UNIVERSITI PUTRA MALAYSIA

NITROGEN FIXATION AND PLANT GROWTH ENHANCEMENT BY BENEFICIAL RHIZOBACTERIA IN ASSOCIATION WITH OIL PALM SEEDLINGS

AMIR HAMZAH BIN AHMAD GHAZALI

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AMIR HAMZAH BIN AHMAD GHAZALI

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Chairman : Associate Professor Dr. Zulkifli Hj. Shamsuddin

Faculty : Agriculture

Nitrogen fertilizer is the most expensive nutrient input in oil palm production with average total estimated cost amounting to RM 470 million year^{-1}. Rapid losses of N fertilizer through leaching further increases the cost on palm oil production and makes the sector less profitable. Recently there is a new finding about an associated N_2 fixing rhizobacteria which could be applied to the oil palm seedling production sector. The beneficial rhizobacteria has been reported to be efficient in N_2 fixation and stimulates plant growth, thus could be used as a biofertilizer and bioenhancer, respectively, for various leguminous and non-leguminous crops. Therefore, a series of experiments were carried out to estimate the N_2 fixing capacity of the rhizobacteria and to observe the effects of inoculation on growth and development of the associated oil palm seedlings.

Results from the initial experiment indicated that *Azospirillum* could contribute 40% of the total N requirement of oil palm plantlets through N_2 fixation process, stimulated total dry matter accumulation and root growth, stimulated uptake and higher concentration of N and K and increased the host photosynthetic rates compared to the

control, after 120 days of planting (D_{120}). The subsequent pot experiment (glasshouse experiment; harvested after 130, 260 and 390 days of planting (D_{130}, D_{260} and D_{390})) demonstrated the potential of *Azospirillum brasilense* Sp 7 and locally isolated *Bacillus sphaericus* UPMB 10 and *Bacillus subtilis* UPMB 13 as a biofertilizer for oil palm seedlings through N_2 fixation (25-50% Ndfa; Nitrogen derived from atmosphere) and stimulated higher concentration of essential nutrients and their accumulation especially N, P and K. The inocula also enhanced the development of roots (root dry weight, volume, primary root numbers and high R/S ratio) and tops (total dry matter, top dry weight, chlorophyll content, leaf area, plant height and stem diameter) of the host plants. Generally, effects of the inoculation at two monthly intervals were more promising during the earlier growth stage (D_{130}-D_{260}) with similar or better response compared to the control with complete N_i fertilization (Sp 7 k +N_i) especially for all root growth observations, top dry weight and total dry matter of the host plants. However, this improvement in plant growth was less than the control with complete N_i fertilization after an intentionally prolonged nursery phase, D_{390}. A duplicated experiment as above was carried out in the field nursery station, FELDA Bukit Mendi, Pahang. The inoculation process showed positive response in enhancing higher concentration and accumulation of N, P and K for the host plants especially at D_{130}, enhanced root growth (root dry weight, volume, primary root number, and R/S ratio) until end of the growth stages (D_{390}) and top growth which includes total dry matter, top dry weight and chlorophyll content of the host plants (until D_{260}).

The *in vitro* N_2 fixation study using (^{15}N) dilution method clearly showed an increase in N_2 fixation capacity (67% Ndfa) for the Sp 7 strain associated with tissue-cultured oil palm plantlets, at D_{56}. Similarly, the locally isolated UPMB 10 and UPMB 13

strains could contribute up to 55% Ndfa. The inoculation process also caused significant increase in total N and higher leaf chlorophyll content of the host plants, although the response was still less compared to the control with complete N_i fertilization. The enhancement in root growth and dry matter production were observed to produce comparable effects to those supplied with complete inorganic-N. All the inocula tested could successfully colonize the root-surface and improve the host plant growth.

The above findings provided evidence that *Azospirillum* and locally isolated *Bacillus* spp. are potentially effective biofertilizers and bioenhancers for sustainable oil palm seedling production. However, more frequent inoculation and higher inoculum size will be required to maintain the inoculum population in the soil especially during a prolonged growth phase of the host plants (D_{390}) under an extended nursery condition.

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

PENGIKATAN NITROGEN DAN PENINGKATAN TUMBESARAN POKOK OLEH RHIZOBAKTERIA BERFAEDAH DALAM ASSOSIASI DENGAN ANAK BENIH KELAPA SAWIT

Oleh

AMIR HAMZAH BIN AHMAD GHAZALI

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Fakulti : Pertanian

Baja nitrogen merupakan sumber nutrien yang paling mahal bagi perusahaan kelapa sawit dan melibatkan purata kos hampir RM 470 juta tahun^{-1}. Kehilangan baja nitrogen melalui proses larut-lesap merupakan antara faktor yang mempengaruhi peningkatan kos pengeluaran kelapa sawit dan seterusnya mengurangkan profit sektor ini. Akhir-akhir ini terdapat penemuan baru berkenaan rhizobakteria pengikat N_2 yang berpotensi untuk diaplikasikan kepada sektor pengeluaran anak benih kelapa sawit. Rhizobakteria berfaedah ini di laporkan amat berkesan bagi proses pengikatan N_2 (bio-baja) dan penggalak tumbesaran pokok (bio-penggalak) bagi beberapa jenis perumah kekacang dan bukan kekacang. Oleh yang demikian, beberapa siri eksperimen telah dijalankan untuk menganggarkan keupayaan pengikatan N_2 dan melihat kesan inokulasi rhizobakteria tersebut terhadap pertumbuhan dan perkembangan anak benih kelapa sawit.

