weight and the [pulp / peel] ratio were computed according to Azizah *et al*. [10]. Only the 2nd and 3rd hands from each bunch were selected for physico-chemical properties since they showed no significant difference in these properties [11]. Sugar content (% Brix), titratable acidity and ascorbic acid in the fruits were determined according to Ranganna [12]. Harvested plants were separated into root, corm, pseudostem and

Table I.
Total accumulation of N, P, K, Ca and Mg (g dry matter per plant) in adult banana plants grown under hydroponic conditions and inoculated with PGPR strains Sp_7 and $UPMB_{10}$ at different levels of fertilizer-N during their growth (n = 3).

Treatments	N	P	K	Ca	Mg
$N_{0\%}$ – PGPR	2.6 c	3.5 c	20 e	1.4 c	1.1 d
$N_{0\%}$ + Sp_7	2.9 c	4.7 c	25 e	1.8 c	1.7 d
$N_{0\%}$ + $UPMB_{10}$	2.7 c	4.0 c	21 e	1.5 c	1.0 d
$N_{33\%}$ – PGPR	76.6 b	36.9 b	300 cd	39.5 b	8.9 c
$N_{33\%}$ + Sp_7	101.2 ab	68.1 a	435 a	64.0 a	16.0 a
$N_{33\%}$ + $UPMB_{10}$	111.7 a	41.6 b	352 b	47.2 ab	11.7 b
$N_{100\%}$ – PGPR	122.0 a	30.9 b	264 d	39.1 b	11.0 bc
$N_{100\%}$ + $UPMB_{10}$	124.6 a	39.9 b	330 bc	43.2 ab	11.1 bc

Means with the same letter in a column do not differ significantly at the 0.05 level by DMRT.

leaves for dry matter computation. From each fresh sample, representative samples were oven-dried at 71 °C for 72 h and weighed. The representative samples were processed and digested for the estimation of N, P, K, Ca and Mg concentrations. The N and P were determined by Autoanalyzer (Technicon II, Technicon Ltd.), while the remaining elements were analyzed by Atomic Absorption Spectrophotometer (Perkin-Elmer, 5100 pc). The collected data were analyzed statistically using the Statistical Analysis System [13].

3. Results

3.1. Plant nutrient uptake

Compared with the control without PGPR, total N accumulation was significantly higher (45%) in plants inoculated by $UPMB_{10}$ and supplied with 33% fertilizer-N *(table I)*. However, plants inoculated by Sp_7 together with 33% fertilizer-N did not increase accumulation of N. Similarly, $UPMB_{10}$ together with 100% fertilizer-N also did not show any increment of N accumulation compared with the control ($N_{100\%}$ – PGPR) *(table I)*.

Application of fertilizer-N and the inoculation process had a positive effect on total accumulation of P *(table I)*. Plants using fertilizer-N, inoculated and uninoculated, accumulated more P up to 33% fertilizer-N; there were no significant differences between the $N_{33\%}$ and $N_{100\%}$ treatments. Plants inoculated with Sp_7 along with 33% N fertilization showed the highest accumulation of P, whereas $UPMB_{10}$ did not show any increment of P uptake. Plants inoculated by Sp_7 together with 33% fertilizer-N showed the highest K accumulation followed by $UPMB_{10}$ with 33% fertilizer-N *(table I)*.

There were no significant differences of total accumulation of Ca in inoculated and uninoculated plants in $N_{0\%}$ conditions *(table I)*. However, the inoculation process, especially with Sp_7 and provided with 33% fertilizer-N significantly increased (62%) the total Ca accumulation. Inoculated plants using 100% fertilizer-N did not show any positive effect on Ca accumulation when compared with the control ($N_{100\%}$ – PGPR).

PGPR inoculation along with 33% N-fertilization significantly stimulated the total Mg accumulation compared with the control ($N_{33\%}$ – PGPR) *(table I)*. Plants inoculated by Sp_7 and $UPMB_{10}$ together with 33% fertilizer-N increased Mg accumulation compared with the control by 80% and 31%, respectively. However, plants inoculated by PGPR along with 100% N did not show any differences.

3.2. Days to flowering

Nitrogen and inoculation showed a remarkable influence on the flowering time of

Table II.
Yield, yield contributing characters and fruit quality of bananas inoculated with PGPR strains Sp_7 and $UPMB_{10}$ grown under hydroponic conditions (n = 3).

Treatments	Days to flowering (day)	Bunch weight (kg·plant^{-1})	No. of hands per bunch	No. of fingers per hand	Finger weight (g)	Finger length (cm)	Finger diameter (cm)	[Pulp /peel] ratio	Sugar content (% Brix)	Titrable acidity (TAA)	Ascorbic acid (mg·100 g^{-1} fresh weight)
$N_{33\%}$ – PGPR	265 a	11.0 b	8.0 a	14.2 a	79 b	11.3 b	3.23 b	2.12 c	16.9 a	1.10 ab	16.6 a
$N_{33\%}$ + Sp_7	247 b	16.6 a	8.3 a	15.5 a	128 a	13.8 a	3.74 ab	2.75 ab	18.6 a	1.31 ab	11.1 b
$N_{33\%}$ + $UPMB_{10}$	244 b	14.8 ab	6.3 b	15.3 a	130 a	14.0 a	3.89 a	2.90 a	19.1 a	1.05 b	11.5 b
$N_{100\%}$ – PGPR	202 c	13.9 ab	7.0 ab	12.3 a	117 a	13.7 a	3.81ab	2.34 bc	18.8 a	1.54 ab	10.9 b
$N_{100\%}$ + $UPMB_{10}$	197 c	15.7 a	8.7 a	13.3 a	119 a	13.2 ab	3.66 ab	2.33 bc	19.4 a	1.77 a	13.4 ab

Means with the same letter in a column do not differ significantly at the 0.05 significant level by DMRT.

bananas *(table II)*. Uninoculated plants provided with 100% fertilizer-N showed at least 2 months early flowering compared with uninoculated plants with 33% fertilizer-N. Similarly, inoculated plants with $UPMB_{10}$ provided with 100% fertilizer-N showed 47 days earliness compared with inoculated plants with 33% fertilizer-N. Inoculation with Sp_7 and $UPMB_{10}$ together with 33% fertilizer-N showed a positive effect on early flowering compared with control ($N_{33\%}$ – PGPR) by 18 and 21 days, respectively. There was no effect of PGPR inoculation with 100% fertilizer-N, which showed only 5 days' earliness compared with the control.

3.3. Fruit yield

PGPR inoculation significantly increased the fruit yield as expressed by bunch weight *(table II)*. Plants inoculated by Sp_7 with 33% fertilizer-N produced significantly the highest bunch yield (16.6 kg) followed by $UPMB_{10}$ with 100% fertilizer-N (15.7 kg). Control plants with 33% fertilizer-N produced the lowest bunch yield (11.0 kg).

3.4. Number of hands

Number of hands was not influenced by the inoculation process and fertilizer-N application *(table II)*. Plants inoculated with $UPMB_{10}$ produced a lower number of hands. The strain Sp_7 also did not show any positive effects on number of hands. However, the number of hands per bunch ranged from 6.3 to 8.7.

3.5. Finger weight

PGPR inoculation greatly stimulated the finger weight *(table II)*. Plants inoculated with Sp_7 and $UPMB_{10}$ and provided with 33% fertilizer-N produced bigger fingers compared with the control ($N_{33\%}$ – PGPR) by 62% and 65%, respectively. However, plants inoculated together with 100% fertilizer-N did not show any increase in finger weight compared with the control ($N_{100\%}$ – PGPR). In the control, finger size increased with the increase in fertilizer-N, while inoculated plants with 33% fertilizer-N showed a similar finger size to the 100% fertilizer-N.

3.6. Finger length and diameter

PGPR inoculation significantly increased the finger length *(table II)*. The longest finger was observed in the plants inoculated by $UPMB_{10}$ with 33% fertilizer-N. In uninoculated plants, finger length increased with fertilizer-N, while inoculated plants showed the longest with 33% fertilizer-N. Similarly, plants inoculated by $UPMB_{10}$ with 33% fertilizer-N produced significantly broader

bananas (larger finger diameter) compared with the control ($N_{33\%}$ – PGPR).

3.7. [Pulp / peel] ratio

[Pulp/peel] ratio increased with fruit maturity and PGPR inoculation significantly stimulated this ratio in bananas *(table II)*. Inoculated plants, especially with $UPMB_{10}$ and provided with 33% fertilizer-N, produced fruit which contained a higher (37% more) [pulp / peel] ratio. Plants using 100% N, with or without inoculation, did not show any differences in the [pulp / peel] ratio.

3.8. Sugar content (% Brix)

Banana fruit sugar content as measured by % Brix ranged from 16.9% to 19.4% (*table II*). There was no effect of PGPR inoculation on % Brix although $UPMB_{10}$ with 33% N-fertilization resulted in 13% more Brix compared with control ($N_{33\%}$). In general, $UPMB_{10}$ showed slightly higher Brix content compared with the others.

3.9. Titratable acidity

Titratable acidity equivalent to malic acid is the most important organic acid in bananas. PGPR inoculation did not influence the malic acid content of bananas *(table II)*. However, the values ranged from 1.05 to 1.77, and plants inoculated by $UPMB_{10}$ and provided with 100% N-fertilization showed the highest values.

3.10. Ascorbic acid content

Ascorbic acid values increase on ripening and the highest ascorbic acid contents are obtained in fully ripened bananas. Inoculation did not influence the ascorbic acid content, whereas uninoculated control showed the highest ascorbic acid content ($N_{33\%}$ – PGPR) *(table II)*.

4. Discussion

Total accumulation of N, P, K, Ca and Mg was heavily influenced by PGPR inoculation. Plants inoculated by $UPMB_{10}$ with 33% fertilizer-N showed a synergistic effect on N accumulation, but not with Sp_7. This might be due to the N_2 fixation capacity of the strain together with the fertilizer-N. Fertilizer-N has the most prominent influence on biological N_2 fixation, which depends on the level of supply. A moderate level of fertilizer-N increases the fixation rate while it declines at high levels. The higher total accumulation of P, K, Ca and Mg nutrients might be due to enhanced root proliferation as well as higher sink demand of nutrients to different plant parts as inoculation stimulated the metabolic activity of the host plant. Higher sink strength of plant nutrients is possibly due to improved physiological and morphological attributes of inoculated plants, namely, plant height, pseudostem base circumference (PBC), leaf chlorophyll content and photosynthetic activity. The increased accumulation of plant nutrients can be attributed to enhanced uptake due to improved root growth of inoculated plants. The higher uptake of plant nutrients in this study is attributable to an increase in ion uptake apparently due to a general increase in root surface area and not due to a specific acceleration of the ion uptake process. The studies clearly indicated that Ca uptake of inoculated plants was not due to enhanced plant growth but also due to increased uptake capacity as evidenced by higher concentration in the root, corm and pulp. The result is supported by other researchers who concluded that *Azospirillum* strains enhanced the uptake of P and K in cereals [14–17].

The inoculation process stimulated early flowering of banana plants. Plants inoculated with $UPMB_{10}$ and supplied with 33% fertilizer-N showed 3 weeks early flowering compared with the control ($N_{33\%}$ – PGPR). However, plants inoculated with $UPMB_{10}$ and provided with 100% fertilizer-N showed only 5 days' earliness compared with $N_{100\%}$ – PGPR. The inoculated plants produced more root growth and enhanced water and nutrient uptake, and improved photosynthetic capacity, which led to better plant growth and development and, consequently, early flowering. Finger number was not increased but finger weight, length and diameter were greatly increased due to inoculation. Plants inoculated with Sp_7 and

$UPMB_{10}$ and supplied with 33% fertilizer-N showed bigger fingers by 62% and 65%, respectively. Finger diameter was heavily influenced by PGPR inoculation while length was not affected to a similar extent. PGPR inoculation was found to exert a positive effect on the [pulp / peel] ratio of bananas. The improved finger size was achieved due to higher uptake of K and Ca since those nutrients are required for improved fruit quality in bananas. The better physical fruit quality of this experiment may be due to improved nutrient uptake as influenced by PGPR inoculation which could be supported by several researchers who found that deficiency of certain nutrient elements, *viz.*, low supply of K, will reduce the translocation of carbohydrate from the leaves to the fruit, and consequently thinner fruit and fragile bunches are produced. Low supply of Ca will produce inferior fruit and peel splits when the fruit is ripened [8].

5. Conclusion

PGPR inoculation with 33% fertilizer-N increased the total nutrient accumulation (N, P, K, Ca and Mg). Inoculation with Sp_7 and with 33% fertilizer-N increased bunch yield, while $UPMB_{10}$ improved the fruit physical attributes, namely, finger weight, length and diameter and [pulp / peel] ratio, besides inducing early flowering by 3 weeks. The results suggested that PGPR strains Sp_7 and $UPMB_{10}$ could be used as bioenhancer and biofertilizer for early, high-yielding and improved banana fruit production in 33% fertilizer-N conditions.

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Producción de bananos de calidad, con fuertes rendimientos, mediante inoculación con rhizobacterias que favorecen el crecimiento de las plantas (PGPR).

Resumen — Introducción. La inoculación de rhizobacterias en el caso de escasas aportaciones de nitrógeno, con una fertilización de por ejemplo un 33% de N, podría permitir obtener un crecimiento de los bananos equivalente a la que se obtiene mediante fertilización con un 100% de N, según se constató en plántulas de bananos desarrolladas bajo cultivo hidropónico. Por ello estudiamos la inoculación de plantas con PGPR junto con una aplicación de abono nitrogenado; y, se evaluaron el papel desempeñado por las cepas de rhizobacterias sobre la acumulación de los elementos nutritivos, así como la fijación del nitrógeno y, consecuentemente, la mejora del rendimiento y de la calidad de los bananos. **Material y métodos**. Se utilizaron dos cepas de PGPR, es decir Sp_7 (*Azospirillum brasilense*) y $UPMB_{10}$ (*Bacillus sphaericus*). El dispositivo experimental se llevó a cabo completamente de forma aleatoria con tres repeticiones. Se experimentaron ocho tratamientos: testigo sin aplicación de abono nitrogenado (N_0) y sin PGPR; N_0 + Sp_7; N_0 + $UPMB_{10}$; $N_{33\%}$ sin PGPR; $N_{33\%}$ + Sp_7; $N_{33\%}$ + $UPMB_{10}$; $N_{100\%}$ sin PGPR y $N_{100\%}$ + $UPMB_{10}$. Una única planta destinada a la plantación, resultante del cultivo *in vitro* del cv. Berangan de banano, fue plantada en una maceta de plástico (4 L) durante 45 días, a continuación fue transferida en un recipiente más grande de polietileno (1000 L) hasta su madurez. Se aplicó una suspensión de 100 mL de Sp_7 o $UPMB_{10}$ en cada recipiente después del trasplante de la planta destinada a la plantación y se repitieron las inoculaciones cada mes. Los frutos se recogieron en fase de madurez después de 80 a 90 días de floración. Tras la maduración, se evaluaron el rendimiento y la calidad de la fruta. **Resultados**. La inoculación con abono de un 33% de N aumentó el contenido total de elementos minerales (N, P, K, CA y Mg). La inoculación de PGPR con abono de un 33% de N aumentó significativamente el rendimiento en regímenes así como los rasgos físicos de la fruta, en concreto, el peso, la longitud y el diámetro de los miembros, así como la relación [pulpa / piel]; por otra parte, este tratamiento indujo una floración avanzada de 3 semanas. **Conclusión**. Los resultados sugieren que las cepas de PGPR, Sp_7 y $UPMB_{10}$, podrían emplearse en tanto que bioestimulador y biofertilizante para una producción mejorada de bananos precoces y con altos rendimientos, en presencia de abono de un 33% de N.

Malasia / *Musa* / nutrición de las plantas / bacteria / abonos nitrogenados / crecimiento / rendimiento / calidad del fruto