UNIVERSITI PUTRA MALAYSIA

GROWTH RESPONSE OF SWEET POTATO (*Ipomoea batatas* L.) TO APPLICATION OF SELECTED INDIGENOUS PLANT GROWTH-PROMOTING RHIZOBACTERIA AND NITROGEN FERTILIZATION

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By

FARZANA YASMIN

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

September 2007

DEDICATED
TO
MY BELOVED HUSBAND
FOR YEARS OF LOVE AND SACRIFICE

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

GROWTH RESPONSE OF SWEET POTATO (*Ipomoea batatas* L.) TO APPLICATION OF SELECTED INDIGENOUS PLANT GROWTH-PROMOTING RHIZOBACTERIA AND NITROGEN FERTILIZATION

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Chairman: Associate Professor Radziah Othman, PhD

Faculty : Agriculture

Sweetpotato requires high amount of fertilizer for commercial cultivation which can lead to increase production cost and environmental pollution. Biofertilizer is globally accepted as an alternative source of chemical fertilizer which improves plant growth through increased uptake of water and mineral nutrients. Laboratory and field experiments were conducted to characterize the beneficial properties of plant growth-promoting rhizobacterial (PGPR) strains isolated from sweetpotato rhizosphere and to determine the effects of rhizobacterial inoculation on the growth and yield of sweetpotato. Performance of the PGPR with different levels of nitrogen fertilizer on plant growth was evaluated under field condition. *In vitro* scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies were conducted to examine the colonization of PGPR strains on roots of sweetpotato plantlets.

Results of the laboratory study showed that 15 rhizobacterial isolates were able to produce indole acetic acid (IAA). The concentration of IAA produced ranged from 3.84 to 13.33 mg L^{-1}. Addition of L-tryptophan (L-TRP) to the bacterial isolates increased the production of IAA ranging from 4.94 to 46.66 mg L^{-1}. Six isolates (40%) were able to solubilize insoluble phosphate as evident by production of clear zone on calcium phosphate medium. All isolates were able to grow in N-free media indicating their abilities to produce nitrogen which ranged from 0.74 to 1.32 ppm. Three of the isolates produced fluorescent pigment on agar plate indicated their abilities to produce siderophores. Four isolates were able to inhibit the fungal pathogens *Rhizoctonia sp.* and *Pythium sp.* The intrinsic antibiotic test showed that all isolates were resistant against Chloramphenicol (10 and 30 µg mL^{-1}), Streptomycin (10 µg mL^{-1}), Kanamycin (5 and 30 µg mL^{-1}), Penicillin (10 µg mL^{-1}) and Tetracyclin (30 µg mL^{-1}). The Biolog identification system identified the rhizobacterial isolates UPMSP2, UPMSP3, UPMSP9, UPMSP10, UPMSP12, UPMSP13, UPMSP18 and UPMSP20 as *Pseudomonas corrugate, Serratia ficaria, Klebsiella terrigena, Erwinia cypripedii, Acinetobacter radioresistens, Pseudomonas maculicola, Paenibacillus pabuli* and *Pseudomonas fuscovaginae,* respectively.

Inoculation of Sepang Oren sweetpotato cultivar with twelve rhizobacteria isolates under glasshouse condition positively affected plant growth. The highest growth was observed on plants inoculated with *Klebsiella* sp. which increased shoot dry weight by 23% compared to control. Five of the isolates

were able to produce sweetpotato storage roots. *Klebsiella* sp and *Erwinia* sp. produced higher storage root yields of 35.15 and 8.22 g plant^{-1}, respectively, compared to the other treatments. PGPR inoculation significantly increased the concentrations and uptake of N, P, K, Ca and Mg in plant tissue, total bacterial populations, soil pH, IAA and nutrients (N, P, K, Ca and Mg) concentrations. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies showed that *Klebsiella* sp*, Erwinia* sp*, Azospirillum brasilense, Bacillus sphaericus* UPMB10 could effectively colonize the sweetpotato root surface and internal region of 7 day old plantlets.

Field inoculation of five bacterial strains in combination with three levels of nitrogen fertilizer (0, 33, and 100 kg N ha^{-1}) in the form of urea significantly ($P<0.05$) influenced growth and storage root yield of Sepang Oren sweetpotato. The highest storage root yield (12.59 kg plot^{-1}) was observed in plants inoculated with *Klebsiella* sp. applied with 33 kg N ha^{-1}. Significant interaction between PGPR inoculation and N fertilization was observed on the uptake of N, K and Ca in shoots. Bacterial Inoculation and N fertilization significantly stimulated the soil bacterial population at various stages of plant growth. The highest population of 2.63X10^{7} CFU g (dry wt.)$^{-1}$ soil was observed in soil inoculated with *Klebsiella* sp. applied with 33 kg N ha^{-1}. However, the population of bacteria in soil declined after the 2nd and 3rd month of inoculation.

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

PERTUMBUHAN KELEDEK (*Ipomoea batatas* L.) KESAN DARI PENGGUNAAN RIZOBAKTERIA PENGGALAK TUMBESARAN TANAMAN ASLI TERPILIH DAN PEMBAJAAN NITROGEN

Oleh

FARZANA YASMIN

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Pengerusi: Profesor Madya Radziah Othman, PhD

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Tanaman keledek memerlukan penggunaan baja yang amat tinggi untuk ditanam secara komersial dan ini mengakibatkan peningkatan kos pengeluaran dan pencemaran alam sekitar. Bajabio kini diterima di peringkat global sebagai sumber alternatif kepada baja kimia yang boleh membantu pertumbuhan pokok dengan meningkatkan pengambilan air dan nutrien. Kajian di makmal dan di lapangan telah dilakukan untuk menentukan ciri-ciri kebaikan penggunaan strain rizobakteria penggalak tumbesaran tanaman (PGPR) yang telah diasingkan dari rizosfera keledek, dan untuk mengkaji kesan inokulasi rizobakteria keatas pertumbuhan dan hasil pengeluaran keledek. Keupayaan PGPR bersama pembajaan nitrogen pada pasas berbeza terhadap pertumbuhan pokok telah dinilai pada keadaan lapangan. Kajian in vitro mikroskop elektron pengimbas (SEM) dan mikroskopi elektron pancaran (TEM) telah dilakukan untuk mengkaji pengkolonian strain PGPR keatas akar anak pokok keledek.

Keputusan kajian menunjukkan 15 isolat rizobakteria boleh menghasilkan asid indol asetik (IAA). Kepekatan IAA yang dihasilkan adalah diantara 3.84 hingga 13.33 mg L^{-1}. Penambahan L-tryptophan (L-TRP) kepada isolat bakteria meningkatkan pengeluaran IAA pada julat kepekatan dari 4.94 hingga 46.66 mg L^{-1}. Enam isolat (40%) berupaya melarutkan fosfat berdasarkan bukti penghasilan zon jernih pada medium kalsium fosfat. Semua isolat boleh tumbuh dalam media bebas N menunjukkan keupayaan menghasilkan nitrogen yang berjulat diantara 0.74 hingga 1.32 ppm. Tiga daripada isolat mengeluarkan pigmen berpendarfluor pada agar yang menunjukkan keupayaan menghasilkan siderofor. Empat isolat berupaya merencat kulat patogen, *Rhizoctonia* sp. dan *Pythium* sp. Kajian antibiotik intrinsik menunjukkan kesemua isolat resistan terhadap Chloramphenicol (10 dan 30 µg mL^{-1}). Streptomisin (10 µg mL^{-1}), Kanamisin (5 dan 30 µg mL^{-1}), Penisilin (10 µg mL^{-1}) dan Tetrasiklin (30 µg mL^{-1}). Sistem identifikasi Biolog telah mengenalpasti isolat rizobakteria UPMSP2, UPMSP3, UPMSP9, UPMSP10, UPMSP12, UPMSP13, UPMSP18 dan UPMSP20, sebagai *Pseudomonas corrugate, Serratia ficaria, Klebsiella terrigena, Erwinia cypripedii, Acinetobacter radiomonas, Pseudomonas maculicola, Paenibacillus pabuli* dan *Pseudomonas fuscovaginae*.

Penginokulatan Kultivar Sepang Oren dengan 12 isolat rizobakteria di dalam rumah kaca mempengaruhi secara positif pertumbuhan pokok. Pertumbuhan pokok yang tertinggi dapat dilihat pada pokok yang diinokulasi dengan *Klebsiella* sp. dengan peningkatan berat kering pucuk sebanyak 23%

berbanding kawalan. Lima daripada isolat berupaya menghasilkan ubi. *Klebsiella* sp. dan *Erwinia* sp. menghasilkan ubi keledek yang tinggi masing-masing, 35.15 dan 8.22 g pokok^{-1}, berbanding rawatan lain. Inokulasi PGPR meningkatkan dengan ketara kepekatan dan pengambilan nutrien N, P, K, Ca dan Mg tisu pokok, jumlah populasi bakteria, pH tanah, kandungan IAA dan nutrien tanah (N, P, K, Ca dan Mg). Mikroskopi elektron pengimbas (SEM) dan mikroskopi elektron pancaran (TEM) menunjukkan bahawa *Klebsiella* sp. *Azospirillum brasilense*, *Bacillus sphaericus* UPMB10 boleh mengkolonisasi secara berkesan permukaan dan kawasan dalaman akar anak pokok berumur 7 hari.

Penginokulatan di lapangan lima strain rizobakteria bersama 3 paras baja nitrogen (0, 33, dan 100 kg N ha^{-1}) dalam bentuk urea telah mempengaruhi dengan signifikan ($P<0.05$) pertumbuhan pokok dan penghasilan ubi keledek Sepang Oren. Penghasilan ubi keledek tertinggi (12.59 kg plot^{-1}) dapat dilihat pada pokok yang telah diinokulasi dengan *Klebsiella* sp. yang ditambah 33 kg N ha^{-1}. Interaksi signifikan diantara inokulasi PGPR dengan pembajaan N telah diperolehi keatas penyerapan nutrien N, K dan Ca dalam pucuk. Inokulasi bakteria dan pembajaan N merangsang dengan ketara populasi bakteria pada pelbagai peringkat pertumbuhan. Populasi bakteria tertinggi sebanyak $2.63X10^7$ CFU g (berat kering)$^{-1}$ tanah dapat dilihat pada tanah diinokulasi dengan *Klebsiella* sp. yang ditambah 33 kg N ha^{-1}. Walau bagaimana pun populasi bakteria dalam tanah merosot selepas 2 dan bulan 3 diinokulasikan.

ACKNOWLEDGEMENTS

With praises and humility, I would like to thank the Almighty Allah for giving me the strength and patience to complete this thesis successfully. I owe a debt of gratitude to many people and institutions for their kind help at various stages of my research work.

Firstly, my sincere gratitude is extended to my honorable supervisor Associate Professor Dr. Radziah Othman, for her untiring and inspiring supervision, critical assessment and helpful suggestions in every step during the preparation of the thesis, and financial help in the form of GRA (Graduate Research Assistantship) from her IRPA project (No. 01-02-04-0097-EA001), which has helped me financially to continue study at UPM. My sincere thanks to Associate Professor Dr. Mohd Said Saad and Associate Professor Dr. Kamaruzaman Sijam, Head of the Department of Plant Protection, for their constant inspiring supervision, critical assessment, valuable advice and fruitful discussions during the preparation of my thesis.

My sincere thanks to Prof. Dr. Azizah Hashim, Prof. Dr. Zulkifli Hj. Shamsuddin, Assoc. Prof. Dr. Halimi Mohd. Saud, Assoc. Prof. Dr. Anuar Abdul Rahim and Dr. Jalloh, Dr. Mahmood Hossain, and Dr. Abdul Baset, for their assistance and suggestion especially on the statistical analysis and experimental design. I would also like to thank all the staff of Department of

Land Management specially, Mr Zul, Mr. Vella, Puan Zarina, Puan Fauziah, Mr. Rafi, Mr. Ho and other colleagues for their help and assistance.

I gratefully acknowledge Mr. Yassin, and Mr. Shameem for whole-hearted cooperation and kind help in every aspect during my study. Furthermore, special thanks to my well wishers Dr. Lutfor Rahman and wife, Dr. Numan and wife, and Mr. Arun for their moral support during my research period. My sincere appreciation goes to my beloved parents and in laws and close relatives for their inspiration and moral support.

I am deeply indebted and acknowledge to my only son Akif Abdullah (Farhan) for his sacrifices and patience particularly final stage of thesis preparation. Finally, no words can express adequately my sense of indebtedness and gratitude to my beloved husband Associate Prof. Dr. Ahasanul Haque, for his love, sacrifices and constant support in every aspect throughout my study. Truly, without his whole-hearted cooperation and help it was impossible for me to complete my Ph.D. degree in UPM, Malaysia.

I certify that an Examination Committee met on 21st of September 2007 to contract the final examination of Farzana Yasmin on his Doctor of Philosophy thesis entitled "Growth Response of Sweet potato (*Ipomoea batatas L*.) to Application Selected Indigenous Plant Growth-Promoting Rhizobacteria and Nitrogen Fertilization" in accordance with Universiti Partanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. The committee members for the candidate are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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FARZANA YASMIN

Date:

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ANOVA	Analysis of Variance
CFU	Colony forming unit
Ca	Calcium
$CaCl_2$	Calcium chloride
$CaHPO_4$	Calcium hydrogen-phosphate
CIP	International Potato Centre
CM Value	Chlorophyll Meter value (measurement on leaf greenness by SPAD meter-502, MINOLTA™)
CRD	Completely Randomized design
DOA	Department of Agriculture
$FeCl_3$	Ferric chloride
IAA	Indole-3-acetic acid
K	Potassium
K_2HPO_4	Dipotassium hydrogen-phosphate
Mg	Magnesium
MOP	Muriate of potash
N	Nitrogen
OD	Optical density
P	Phosphorous
PDA	Potato dextrose agar
PDYA	Potato dextrose yeast-extract agar
PGPR	Plant Growth-Promoting Rhizobacteria
PSB	Phosphate solubilizing bacteria

R/S	Root to Shoot ratio
RCBD	Randomized complete block design
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope
TSP	Triple Super Phosphate
TRP	Tryptophen