UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF KAEMPFEROL AND NUTRIENTS ON ARBUSCULAR MYCORRHIZAL COLONIZATION AND GROWTH OF OIL PALM SEEDLINGS

NUR WALIDAH BINTI AHMAD

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By

NUR WALIDAH BINTI AHMAD

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

September 2017

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DEDICATION

To

My husband

Sharazul Fikri Mohd Salleh

My parents

Ahmad Musa
Aishah Ibrahim

And

Family
Friends

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

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September 2017

Chairman : Associate Professor Radziah Othman, PhD
Institute : Tropical Agriculture and Food Security

Arbuscular mycorrhizal fungi (AMF) can form potentially beneficial associations with the roots of more than 80% of terrestrial plants. Plant roots release a wide range of compounds which are involved in complex communication processes in the rhizosphere. One of the alternative methods to decrease fertilizer application in oil palm is via AMF inoculation. Flavonoid exudates of oil palm roots can be important in the interaction between oil palm and AMF. This study aims to characterize the flavonoid in oil palm root exudates and determine the influence of this flavonoid on AMF colonization of oil palm roots. A series of experiments were thus conducted under laboratory and glasshouse conditions with the following objectives: i) to characterize the flavonoid secreted from oil palm root exudates; ii) to determine the effect of root flavonoids on the establishment of AMF in oil palm roots; and iii) to determine the effect of nitrogen and phosphorus on root exudate compositions, AMF colonization and oil palm growth. Oil palm root exudates were screened for flavonoid compounds using liquid chromatography. Kaempferol was identified as the flavonoid existing in the oil palm root exudates. The effect of kaempferol on the establishment of AMF in oil palm roots was then evaluated under glasshouse conditions, followed by study on the effects of nitrogen and phosphorus on flavonoid production. Exogenous kaempferol was applied in four concentrations (0, 2.5, 5.0 and 10.0 ppm) in two mycorrhiza treatments (*Glomus mosseae* and mixed AM species) and four fertilizer treatments (P0N0, P0N1, P1N0 and P1N1). The results showed that addition of kaempferol positively affected infection of *G. mosseae*, but not infection of the mixed AM species that contained a mixture of *G. mosseae* and *Scutelospora sp.* Hoagland nutrient solution was applied as the nutrient source. At sixth week of growth, the roots were harvested from the soil, and debris and soil particles washed off. The root development was observed through a root scanner. The results showed that kaempferol was the dominant flavonoid produced by the oil

palm roots. The highest amount of flavonoid (42.57 mg mL^{-1}) was secreted at 31 days of growth. Oil palm seedlings inoculated with *G. mosseae* in the presence of 10 ppm kaempferol showed improved growth compared to the plants inoculated with mixed AM species, in the treatments over a six week period. The second experiment proved that nitrogen and phosphorus fertilizers influenced AM colonization and growth of oil palm seedlings. The application of fertilizer resulted in this effect when the complete fertilizer requirement was applied. Plant growth was affected by *G. mosseae at* 10 ppm. The availability of P affects the plant biomass, in contrast to N. When complete fertilizer was applied, the *G. mosseae* inoculated plants showed the highest root infection at 10 ppm kaempferol, with 96.11 % infection. The N uptake was significant when complete fertilizer was given to the *G. mosseae* inoculated plants and non-inoculated plants, at 3.18 mg/plant (10 ppm) and 3.26 mg/plant (0 ppm), respectively. Application of AMF and kaempferol significantly affected oil palm root development.

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

PENGARUH KAEMPFEROL DAN NUTRIEN PADA KOLONISASI KULAT ARBUSKULAR MIKORIZA DAN PERTUMBUHAN ANAK KELAPA SAWIT

Oleh

NUR WALIDAH BINTI AHMAD

September 2017

Pengerusi : Professor Madya Radziah Othman, PhD
Institut : Pertanian Tropika dan Sekuriti Makanan

Kulat arbuskular mikoriza (AMF) boleh membentuk gabungan yang berpotensi, yang memberi manfaat kepada lebih 80% akar tumbuhan daratan. Akar tumbuhan merembeskan sebilangan besar sebatian yang terlibat dalam proses komunikasi kompleks di persekitaran akar. Salah satu daripada kaedah alternatif untuk mengurangkan penggunaan baja di kelapa sawit adalah melalui inokulasi AMF. Rembesan flavonoid dari akar kelapa sawit boleh menjadi penting dalam interaksi antara kelapa sawit dan AMF. Kajian ini bertujuan untuk mencirikan flavonoid dalam akar kelapa sawit dan menentukan pengaruh flavonoid ini terhadap kolonisasi AMF pada akar kelapa sawit. Satu siri percubaan telah dijalankan di bawah keadaan makmal dan rumah kaca dengan objektif berikut: i) mencirikan flavonoid yang dirembeskan dari rembesan akar kelapa sawit; ii) untuk menentukan kesan flavonoid akar pada pertumbuhan AMF dalam akar kelapa sawit; dan iii) untuk menentukan kesan nitrogen dan fosforus pada komposisi rembesan akar, kolonisasi AMF dan pertumbuhan kelapa sawit. Rembesan akar kelapa sawit ditapis untuk sebatian flavonoid menggunakan cecair kromatografi. Kaempferol dikenalpasti sebagai flavonoid yang ada dalam rembesan akar kelapa sawit. Kesan kaempferol pada pertumbuhan AMF dalam akar kelapa sawit kemudiannya dinilai di bawah keadaan rumah kaca, diikuti oleh kajian mengenai kesan nitrogen dan fosfor pada pengeluaran flavonoid. Kaempferol luaran digunakan dalam empat kepekatan (0, 2.5, 5.0 dan 10.0 ppm) dalam dua rawatan mycorrhiza (*Glomus mosseae* dan spesis AM campuran) dan empat rawatan baja (P0N0, P0N1, P1N0 dan P1N1). Hasilnya menunjukkan bahawa penambahan kaempferol positif dalam jangkitan *G. mosseae*, tetapi tidak jangkitan spesies AM campuran yang mengandungi campuran *G. mosseae* dan *Scutelospora sp*. Nutrien Hoagland lengkap digunakan sebagai sumber nutrien. Pada minggu keenam pertumbuhan, akar dituai dari tanah, dan serpihan dan zarah tanah dibasuh. Perkembangan akar diperhatikan melalui pengimbas akar.

Hasilnya menunjukkan bahawa kaempferol adalah flavonoid dominan yang dihasilkan oleh akar kelapa sawit. Jumlah flavonoid tertinggi (42.57 mg mL^{-1}) direkodkan pada hari 31 pertumbuhan. Anak benih kelapa sawit yang diletakkan dengan *G. mosseae* dengan 10 ppm kaempferol menunjukkan peningkatan yang lebih baik berbanding kelapa sawit yang diletakkan dengan spesies AM campuran, dalam rawatan sepanjang tempoh enam minggu. Eksperimen kedua membuktikan bahawa baja nitrogen dan fosforus mempengaruhi kolonisasi AM dan pertumbuhan anak kelapa sawit. Penggunaan baja menghasilkan kesan ini apabila keperluan baja lengkap digunakan. Pertumbuhan kelapa sawit dipengaruhi oleh *G. mosseae* pada 10 ppm kaempferol. Ketersediaan P memberi kesan kepada biomas tumbuhan, berbeza dengan N. Apabila baja lengkap digunakan, tumbuhan yang diletakkan *G. mosseae* menunjukkan jangkitan akar tertinggi pada 10 ppm kaempferol, dengan jangkitan 96.11%. Pengambilan N adalah penting apabila baja lengkap diberikan kepada tumbuhan dan tanaman yang tidak diletakkan *G. mosseae*, masing-masing pada 3.18 mg/ tumbuhan (10 ppm) dan 3.26 mg/ tumbuhan (0 ppm). Penggunaan AMF dan kaempferol ketara mempengaruhi pertumbuhan akar kelapa sawit.

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I certify that a Thesis Examination Committee has met on 20 September 2017 to conduct the final examination of Nur Walidah binti Ahmad on her thesis entitled "Influence of Kaempferol and Nutrients on Arbuscular Mycorrhizal Colonization and Growth of Oil Palm Seedlings" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Hamdan bin Jol, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Puteri Edaroyati binti Megat Wahab, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Amir Hamzah bin Ahmad Ghazali, PhD
Associate Professor
Universiti Sains Malaysia
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 April 2018

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

Radziah Othman, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Kamaruzaman Sijam, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
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Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Kamaruzaman Sijam

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LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
AM	Arbuscular Mycorrhizal
IAA	Indoleacetic Acid
FFB	Fresh Fruit Bunch
WWF	World Wildlife Fund
CoA	Coenzyme A
PGPR	Plant Growth Promoting Rhizobacteria
VAM	Vesicular Arbuscular Mycorrhiza
AMT1	Ammonium Transmembrane Transporter
ATP	Adenosine triphosphate
MPOB	Malaysian Palm Oil Board
L	Litre
HPLC	High Perfomance Liquid Chromatography
UV	Ultra Violet
DAD	Diode Array Detector
NH4F	Ammonium fluoride
SPAD	Soil Plant Analysis Division: Chlorophyll meter
PTFE	Polytetrafluoroethylene

CHAPTER 1

INTRODUCTION

Malaysia is one of the leading countries in producing palm oil in the world, which currently produces about 21,000,000 metrics tonnes of palm oil annually (globalpalmoilproduction.com, 2017). Several factors affect the growth and yield of oil palm, which include temperature, annual precipitation and number of months which receive less than 100 mm of precipitation (Johannes *et al.*, 2016).