Keputusan daripada kajian awal menunjukkan bahawa proses inokulasi *Azospirillum* dapat menyumbang sehingga 40% daripada jumlah keseluruhan N yang

diperlukan oleh anak benih kelapa sawit melalui proses pengikatan N_2, meningkatkan berat kering total pokok dan pertumbuhan akar, meningkatkan kadar pengambilan N dan K dan peningkatan kadar fotosintesis perumah berbanding kawalan selepas ditanam selama 120 hari (D_{120}). Kajian berpasu berikutnya (rumah kaca; pengumpulan data pada 130, 260 dan 390 hari selepas penanaman (D_{130}, D_{260} and D_{390})) menunjukkan potensi *Azospirillum brasilense* Sp 7, isolat tempatan *Bacillus sphaericus* UPMB 10 dan *Bacillus subtilis* UPMB 13 sebagai bio-baja untuk anak benih kelapa sawit berdasarkan 25-50% kadar pengikatan N_2 (%Ndfa) dan meningkatkan kadar pengambilan dan pengumpulan nutrien penting terutamanya N, P dan K. Inokulum yang digunakan juga dapat menggalakkan perkembangan akar (berat kering akar, isipadu, bilangan akar primer dan nisbah akar/pucuk 'R/S ratio' yang lebih tinggi) dan pucuk (berat kering total, berat kering pucuk, kandungan klorofil, keluasan daun, ketinggian pokok dan diameter batang) perumah. Secara amnya, kesan inokulasi adalah lebih baik pada peringkat awal pertumbuhan (D_{130}-D_{260}) selepas beberapa siri proses inokulasi pada kekerapan setiap dua bulan dengan kesan yang setara dan lebih baik berbanding kawalan dengan pembajaan penuh N (Sp 7 k +N_i) terutamanya bagi perkembangan pertumbuhan akar pokok, berat kering pucuk dan berat kering total perumah. Walau bagaimanpun kesan positif tersebut mulai manurun berbanding kawalan dengan pembajaan penuh N_i selepas fasa penanaman di tapak semaian dilanjutkan sehingga D_{390}. Satu kajian yang sama seperti kajian yang di atas telah dijalankan di ladang tapak semaian kelapa sawit, FELDA Bukit Mendi, Pahang. Proses inokulasi yang dijalankan telah menunjukkan kesan positif bagi pengumpulan dan pengambilan N, P dan K untuk perumah terutamanya pada D_{130}, meningkatkan pertumbuhan akar (berat kering akar, isipadu, bilangan akar primer dan nisbah akar/pucuk 'R/S ratio' yang lebih tinggi) sehingga D_{390}. Pertumbuhan pucuk

perumah juga meningkat dengan proses inokulasi tersebut terutamanya jumlah berat kering, berat kering pucuk dan kandungan klorofil perumah (sehingga D_{260}).

Kajian berikutnya, melibatkan kajian pengikatan N_2 *in vitro* ('^{15}N isotope dilution method') yang menunjukkan keupayaan pengikatan N_2 sehingga 67% Ndfa bagi inokulasi Sp 7 ke atas tisu kultur kelapa sawit pada D_{56}. Sementara inokulasi isolat tempatan strain UPMB 10 dan UPMB 13 dapat menyumbang sehingga 55% Ndfa. Proses inokulasi tersebut juga dapat meningkatkan kandungan N total dan klorofil perumah walaupun kesannya masih rendah berbanding kawalan dengan pembajaan penuh N_i. Peningkatan pertumbuhan akar dan berat kering perumah adalah sama seperti kawalan dengan pembajaan penuh N_i. Kesemua inokula yang dikaji menunjukkan keupayaan kolonisasi permukaan akar tisu kultur kelapa sawit yang seterusnya membantu pertumbuhan perumahnya.

Penemuan di atas dapat membuktikan bahawa *Azospirillum* dan isolat tempatan *Bacillus* spp. mempunyai potensi yang baik sebagai penggalak (bio-baja dan bio-penggalak) pertumbuhan lestari anak benih kelapa sawit. Kekerapan inokulasi yang lebih tinggi dan saiz inokulum yang lebih besar adalah amat diperlukan untuk mengekalkan populasi inokula yang tinggi di dalam tanah untuk tempoh pertumbuhan perumah yang lebih lama (D_{390}) di tapak semaian.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES