UNIVERSITI PUTRA MALAYSIA

BENEFICIAL EFFECTS OF RHIZOBACTERIAL INOCULATION ON NUTRIENT UPTAKE, GROWTH AND YIELD OF BANANA (MUSA SPP. CV. 'BERANGAN')

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By

MD. ABDUL BASET MIA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of Requirement for the Degree of Doctor of Philosophy

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DEDICATED TO
MY MOTHER, UNCLE ABDUS SHAHID
AND
DEPARTED SOUL OF MY FATHER

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Faculty : Agriculture

Banana, an important fruit crop, requires high amounts of N-fertilizers for commercial cultivation, which is costly and can be hazardous to the environment when used excessively. Biofertilizer is globally accepted as an alternative source of nitrogen fertilizer and can substantially supplement the N requirement while enhancing the uptake of water and mineral nutrients of crop plants.

A series of research study involving six experiments were conducted to observe the effects of PGPR inoculation on root stimulation and colonization, nutrient absorption, growth, yield and fruit quality of bananas (*Musa* spp cv 'Berangan', AA type) under hydroponics condition.

In the preliminary study, the effects of inoculation with two PGPR strains, Sp7 (*Azospirillum brasilense*) and UPMB10 (*Bacillus sphaericus* UPMB10), on plant growth and N accumulation of banana plantlets were observed under N-free hydroponics condition for 45 days. A marked increase in root growth namely length (33-44%), volume

(76-168%) and mass (137-141%) were recorded due to the PGPR inoculation besides a higher shoot growth (123-202%) and N yield (94-144%).

An *in vitro* electron microscopy study was conducted to observe the pattern of colonization of PGPR strains Sp7 and UPMB10 on roots of banana plantlets. This study demonstrated that both strains could effectively colonize the banana roots and more bacterial cells were present in the root hair proliferation zone.

In the growth study with banana plantlets under hydroponics condition using 4.0 L plastic pots, 150 ppm fertilizer-N was found to be optimum for the 45 days period. Another study with similar conditions was undertaken to observe the synergistic effect of minimal fertilizer-N supply (33% of the total N requirement) and PGPR inoculation on root growth and nutrient uptake of banana plantlets. The results showed that inoculation by UPMB10 with minimal fertilizer-N supply increased the primary root elongation and secondary root initiation and subsequently increased the root mass. The same treatment also increased N concentration in pseudostem and leaves and Ca concentration in roots. The total accumulation of N, P, K, Ca and Mg was 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.

A subsequent experiment with larger containers (1000 L) was conducted to observe the effect of PGPR inoculation on plant growth, nutrient uptake, yield and fruit quality of bananas at different levels of N-fertilization. The results showed that inoculation together with 33% N improved the bioenhancing activity by increasing root and shoot growth, and photosynthetic rate (25%). As observed in the earlier study with smaller pots (4.0 L), the N concentration in the pseudostem was increased. PGPR inoculation with 33% fertilizer-N also increased the Ca uptake capacity resulting in higher Ca concentration in root, corm and pulp but increased the Mg concentration in the root

only. In addition, the total accumulation of nutrients was heavily influenced by PGPR inoculation due to enhanced root proliferation. PGPR inoculation greatly increased the bunch yield (35-51%) and physical fruit attributes namely finger weight (62-65%), finger length (22-24%), grade and pulp/peel ratio. Plants also flowered three weeks earlier in PGPR-inoculated plants.

PGPR strains Sp7 and UPMB10 were evaluated for their N_2 fixing capacities in association with banana roots by acetylene reduction assay (ARA) and ^{15}N isotopic dilution technique using 4 L pots for 45 days. The results conclusively showed that roots of PGPR-inoculated plants produced higher ARA activities (129 ηmole plant^{-1} hour^{-1}). Inoculated plants together with the least fertilizer-N supply (3.2 ppm, 2.13% of the total plant N requirement) showed the highest amount of nitrogen derived from atmosphere (Ndfa ; 37-39%) while those with higher inorganic-N fertilizer (50 ppm, 33% of the total N requirement) showed the lowest Ndfa (5%). However, PGPR inoculation with 20 ppm fertilizer-N (13% of the N requirement) produced a synergistic effect on N_2 fixation with a relatively higher Ndfa and amounts of fixed-N_2 (12.4 to 12.5 % Ndfa; 10.26-10.85 mg plant^{-1}). However, the nitrogen fixation capacity between the strains was not significantly different.

The findings from the above studies demonstrated that PGPR strains (Sp7 and UPMB 10) inoculation with minimal fertilizer-N supply are effective as a bioenhancer and biofertilizer to fix N_2 and increase plant growth, nutrient uptake, yield and fruit qualities of bananas under hydroponics condition.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN INOKULASI RHIZOBAKTERIA TERHADAP PENGAMBILAN NUTRIEN, PERTUMBUHAN POKOK DAN HASIL BUAH TANAMAN PISANG (*MUSA* SPP. CV. 'BERANGAN')

Oleh

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Pisang merupakan tanaman buah-buahan yang penting dan memerlukan sejumlah besar baja N untuk penanaman komersil. Kos baja N adalah tinggi dan bagi N ini boleh mencemarkan alam sekitar sekiranya digunakan secara berlebihan. Konsep biobaja telah diterima di seluruh dunia sebagai sumber alternatif bagi baja nitrogen dan dapat menampung keperluan N dan meningkatkan pengambilan air serta nutrien bagi pelbagai tanaman.

