UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ROOT EXUDATES ON SPECIFIC DIAZOTROPH-RICE GENOTYPE ASSOCIATION

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EFFECTS OF ROOT EXUDATES ON SPECIFIC DIAZOTROPH-RICE GENOTYPE ASSOCIATION

By

UMME AMINUN NAHER

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

SEPTEMBER 2009

DEDICATED

to

My son
Azmaine Iqtidar
my husband
Mesbahuddin Ahmed
my parents
Syed Ahmed Ali
Amina Ahmed
and
Family

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

EFFECTS OF ROOT EXUDATES ON SPECIFIC DIAZOTROPH-RICE GENOTYPE ASSOCIATION

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UMME AMINUN NAHER

September 2009

Chairman: Associate Professor Radziah Othman, PhD

Faculty : Agriculture

Diazotrophs are known to utilize root exudate carbon compounds, form natural associations with rice plants and subsequently fix nitrogen. The specific association can be influenced by the bacterial strains and rice genotypes. A series of experiments were conducted in laboratory and glasshouse conditions with the following objectives; (i) to isolate and characterize the indigenous diazotrophs, (ii) to determine the root exudate sugars and amino acids of different rice genotypes (iii) to determine the utilization of root exudates sugars by the diazotrophs (*Rhizobium* sp. and *Corynebacterium* sp.) during colonization and (iv) to determine the effects of specific sugars on plant-diazotrophs associations, biological nitrogen fixation and growth of different rice genotypes. The diazotrophs were isolated from Tanjong Karang rice irrigation project area using N-free semi-solid media and the nitrogenase enzyme activity was determined by Acetylene Reduction Assay (ARA) technique. The isolated diazotrophs were identified using Biolog Identification method. Root exudates sugars and amino acids of the three rice genotypes (Mahsuri, Mayang Segumpal and MR219) were determined using high

performance liquid chromatography (HPLC). The root (rhizosphere and endophytic) colonization were visually observed by using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The diazotroph–plant association and biological nitrogen fixation (BNF) was estimated using ^{15}N dilution technique in glasshouse condition. Results showed that the indigenous diazotrophic populations were significantly ($P<0.01$) influenced by soil types, plant age and rice varieties. Bacterial populations were significantly higher in soil (1.8-2.2 x 10^6 cfu g^{-1}soil) and rhizosphere (1.4-4.2 x 10^7 cfu g^{-1} root) of rice grown in Organic Clay & Muck, Bakau, Sedu and Serong soil series. Molecular analysis indicated a diverse group of diazotrophic strains were present in the different soil types. Ten of the strains were identified by the Biolog Identification method as *Rhizobium*, *Burkholderia* and *Corynebacterium* spp.. Biochemical tests of 19 isolates showed that these strains were positive for N_2 fixation, capable of degrading cellulose and able to produc high amounts of indoleacetic acid (IAA) which ranged from 15 to 69 mg L^{-1}. The diazotrophs exhibited differences in the specific growth rate, generation time, and utilized mono and disaccharide sugars as sole energy sources. A total of seven sugars and 16 amino acids were determined from rice root exudates. The concentration of root exudate sugars, amino acids and their release patterns differed significantly among rice genotypes. Mahsuri released the highest root exudate sugars (25.73%) followed by MR219 (23.14%) and Mayang Segumpal (20.85%). Inoculated plants produced different amounts of sugars and amino acids compared to non-inoculated plants. Mahsuri inoculated with *Corynebacterium* sp. (Sb26) released the highest amount of fructose and arabinose, while Mayang Segumpal inoculated with *Rhizobium* sp. (Sb16) produced the highest amount of sucrose in the root exudate. All rice genotypes produced significantly higher amounts of glycine and

isoleucine in root exudates as compared to other amino acids. In general, plants inoculated with Sb16 produced higher amounts of total sugars and amino acids in their root exudates compared to those inoculated with Sb26. A significant relationship was observed between diazotrophic populations and utilization of root exudates sugars and amino acids in the rice genotypes. The Sb16 strain utilized higher amounts of sugars and stimulating higher rhizosphere population compared to Sb26 strain. The diazotrophs were able to colonize and proliferate endophytically in the rice roots. SEM micrographs showed the occurrence of bacterial colonization on surfaces of primary and lateral roots, root hair zone, lateral root junction, in crevices and root tips. TEM view of roots revealed the presence of diazotroph in the intercellular spaces of cortical parenchyma, within epidermis, inner cortex, and near vascular tissue. The results of *invitro* and glasshouse study using ^{15}N dilution studies showed that Mayang Segumpal inoculated with Sb16 and applied with galactose significantly increased plant-N content and fixed 42 % of atmospheric N (Ndfa). This association increased 147-245 % plant biomass compared to non-inoculated control and 8-52 % over 60 kg ha^{-1} N-fertilizer application.The study proved that diazotroph inoculation enhanced root exudate sugar production and provides specific sugars for specific diazotroph-rice plant association. Application of galactose and arabinose as external carbon source enhanced the growth and N_2 fixation activity of the *Rhizobium* sp. (Sb16) and *Corynebacterium* sp. (Sb26), respectively. The association of Mayang Segumpal with Sb16 and MR219 rice with Sb26 significantly improved nitrogen fixation and subsequently plant growth.

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

KESAN EKSUDAT AKAR KE ATAS GABUNGAN ANTARA DIAZOTROF KHUSUS-GENOTIP PADI

Oleh

UMME AMINUN NAHER

September 2009

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Diazotrof diketahui menggunakan kompaun karbon dalam eksudat akar, membentuk penyatuan semulajadi dengan pokok padi dan seterusnya mengikat nitrogen. Penyatuan khusus boleh dipengaruhi oleh strain bakteria dan genotip padi. Beberapa siri eksperimen telah dijalankan di dalam makmal dan di rumah kaca dengan objektif-objektif berikut; (i) untuk mengasingkan dan mencirikan diazotrof asal, (ii) untuk menentukan gula eksudat akar dan asid amino pada genotip padi berbeza, (iii) untuk menentukan penggunaan gula eksudat akar oleh diazotrof (*Rhizobium* sp. and *Corynebacterium* sp.) semasa kolonisasi dan (iv) untuk menentukan kesan gula khusus ke atas penyatuan tanaman-diazotrof, pengikatan nitrogen biologikal dan pertumbuhan genotip padi berbeza. Diazotrof telah dipencilkan dari kawasan projek pengairan padi Tanjong Karang menggunakan media separa pepejal bebas nitrogen dan aktiviti enzim nitrogenase ditentukan oleh teknik asai penurunan asetilena (ARA). Diazotrof tersebut telah dikenalpasti menggunakan kaedah pengecaman Biolog. Gula eksudat akar dan asid amino tiga genotip padi (Mahsuri, Mayang Segumpal dan MR219) telah ditentukan dengan menggunakan kromatografi cecair berkeupayaan tinggi (HPLC). Pengkolonian bakteria pada akar (rhizosfera dan endofitik) telah diperhati secara visual menggunakan

Mikroskop Pengimbas Elektron (SEM) dan Mikroskop Pemancar Electron (TEM). Pengikatan nitrogen biologikal (BNF) pada penyatuan bakteria-tanaman telah ditentukan dengan menggunakan kajian pencairan ^{15}N dalam rumah kaca. Keputusan kajian menunjukkan populasi diazotrof asal dipengaruhi dengan signifikan ($P<0.01$) oleh jenis tanah, umur tanaman dan varieti padi. Populasi bakteria adalah lebih tinggi secara signifikan dalam tanah (1.8-2.2 x 106 cfu g-1 tanah) dan rhizosfera (1.4-4.2 x 107 cfu g^{-1} akar) pertumbuhan padi dalam siri tanah *Organic Clay & Muck*, Bakau, Sedu dan Serong. Analisis molekular menunjukkan pelbagai kumpulan strain bakteria hadir dalam jenis tanah berlainan. Sepuluh strain telah dikenalpasti oleh kaedah Biolog sebagai *Rhizobium*, *Burkholderia* dan *Corynebacterium* spp. Ujian biokimia ke atas 19 isolat menunjukkan bahawa strain-strain ini adalah positif pada pengikatan N_2, berkebolehan menghancurkan selulosa dan berupaya menghasilkan jumlah asid indolasetik (IAA) yang tinggi pada julat antara 15 hingga 69 mg L^{-1}. Diazotrof telah menunjukkan perbezaan pada kadar pertumbuhan tentu, masa generasi dan penggunaan gula mono dan disakarida sebagai sumber tenaga utama. Sejumlah tujuh gula dan 16 asid amino telah ditentukan daripada eksudat akar padi. Kepekatan gula eksudat akar, asid amino dan corak pembebasannya berbeza dengan ketara antara genotip padi. Mahsuri menghasilkan gula eksudat tertinggi (25.73%), diikuti dengan MR219 (23.14%) dan Mayang Segumpal (20.85%). Pokok yang diinokulasi menghasilkan kandungan gula dan asid amino yang berbeza berbanding pokok yang tidak diinokulasi. Mahsuri yang diinokulasi dengan *Corynebacterium* sp. (Sb26) telah membebaskan kandungan fruktosa dan arabinosa tertinggi, manakala Mayang Segumpal yang diinokulasi dengan *Rhizobium* sp. (Sb16) telah menghasilkan kandungan sukrosa yang tertinggi dalam eksudat akar. Semua varieti padi telah menghasilkan kandungan glisina dan isoleusina yang ketara tinggi dalam eksudat akar berbanding dengan asid amino yang lain. Pada amnya, pokok yang diinokulasi dengan Sb16 telah menghasilkan jumlah gula dan asid amino yang lebih

tinggi dalam eksudat akar berbanding dengan pokok yang diinokulasi dengan Sb26. Interaksi signifikan dapat dilihat di antara populasi diazotrof dengan penggunaan gula dan asid amino pada tiga jenis varieti padi. Sb16 menggunakan lebih banyak gula dan menghasilkan lebih tinggi populasi rhizosfera berbanding Sb26. Perbezaan penggunaan gula oleh diazotrof dapat dilihat untuk kesemua tiga varieti padi. Strain Sb16 menggunakan jumlah gula yang lebih tinggi dan menggalakkan populasi bakteria pada rhizosfera yang lebih tinggi berbanding strain Sb26. Diazotrof mampu mengkolonisasi dan bercambah secara endofitik dalam akar padi. SEM menunjukkan pengkolonian bakteria di atas permukaan akar primer dan akar sisi, zon akar rerambut, persimpangan pengeluaran akar sisi, di celahan dan hujung akar. TEM menunjukkan kehadiran diazotrof di ruang interselular pada parenkima kortikal, di antara epidermis, korteks dalam dan saluran tisu vaskular. Keputusan kajian dalam makmal dan rumah kaca dengan menggunakan kajian pencairan ^{15}N menunjukkan bahawa Mayang Segumpal yang diinokulasi dengan Sb16 dan diberi galaktosa meningkatkan dengan signifikan kandungan N-pokok dan mengikat 42% N atmosfera (Ndfa). Penyatuan ini telah meningkatkan 147-245% biomas pokok berbanding pokok yang tidak diinokulasi dan 8-52% berbanding pokok yang diaplikasi dengan 60 kg ha^{-1} baja N. Kajian ini membuktikan bahawa inokulasi diazotrof meningkatkan pengeluaran gula eksudat akar dan membekalkan gula khusus untuk penyatuan khusus diazotrof-pokok padi. Aplikasi galaktosa dan arabinosa sebagai sumber karbon luaran telah masing-masing meningkatkan pertumbuhan dan aktiviti pengikatan nitrogen *Rhizobium* sp. (Sb16) dan *Corynebacterium* sp. (Sb26). Penyatuan padi Mayang Segumpal dengan Sb16 dan padi MR219 dengan Sb26 meningkatkan dengan ketara pengikatan nitrogen dan seterusnya pertumbuhan pokok.

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I certify that an Examination Committee has met on 15th September, 2009 of viva voce to conduct the final examination of Umme Aminun Naher on her Doctor of Philosophy thesis entitled "Effect of Root Exudates on Specific Diazotroph-Rice Genotypes Association" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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

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Date: 16th November 2009

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.

UMME AMINUN NAHER

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES