UNIVERSITI PUTRA MALAYSIA

NITROGEN FIXATION AND VEGETATIVE GROWTH OF IMMATURE OIL PALM INOCULATED WITH *BACILLUS SPHAERICUS* (STRAIN UPMB-10)

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FP 2008 2

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2008

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

April 2008

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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Chairman: Professor Zulkifli Hj. Shamsuddin, Ph.D.

Faculty: Agriculture

Inorganic-N fertilizer is the most widely used input in agriculture and it is a major factor that limits crop growth and yield. However, excessive use of inorganic nitrogenous fertilizer could lead to hazardous effect to the agro-environment. Besides that, it would also increase total production cost of the agricultural sector especially in the oil palm industry, the largest income generator to Malaysian agricultural economy. Rapid losses of fertilizer-N through leaching would further increase the cost on oil palm production and make the sector less profitable. In recent times, there is an increased attention to biological nitrogen fixation (BNF) as an alternative or supplement to nitrogenous fertilizers through application of associative N_2-fixing rhizobacteria which could be applied to oil palm. These beneficial plant growth promoting rhizobacteria (PGPR) have been reported to be efficient in fixing atmospheric nitrogen and stimulating growth of leguminous and non-

leguminous crops. Thus, it could be used as a biofertilizer and bioenhancer for most crops. However, there has been no report on the beneficial effect of PGPR on early growth of young oil palms under field conditions. A field trial using ^{15}N isotope was conducted to estimate the N_2 fixing capacity of a single strain N_2-fixing rhizobacterium, *Bacillus sphaericus* UPMB-10, and assess its effects on early growth of young oil palms. Results from a 240-day (D_{240}) field experiment indicated that the *Bacillus sphaericus* rhizobacterial strain UPMB-10 is a potential biofertilizer for oil palm seedlings with a N_2 fixing capacity of 63% Ndfa (N derived from atmosphere) (78 g N_2 fixed palm^{-1}) of the total N requirement of young oil palms (equivalent to 12 kg N ha^{-1} at 148 palms ha^{-1}) over 8 months. *B. sphaericus* UPMB-10 inoculation stimulated higher N content (125 g palm^{-1}) as compared to the uninoculated control (90 g palm^{-1}) and also promoted a total dry matter accumulation of 11 kg palm^{-1} in comparison to the control, 8 kg palm^{-1}. Present study also showed significant differences in two of the four physical parameters (leaflet numbers, rachis lengths, cross-sections of petioles and leaf area) of vegetative growth of young oil palm inoculated with strain UPMB-10 at D_{120}. *B. sphaericus* UPMB-10 inoculation stimulated leaflet numbers and leaf area at 123.4 ± 2.5 and 1.57 ± 0.05 m^2, respectively, as compared to Uninoculated + 2/3N_i palms at 115.6 ± 2.2 and 1.43 ± 0.05 m^2, respectively, at D_{120}. However, no significant response was observed at D_{240}. Thus, the study showed that the significant total N_2 fixed does not correlate significantly with physical parameters at the later stage (D_{240}) of vegetative growth. This phenomenon is caused by unstable development of immature oil palm under field planting. This was also true for dry matter and Total-N yield analyses at D_{240} which were not statistically significant although they showed incremental trends. The frond (leaflets plus rachis) of the young oil palm is the most likely vegetative component to be adopted as representative of the whole palm for future N_2 fixation studies based on the % Ndfa values of the frond (70%) and whole palm (63%) which did not deviate significantly. This new approach will make such field studies more cost effective and time saving. *B. sphaericus* UPMB-10 inoculation is more effective in delivering N to the immature palms than nitrogenous fertilizer. The reduction (63%) in inorganic-N fertilizer rates due to the incorporation of the inoculum and the resultant N_2 fixation could subsequently reduce the cost of nitrogenous fertilizer. The above findings provided evidence that locally isolated *B. sphaericus* UPMB-10 has the potential to effectively supplement inorganic-N fertilizer for sustainable cultivation of young oil palm.

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

PENGIKATAN NITROGEN DAN PERTUMBUHAN VEGETATIF KELAPA SAWIT BELUM MATANG DIINOKULAT DENGAN *BACILLUS SPHAERICUS* (STRAIN UPMB-10)

Oleh

ZAKRY FITRI BIN AB. AZIZ

April 2008

Pengerusi: Profesor Zulkifli Hj. Shamsuddin, Ph.D.

Fakulti: Pertanian

Baja N bukan organik merupakan input yang digunakan secara meluas dalam pertanian dan ia merupakan faktor utama mengekang pertumbuhan dan hasil tanaman. Walau bagaimanapun, penggunaan baja N bukan organik yang berlebihan akan memberi kesan yang memudaratkan kepada persekitaran alam pertanian. Di samping itu, ia juga meningkatkan jumlah kos pengeluaran sektor pertanian terutamanya dalam industri kelapa sawit, penyumbang terbesar kepada ekonomi pertanian Malaysia. Kehilangan baja N yang cepat menerusi larutlesap meningkatkan lagi kos pengeluaran minyak sawit menyebabkan sektor ini kurang menguntungkan. Pada masa kini, perhatian telah diberikan kepada pengikatan nitrogen biologi sebagai alternatif atau penambahan kepada baja-baja nitrogen menerusi aplikasi rhizobakteria assosiatif pengikat N_2 yang boleh digunakan untuk kelapa sawit. Rhizobakteria penggalak pertumbuhan tanaman (PGPR) yang

berfaedah telah dilaporkan bertindak secara cekap dalam pengikatan nitrogen dari atmosfera dan menggalakkan pertumbuhan tanaman kekacang dan bukan kekacang. Justeru itu, ia boleh digunakan sebagai biobaja dan biopenggalak untuk kebanyakan tanaman. Walau bagaimanapun, tiada terdapat laporan kesan berfaedah PGPR ini ke atas pertumbuhan awal kelapa sawit muda di lapangan. Satu ujian lapangan menggunakan isotop ^{15}N dijalankan untuk menganggarkan kapasiti pengikatan N_2 oleh satu strain rhizobakteria pengikat N_2, *Bacillus sphaericus* UPMB-10, dan menilai kesan-kesannya ke atas pertumbuhan awal kelapa sawit muda. Keputusan kajian lapangan pada hari ke-240 (D_{240}) menunjukkan *Bacillus sphaericus* strain rhizobakteria UPMB-10 adalah biobaja berpotensi untuk anak benih kelapa sawit dengan kapasiti pengikatan N_2 sebanyak 63% Ndfa (N dari atmosfera) (78 g N_2 diikat pokok^{-1}) daripada jumlah N yang diperlukan kelapa sawit muda (bersamaan 12 kg N ha^{-1} bagi 148 pokok ha^{-1}) selama 8 bulan. Inokulasi *B. sphaericus* UPMB-10 merangsangkan kandungan N lebih tinggi (125 g pokok^{-1}) berbanding dengan kawalan tanpa diinokulasi (90 g pokok^{-1}) dan juga meningkatkan jumlah bahan kering kepada 11 kg pokok^{-1} berbanding dengan kawalan pada 8 kg pokok^{-1}. Kajian ini menunjukkan perbezaan yang bererti pada parameter-parameter fizikal (bilangan bilah daun, panjang rakis, keratan rentas petiol dan luas daun) pertumbuhan vegetatif kelapa sawit muda yang diinokulat dengan strain UPMB-10 pada D_{120}. Inokulasi *B. sphaericus* UPMB-10 merangsang bilangan bilah daun dan luas daun masing-masing kepada 123.4 ± 2.5 dan 1.57 ± 0.05 m^2 berbanding pokok tak diinokulat + 2/3N_i pada 115.6 ± 2.2 dan 1.43 ± 0.05 m^2 pada D_{120}. Walau bagaimanapun, tiada tindakbalas bererti pada pemerhatian D_{240}. Maka, kajian menunjukkan jumlah pengikatan N_2 yang signifikan tidak menunjukkan hubungan yang bererti kepada paramater fizikal pertumbuhan vegetatif pada peringkat seterusnya (D_{240}). Fenomena ini disebabkan oleh perkembangan pokok kelapa sawit belum matang yang masih tidak stabil di ladang. Hal ini juga terbukti pada analisis berat kering dan kandungan jumlah N pada D_{240} yang tidak bererti secara statistik, walaupun purata menunjukkan aliran meningkat. Pelepah daun (bilah daun dan rakis) kelapa sawit muda adalah komponen vegetatif yang sesuai untuk digunakan sebagai komponen yang setara dengan keseluruhan kelapa sawit untuk kajian pengikatan N_2 pada masa depan, berasaskan kepada nilai % Ndfa pelepah daun (70%) dan keseluruhan pokok (63%) yang tidak tersisih secara bererti. Pendekatan baru ini akan membuatkan kajian lapangan lebih kos efektif dan menjimatkan masa. Kajian juga mendapati bahawa inokulasi *B. sphaericus* UPMB-10 adalah lebih efektif menyalurkan N kepada kelapa sawit muda berbanding N bukan organik. Pengurangan kadar baja N bukan organik (63%) kesan daripada inokulum dan hasil pengikatan N_2 akan dapat mengurangkan kos baja nitrogen. Hasil daripada kajian menunjukkan bahawa isolat tempatan *B. sphaericus* UPMB-10 berpotensi untuk menampung baja N bukan organik secara efektif bagi kelestarian penanaman kelapa sawit.

ACKNOWLEDGEMENTS

My truthful appreciation is extended to Professor Dr. Zulkifli Hj. Shamsuddin, Associate Professor Dr. Anuar Abd. Rahim, Dr. Zin Zawawi Hj. Zakaria and Dr. Khairuddin Abd. Rahim for their invaluable guidance, advice and comments during the planning and execution of this study and in the preparation of this manuscript.

Special appreciation is also extended to the staff of the Golden Hope Research Centre especially Ms. Zuelida Idayu Zulkifli for arrangements of the field trial. Grateful appreciation is also extended to Mr. Ghooi (Tangkah Estate Manager) and Mr. Tan Kim Chai for providing and supporting field area preparation and maintenance and to the following staff: Mrs. Latiffah Norddin, Mr. Abdul Razak Ruslan and Mr. Ahmad Nazrul Abd. Wahid of Agrotechnology and Bioscience Division, Malaysian Nuclear Agency (Nuclear Malaysia) (formerly Malaysian Institute for Nuclear Technology Research (MINT) for technical assistance in ^{15}N field application and assays, to MINTec-SINAGAMA, Nuclear Malaysia for gamma irradiation services.

Gratitude is also expressed to all lecturers at Department of Land Management, UPM, Mrs. Zarinah Basir, Mr. Dzulkifli Duaji, Mr. Alias Tahar, Mr. Junaidi Jaafar, Mr. Abd. Rahim Utar, Mr. Jamil Othman and my colleagues Dr. Premalatha Pakirisamy, Illani Zuraihah Ibrahim, Mohd Fauzi, Khairul Muna, Azuan Sukri and others for their continuous comments, advice and assistance throughout the study.

The research project was made feasible by the financial support from Ministry of Science, Technology and Innovation for research funding (IRPA (EA): 09-02-04-0497-EA001) and a scholarship from Universiti Putra Malaysia under the Tutorship Scheme.

I am deeply indebted to my wife Masnindah Malahubban, my daughter Zunah Farwizah, my parents and all my in-laws for their sacrifices and patience during the course of my graduate study.

I certify that an Examination Committee has met on 21 April 2008 to conduct the final examination of Zakry Fitri bin Ab. Aziz on his Master of Science thesis entitled "Nitrogen Fixation and Vegetative Growth of Immature Oil Palm Inoculated with *Bacillus sphaericus* (strain UPMB-10)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Master of Science.

Members of the Examination Committee were as follows:

Mohd. Rafii Yusop, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Zakaria Wahab, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Halimi Mohd. Saud, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Abdul Jalil Abdul Kader, Ph.D.
Professor
Pusat Pengurusan Penyelidikan dan Persidangan
Universiti Sains Islam Malaysia
(External Examiner)

____

HASANAH MOHD. GHAZALI, Ph.D.
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Zulkifli Hj. Shamsuddin, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Anuar Abd. Rahim, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Zin Zawawi Hj. Zakaria, PhD
Senior Research Fellow
Biology Division
Malaysian Palm Oil Board
(Member)

Khairuddin Abd. Rahim, PhD
Senior Research Officer
Bioscience and Agrotechnology Division
Malaysian Nuclear Agency (Nuclear Malaysia)
(formerly Malaysian Institute for Nuclear Technology Research)
(Member)

AINI IDERIS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 July 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

ZAKRY FITRI BIN AB. AZIZ

Date: 7 May 2008

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

PGPR	Plant Growth Promoting Rhizobacteria
BNF	Biological Nitrogen Fixation
Uninoculated - N_i	killed (Gamma-irradiated) *Bacillus sphaericus* UPMB-10, without inorganic-N fertilizer
Uninoculated + 2/3N_i	killed (Gamma-irradiated) *Bacillus sphaericus* UPMB-10, 2 per 3 inorganic-N fertilizer
Inoculated + 2/3N_i	*Bacillus sphaericus* UPMB-10, 2 per 3 inorganic-N fertilizer
Uninoculated + N_i	killed (Gamma-irradiated) *Bacillus sphaericus* UPMB-10, with inorganic-N fertilizer
D_{120}, D_{240}	Days after inoculation (120 and 240)
N_i	Inorganic-N fertilizer
% ^{15}N a.e.	Percentage of ^{15}N atom excess
% ^{15}N $a.e._{corr.}$	Percentage of ^{15}N atom excess corrected
% Ndfa	Percentage of Nitrogen derived from atmosphere
% Ndff (labelled)	Percentage of Nitrogen derived from labelled fertilizer
% Ndff (fertilizer)	Percentage of Nitrogen derived from unlabelled fertilizer
% Ndfs	Percentage of Nitrogen derived from soil
N_2 fixed, g $palm^{-1}$	Total amount of N_2 fixed, gram per palm
WAE	Weighted atom excess
SEM	Standard error of mean
ANOVA	Analysis of Variance
N	Nitrogen
P	Phosphorous
K	Potassium

Mg	Magnesium
GOPF	Ground Oil Palm Frond
cfu	Colony Forming Unit

CHAPTER 1

INTRODUCTION

Oil palm is the major agricultural crop in Malaysia with 4.17 million hectares planted by 2006 (Wahid, 2007), or about two-thirds the national agricultural area. However, over the years, its profitability has been eroded by the rising cost of production. One of the major costs is the inorganic fertilizer input, of which nitrogen, the most used and the most expensive per unit weight, is the major component. At a typical recommended rate of 1.0 kg N/mature palm/year (Corley and Tinker, 2003) and an average urea (46% N) price of RM1,475/tonne (IRM, 2008), the cost to fertilize one hectare (148 palms) would be RM475 for the N-input alone, and this inorganic fertilizer cost is ever increasing. Another concern with mineral fertilization is the effect of its excessive application on the ecosystem and pollution of the ground water, and soil acidification. Therefore, any possible alternatives to N fertilization would be greatly welcomed.

Associative biological nitrogen fixation (BNF) by bacteria (e.g. *Azospirillum* and *Bacillus* spp.) with non-leguminous crops is seriously being considered in attempts to develop sustainable agriculture. This low-input biotechnology can be exploited to promote environmentally friendly crop production (Barea *et al*., 2005). BNF can eliminate or, at least, reduce N_i fertilization and reduce the cost of crop production. This would be especially attractive in the tropics where there is a more urgent need to produce more food with more limited resources to do so (Cocking, 2000).

The obvious question in Malaysia is to apply BNF to oil palm to make palm oil production more sustainable and environmentally friendly (Dobereiner and Baldani, 1998; Shamsuddin *et al*., 2000). Amir (2001) found that a locally isolated N_2-fixing rhizobacterium, *Bacillus sphaericus* UPMB-10, promoted the growth of inoculated oil palm seedlings *in vitro* (laboratory and glasshouse). Similar evidence was also observed in the nursery (Amir *et al*., 2005). However, no *in vivo* work has been reported, and such studies would be required for concrete evidence of N_2 fixation (James, 2000) and real increase in growth or yield under field conditions. As a step forward, this study seeks to assess the effect of *Bacillus sphaericus* UPMB-10 inoculation on N_2 fixation, N partitioning and dry matter production by young, immature oil palm (from field planting of seedlings until 30 months old) in the field. The specific objectives are to:

1. Quantify the amount of N_2 fixed by *Bacillus sphaericus* UPMB-10 inoculated on young, immature oil palm.
2. Assess the effect of *Bacillus sphaericus* UPMB-10 inoculation on the vegetative growth and dry matter production of the palms.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm (*Elaeis guineensis* Jacq.)

The oil palm belongs to the family *Palmaceae* and the genus *Elaeis.* There are two important species in the genus *Elaeis* - *E. guineensis* (Jacq.), the African oil palm and *E. olifeira,* the South American oil palm. By far the most important species economically is the former which, unless stated otherwise, will be the only one discussed hereinafter. The natural habitat of the oil palm is the humid tropics – a narrow belt from 10^{o}N to 10^{o}S straddling the equator and at low altitude (below 500 m). The optimum temperature is 22°C to 32°C. It requires a high rainfall of 1,800 – 2,500 mm but, more importantly, evenly distributed throughout the year with no marked and severe dry periods (Corley and Tinker, 2003).

The plant is a monocotyledon of the Order *Spadiciflorae* with a single growing point from which fronds emerge in a regular sequence at a rate of 20-26 a year. It is monoecious, generally requiring cross-pollination as it produces separate male and female inflorescences from axillary buds in alternate phases. The fruits (drupes) are borne in branches and consist of an orange-colored mesocarp which contains 'palm oil', a hard lignified shell (endocarp) and a white kernel containing 'kernel oil'. The number of fruits per bunch varies from 50 to 100 in the small bunches of young palms to 1000-3000 in the larger bunches from older palms. Individual bunches weigh from less than 1 kg to 20-50 kg. The economic life of oil palm is about 20-25 years, above which it becomes too tall for harvesting (Corley and Tinker, 2003).

The oil palm seed requires three months to germinate and establish itself as a young seedling capable of nutrient absorption from the soil and photosynthesis. After three to four months, the base of the stem of seedling becomes a swollen ‘bulb’ and the first true primary roots emerge at an angle of 45° from it. These primary roots are thicker than the radicle. Secondary roots grow out in all directions. Subsequently, the leaves become successively larger and change in shape. The first few leaves are lanceolate with a midrib to half their lengths; two veins proceed from the end of this midrib to the tip of the leaf. In later leaves, a split appears between these veins and the leaf becomes bifurcate. This type of leaf is quickly followed by leaves in which splits divide the laminae between the veins into leaflets or pinnae, although the latter are still joined to one another at the apex. Then the leaflets become entirely detached, the tip of the leaflet being the last to become entirely unattached (Corley and Tinker, 2003).

The main product is palm oil, which is expressed from the fruit mesocarp, palm kernel oil, expressed from the kernel (nut), and palm kernel cake, the residue of the kernel after oil expression. The two oils have different chemical compositions and, therefore, different chemical and physical characteristics, and are used in different applications. Palm oil mainly contains (saturated) palmitic acid and (monounsaturated) oleic acid, and is