For high yield production, high amount of fertilizers is required. Excessive amounts of fertilizers, especially inorganic nitrogen and phosphorus, may not be taken up by the plant efficiently and may be lost through leaching process, escaping into the natural ecosystem and causing alterations to the natural ecosystem (Phosri *et al*., 2010). The increasing chemical fertilizer prices also require producers and agriculturalists to make efforts to reduce costs by increasing the efficiency in chemical fertilizer application in oil palm plantations as well as identifying other nutrient sources that can be readily and reliably used to support plant growth, including bio fertilizers (Taryo *et al.*, 2006, Timothy, 2014).

High levels of chemical fertilizers have numerous disadvantages in relation to plant growth and the environment. The use of high inorganic nitrogen fertilizers can lead to nitrous oxide production, resulting in increase in temperature and losses of N through leaching in the form of nitrates and volatilization of ammonia. The usage of biofertilizers is becoming of significant importance as an alternative to reduce the detrimental effects of chemical fertilizers, thus reducing the harmful effects to the environment and humans (Azlin *et al*., 2009).

Plant roots release an enormous range of prospectively valuable compounds which are involved in complex communication processes in the rhizosphere. Flavonoids are one of such compound released as root exudates. They are one of the largest groups of polyphenolic compounds and plant secondary metabolites, and they play an important role in plant development, as defence and signalling compounds in reproduction, pathogenesis and symbiosis, such as that in the legumes and Rhizobium symbiosis (Eva *et al*., 2006). A number of flavonoids exhibit a strong stimulatory effect on AMF hyphal growth and this seems dependent upon the chemical structure of the compound itself. Flavonoids are well known to have interactions with microbes, such as that in nitrogen fixing (Steinkellner *et al*., 2007).

The most significant rhizosphere mutualisms mentioned are between mycorrhizae or rhizobacteria and plants. Mycorrhizae involved in improving uptake of macro and micronutrients, help in the beneficial alterations of plant growth promoting rhizobacteria (PGPR) and in proliferating tolerance to stresses by affecting water

relations and pathogen resistance. Further, the arbuscular mycorrhiza fungi (AMF) provide other benefits to their host plants. They are able to improve mineral nutrition as well as increase tolerance to water stress, encourage greater resistance to pathogens and lessen sensitivity to toxic substances that exist in the soil. The mutualistic symbiosis of most land plants with AMF has been revealed to favor water and mineral nutrition and to increase resistance to abiotic and biotic stresses (Lioussanne, 2010).

As many agricultural chemicals are implicated in human toxicity and negative environmental impacts, biofertilizers, such as microbial inoculants and mycorrhiza, can be used instead to promote plant growth and productivity (Mia *et al*., 2010). Arbuscular mycorrhizal fungus has the ability, similar to other microorganisms such as phosphate-solubilizing bacteria and nitrogen fixing bacteria, to ensure that oil palm cultivation becomes sustainable and help to decrease the effect of chemicals on biodiversity (Phosri *et al*., 2010, Naher *et al*., 2013). The AMF symbiosis works by AMF penetrating into root cortical cells and subsequently its hyphae proliferate into the soil to get mineral nutrients and water, other than contributing to improve the soil structure (Sajid *et al.*, 2014).

Colonization by AMF can be influenced by the compounds present in the plant's rhizosphere. Plant roots release a broad range of compounds which are involved in the complicated interaction processes in the rhizosphere. The root exudates comprise of numerous phenols, organic acids, dead cell lysates and sloughed off roots. Flavonoids are key indicator molecules for plant microbe interactions in soil, such as AMF, nitrogen-fixing rhizobia and fungal pathogens such as the *Fusarium* species (Steinkellner *et al*., 2007). Flavonoid is able to influence AMF by increasing the level of AMF colonization (Scervino *et al*., 2005). These studies have also described the effects of flavonoids on hyphal differentiation, hyphal growth, and root colonization (Steinkellner *et al*., 2007; Scervino *et al*., 2005). Nevertheless, there are few reports that focus on evaluating the interactions of each flavonoid with AMF (Steinkellner *et al*., 2007).

This study aimed to:

i) Determine the effect of root flavonoids on the establishment of AMF in oil palm roots and
ii) ii) Determine the effect of phosphorus and nitrogen on root exudate compositions, AMF colonization and oil palm growth.

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