Enam siri eksperimen telah dijalankan untuk menilai kesan inokulasi PGPR terhadap perkembangan dan kolonisasi akar, penyerapan nutrien, pertumbuhan hasil dan kualiti buah tanaman pisang berangan (*Musa* spp. jenis AA) yang ditanam secara hidroponik.

Kajian awal melibatkan inokulasi strain PGPR Sp7 (*Azospirillum brasilense*) dan UPMB10 (*Bacillus sphaericus* UPMB10) terhadap pertumbuhan pokok dan pengumpulan N pada kultur tisu pisang dalam keadaan hidroponik (tanpa N) selama 45 hari. Inokulasi PGPR tersebut telah meningkatkan pertumbuhan akar terutamanya panjang akar (33-44%), isipadu (76-168%) dan berat akar (137-141%) serta

peningkatan pertumbuhan pucuk (123-202%) dan kandungan jumlah N (94-144%) yang tinggi juga diperhatikan.

Kajian *in vitro* kolonisasi akar menggunakan mikroskop elektron untuk melihat corak kolonisasi Sp7 dan UPMB10 pada akar pisang kultur tisu telah dijalankan. Kajian ini menunjukkan bahawa kedua-dua strain mampu mengkolonisasi akar terutamanya pada zon pembentukan akar rerambut.

Dalam kajian pertumbuhan pisang kultur tisu menggunakan kaedah hidroponik dan pasu plastik 4.0 L, 150 bsj baja-N didapati optimum untuk tanaman selama 45 hari. Satu lagi kajian inokulasi PGPR dalam keadan yang sama ke atas pisang kultur tisu telah dijalankan bersama pengunaan kandungan N yang rendah (33% daripada keperluan N keseluruhan; 150 ppm N) untuk menilai kesan sinergistik di antara kandungan N rendah dan inokulasi PGPR terhadap pertumbuhan akar dan pengambilan nutrien oleh pokok pisang. Keputusan menunjukkan bahawa inokulasi UPMB10 dan kandugan N yang minima dapat meningkatkan kadar pemanjangan akar primer, pencetusan akar sekunder dan seterusnya meningkatkan berat akar. Rawatan yang sama juga dapat meningkatkan kepekatan N di dalam pseudostem dan daun serta kepekatan Ca dalam akar. Jumlah pengumpulan keseluruhan P, K, Ca dan Mg juga meningkat akibat peningkatan pertumbuhan pokok. Berat kering keseluruhan rawatan ini sama seperti aplikasi 100% baja N.

Kajian berikutnya melibatkan penanaman pokok pisang di dalam bekas 1000 L untuk menilai kesan inokulasi PGPR dan paras N yang berbeza terhadap pertumbuhan, pengambilan nutrien, hasil dan kualiti buah pisang. Keputusan eksperimen ini menunjukkan bahawa inokulasi PGPR dengan 33% N dapat meningkatkan aktiviti

penggalak pertumbuhan biologi tanaman yang meliputi peningkatan pertumbuhan akar dan daun serta kadar fotosintesis (25%). Kesan yang sama dalam pseudostem turut dilihat sama seperti eksperimen sebelum ini yang menggunakan pasu yang lebih kecil (4.0 L). Inokulasi PGPR dan 33%N juga meningkatkan pengambilan Ca dan menyebabkan peningkatan kepekatan Ca di akar, umbi dan pulpa tetapi meningkatkan kepekatan Mg di akar sahaja. Di samping itu, pengumpulan jumlah nutrien juga dipengaruhi oleh inokulasi PGPR yang meningkatkan pembentukan akar. Inokulasi PGPR juga telah merangsang pengeluaran hasil dan kualiti buah terutamanya berat tandan (35-51%) dan sisir (62-65%) serta panjang (22-24%), kualiti dan nisbah pulpa/kulit buah pisang. Pisang yang diinokulasi juga telah mengeluarkan bunga tiga minggu lebih awal daripada rawatan kawalan. Keupayaan strain PGPR Sp7 dan UPMB10 untuk mengikat N_2 juga telah dinilai dengan menggunakan teknik asai penurunan asetilin (ARA) dan teknik pencairan isotop ^{15}N di dalam pasu 4 L selama 45 hari. Keputusannya, PGPR menunjukkan aktiviti ARA yang tinggi (129nm/pokok/jam) di dalam akar kultur tisu pisang. Rawatan yang diinokulasi dan diberi bekalan baja inorganik N terendah (3.2 ppm, 2.13% dari keperluan N keseluruhan) menunjukkan kandungan pengikatan N_2 (Ndfa) yang tertinggi (37-39%). Rawatan yang dibekalkan dengan kandungan baja nitrogen inorganik yang tinggi sekali (50 ppm, 33% dari keperluan N keseluruhan) pula menunjukkan Ndfa yang terendah (5%). Namun demikian, rawatan dengan bekalan baja inorganik N sebanyak 20 ppm (13% dari keperluan N keseluruhan) menghasilkan kesan sinergistik ke atas pengikatan N_2 dengan Ndfa yang tinggi (12.4 hingga 12.5%; 10.26-10.85 mg pokok^{-1}). Walau bagaimana pun

tiada perbezaan yang bermakna diantara keupayaaan pengikatan N_2 kedua-dua strain tersebut.

Kajian ini menunjukkan penggunaan strain PGPR (Sp7 dan UPMB10) dengan penambahan sejumlah kecil baja N adalah berkesan sebagai bio-penggalak dan biobaja (pengikat N_2) dan seterusnya meningkatkan pertumbuhan perumah, hasil buah serta kualiti fizikal tanaman pisang.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES