UNIVERSITI PUTRA MALAYSIA

NUTRIENT UPTAKE BY RICE PLANT INOCULATED WITH MICROAEROPHILIC RHIZOBACTERIA ISOLATED FROM SELECTED RICE SOILS

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By

NEO SYE PENG

Thesis Submitted to The School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of The Requirement for The Degree of Master of Science

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UPM

Dedicated

to

my

family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement of the degree of Master of Science

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February 2002

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The major problem of rice cultivation is low efficiency of N fertilizer and increasing production costs due to the rising price of N fertilizer. Nitrogen fertilizer inputs constitute a high proportion of the price of production. The biological nitrogen fixation technology by inoculating plants with diazotrophic rhizobacteria is an alternative that would subsequently reduces cereal production cost.

The study consisted of two experiments. Experiment I was the isolation of microaerophilic rhizobacterial strains from rice soil. Experiment II was a glasshouse experiment to study the effect of selected microaerophilic rhizobacteria on rice nutrient uptake under glasshouse condition.

From the isolation procedures, 62 rhizobacterial isolates were collected from different rice soils. Twenty-six or 41.93% isolates showed positive results in nitrogen-free media test. Among the positive strains, six isolates

(designated as E18, E23, E38, E40, E44 and E47) with comparatively the highest c.f.u. were studied under the microscope and applied as inoculant in subsequent glasshouse experiment.

In the glasshouse experiment, a factorial experiment comprising 6 strains X 5 concentrations of nitrogen input with 5 replications were set up giving a total of 150 pots.

Rice plants inoculated with rhizobacteria E44 showed the tendency to increase nitrogen content, rhizobacteria E38 tends to increase plant phosphorus content, and rhizobacteria E40 inoculation tends to increase dry weight of rice plants. Meanwhile, inoculation of rhizobacteria E40 showed the tendency to increase shoot magnesium content while rhizobacteria E23 tends to increase root magnesium content. There was no obvious result that shows either potassium and calcium uptake of rice plant were promoted by inoculation with the isolated rhizobacteria.

In conclusion, rhizospheres of rice cultivation areas were found to have high populations of microaerophilic rhizobacteria that have the potential to be diazotrophs. The selected rhizobacterial strains respectively showed the trend to promote plant growth (in term of total dry weight) and nutrient (nitrogen, phosphorus, potassium, calcium and magnesium) uptake of rice plants. Therefore, they could have the potential to be applied as biofertilizer or bioenhancer.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGAMBILAN NUTRIEN OLEH POKOK PADI YANG DIINOKULASI RHIZOBAKTERIA MIKROAEROFILIK YANG DIPEROLEHI DARIPADA TANAH PADI TERPILIH

Oleh

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Masalah utama dalam penanaman padi adalah kadar keberkesanan baja nitrogen yang rendah dan kos pengeluaran yang melambung oleh sebab peningkatan harga baja nitrogen. Penggunaan baja nitrogen merangkumi sebahagian besar kos pengeluaran. Teknologi pengikatan nitrogen secara biologi yakni penginokulatan pokok dengan rhizobakteria diazotrofik merupakan satu pilihan yang boleh mengurangkan kos pengeluaran bijirin.

Dua kajian telah dijalankan. Kajian I adalah pengasingan rhizobakteria mikroaerofilik daripada tanah sawah padi. Kajian kedua pula adalah pengajian kesan rhizobakteria mikroaerofilik yang terpilih ke atas pengambilan nutrien oleh pokok padi di rumah kaca.

Daripada prosedur pengasingan, 62 rhizobakteria telah diperolehi daripada tanah-tanah sawah padi. Dua puluh enam atau 41.9% rhizobakteria terperoleh menunjukkan keputusan positif dalam kajian media tanpa nitrogen.

Di kalangan rhizobakteria yang berkeputusan positif, enam rhizobakteria (dinama sebagai E18, E23, E38, E40, E44 and E47) yang berkecenderungan untuk menghasilkan koloni yang paling banyak telah dipilih dan digunakan sebagai inokulan dalam kajian rumah kaca. Dalam kajian rumah kaca, satu rekabentuk berfaktoran dengan 6 isolat X 5 kepekatan nitrogen dan 5 replikasi yang berjumlah 150 pasu telah dijalankan.

Dalam analisis statistik, padi yang diinokulasi dengan rhizobakteria E44 menunjukkan kecenderungan meningkat kandungan nitrogen, rhizobakteria E38 cenderung untuk meningkatkan kandungan phosphorus dan rhizobakteria E40 untuk berat kering pokok padi. Sementara itu, inokulasi rhizobakteria E40 didapati bercenderung untuk meningkatkan kandungan magnesium pucuk manakala rhizobakteria E23 untuk magnesium akar. Tidak ada keputusan yang ketara menunjukkan peningkatan pengambilan kedua-dua nutrien kalium dan kalsium oleh padi yang diinokulasi dengan rhizobakteria.

Kesimpulannya, rizosfera kawasan sawah padi didapati mengandungi populasi rhizobakteria mikroaerofilik yang tinggi dan berpotensi diazotrof. Rhizobakteria yang terpilih didapati masing-masing berkecenderungan meningkatkan tumbesaran padi (dari segi berat kering) and pengambilan nutrien (nitrogen, fosforus, kalium, kalsium dan magnesium) yang tertentu. Oleh itu, rhizobakteria tersebut berpotensi dijadikan "biofertilizer" atau "bioenhancer".

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I certify that an Examination committee met on 18th February 2002 to conduct the final examination of Neo Sye Peng on his Master of Science thesis entitled "Nutrient Uptake by Rice Plant Inoculated with Microaerophilic Rhizobacteria Isolated from Selected Rice Soils" in the accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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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.

NEO SYE PENG

Date: 16. 4. 2002

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LIST OF ABBREVIATIONS

FAO	=	Federal Agriculture Organization
BNF	=	Biological Nitrogen Fixation
MARDI	=	Malaysia Agriculture Research and Development Institute
Ndfa	=	Nitrogen derived from air (atmosphere)
ARA	=	Acetylene reduction assay
Nfb	=	Nitrogen-free semisolid agar
c.f.u.	=	Colonies forming unit
TEM	=	Transmission electron microscopy
AA	=	Autoanalyser
AAS	=	Atomic absorption system
SAS	=	Statistical Analysis System

CHAPTER 1

INTRODUCTION

Nitrogen is always the major factor limiting the yield of lowland rice in tropical area. Yoshida (1981) reported that the efficiency of N fertilizer in lowland rice cultivation in the tropics is low. Field studies with flooded rice have shown that about 70% of the applied nitrogen can be lost due to denitrification, ammonia volatilization (Freney *et al.*, 1990; Buresh *et al.*, 1993), and leaching or run-off (Schinier *et al.*, 1988; De Datta and Buresh, 1989).

Kundu and Ladha (1995) stated that to feed the increasing global population, the world's annual rice production must increase from the present 520 x 10^6 tons to 760 x 10^6 tons by the year 2020. In addition, it is estimated that twice as much fixed nitrogen will be required for cereal crop production by the year 2020 to meet the food requirements of increasing human populations (BNF, 1997).

To maintain rice sufficiency with diminishing rice-area production and reducing inorganic fertilizer inputs, alternative and sustainable sources of fertilizer is required (People *et al.*, 1995).

In Malaysia, the land area for rice cultivation is decreasing. The statistic obtained from Federal Agriculture Organization (FAO) web site (2000) showed that there was 698,624 hectares for rice cultivation area in Malaysia

in 1994. However, the cultivation area has decreased to 692, 389 hectares in 2000. To increase rice production from the same or even less land area, productivity (yield ha^{-1}) must be enhanced.

Nitrogen fixing bacteria, or diazotrophs, are the beneficial bacteria that exist in the soil which can colonize, either entophytic or epiphytic, and contribute to biological nitrogen to wetland rice crops (Kundu and Ladha, 1995). Compared with industrial nitrogen fixation, biological nitrogen fixation (BNF) is sustainable and can be less polluting and cheaper. Biological nitrogen fixation has the advantages of lower cost, reduced production of greenhouse gases like carbon dioxide and less nitrate contamination of underground water.

In recent years, the use of plant growth promoting rhizobacteria (PGPR) has been used in rice cultivation (Malik *et al*., 1997) and has been shown to increase rice yield (straw and grain) with minimal inputs from chemical fertilizers.

So far, only few studies have been carried out on the effects of BNF on rice cultivation in Malaysia. Furthermore, the reports mostly emphasized only on N uptake and subsequent effects of plant responses. Therefore, besides N uptake, the aim of this study is to investigate if there are any other beneficial effects contributed by diazotrophic inoculants.

The objectives of this research are:

1. To isolate local potential microaerophilic bacteria strains from rice soils.
2. To investigate the effect of selected microaerophilic strains on rice mineral uptake under glasshouse condition.

CHAPTER 2

LITERATURE REVIEW

Rice

Rice, or paddy, *Oryza sativa* of family Gramineae, is the only crop for wet area. Watanabe (1986) mentioned that flooding favours rice growth environments by bringing the soil pH near to neutral that would making nutrients like P and Fe more available and depressing soil organic matter decomposition.

The low concentration of oxygen under the surface of the water would stimulate nitrogen fixation, depressing the outbreak of soil-borne disease and suppressing weeds, especially those of C4 type (Tanaka, 1976).

The rice cultivation area at the same time also could be acting as a water reservoir and preventing soil erosion. Meanwhile, the irrigation water would supply additional nutrients.

Rice is unarguably the most essential cereal crop in the world. It feeds over 2 billion people, particularly in Asia, Africa and Latin America (Ladha *et al.*, 1997). And, the tropical lowland rice cultivation is responsible for 86% of the total world rice crop (James e*t al.*, 1999).

In 1994, world rice production was around 535 million tonnes. More than 50 countries throughout the world contributed to that total with an annual production of at least 100 thousand tones of rice grains. Asian farmers produced 91% of the total. Worldwide, about 79 million hectares of rice is grown under irrigated condition, 36 million hectares is rainfed and around 11.4 million hectares of rice cultivation area in South and Southeast Asia are subject to uncontrolled flooding (Riceweb, 2001).

Average rice yields in tropical area were approximately 2.3 t ha^{-1} overall, and some were as low as 1.3 t ha^{-1} for the more flood-prone lands (IRRI, 1997).

Rice in Malaysia

Since the establishment of MADA, the most widely planted rice varieties were MR84 (58%), MR167 (21%), MR185 (15%) and other varieties (6%) with yield of 1.4 t to 6.8 t per hectare, respectively (Agrolink, 1999).

Recently, Malaysia Agriculture Research and Development Institute (MARDI) successfully produced two new varieties of rice: MRQ50 and MR211. These two varieties were announced to have the potential in producing high yield per hectare that subsequently will increase the income per farmer.