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MYCORRHIZAL SYMBIOSIS FOR ENHANCEMENT OF NURSERY GROWN TECTONA GRANDIS L. AND GMELINA ARBOREA ROXB.

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By

NOOR FAIQOH MARDATIN

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

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Arbuscular mycorrhizal (AM) fungi are soil fungi which form mutualistic associations with plant roots which often promote plant growth through increased mineral nutrient acquisitions from the highly weathered tropical soils. In the field, seedlings infected with mycorrhiza often survive and grow better than the controls. Thus this study aims to evaluate the effectivity of AM in affecting growth of two forest plantation species i.e. *Tectona grandis* and *Gmelina arborea* seedlings. The effectivity of these AM inocula were assessed using both quantitative and qualitative analyses.

This study involved the use of two types of AM designated as MM and IM from different origin. The effectivity of these AM inocula were initially evaluated based on standard protocols; spore count and MPN bioassay. Following that, effectivity of these inocula were also assessed based on growth performance of *T. grandis* and *G. arborea*. The seedlings were grown in a controlled environment in the glasshouse for 21 weeks and growth measurements were taken every three weeks. This nursery experiment adopted a randomised complete factorial design of three media and four inocula treatments with six replications. In addition, isozyme analysis and root colonisation for host preference were conducted to support this study.

Laboratory assessment of inocula indicated lower spore number (24 spores per 10 g inoculum) and MPN bioassay (87.5 propagules per g inoculum) in MM. Inoculum MM comprised three spore types from genera *Glomus*, *Gigaspora*, and *Scutellospora*. On the other hand, IM recorded a higher spore number (55 spores per 10 g inoculum) and MPN bioassay (325.0 propagules per g inoculum). Eventhough, IM comprised three spore types as in MM, only two were similar, the exception was *Acaulospora*.

Generally, the growth responses of the seedlings were affected significantly by media, AM inocula and their interactions. It was found

that both inocula (MM and IM) responded similarly (no significant difference) but when compared to their controls they showed significant difference at ($P<0.05$) in all growth parameters measured. Morphological observations also revealed that the AM treated seedlings were more vigorous than their controls. Inoculum IM showed significantly higher mean values of photosynthetic rate (4.96 and 4.72 mol/m²/s) and chlorophyll content (358.33 and 689.11 SPAD porometer) for *T. grandis* and *G. arborea* respectively than MM. Zymogram patterns revealed by isozyme analysis of alkaline phosphatase (ALP) indicated the presence of IM spores in the roots of these seedlings. Root colonisation of host preferences also indicated that MM or IM has same host preference. From this study it can be concluded that AM fungi have the potentials to improve the growth and physiological characteristics of these seedlings.

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

SIMBIOSIS MIKORIZA DALAM MENINGKATKAN PERTUMBUHAN SEMAI *TECTONA GRANDIS* L AND *GMELINA ARBOREA* ROXB.

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Kulat arbuskul mikoriza (AM) merupakan kulat tanah yang mempunyai simbiosis saling menguntungkan dengan akar-akar tanaman, yang berupaya meningkatkan pertumbuhan menerusi peningkatan penyerapan unsur nutrien dari tanah daerah tropika yang terluluhawa. Di lapangan, anak benih yang bermikoriza menunjukkan pertumbuhan yang lebih baik dan dapat hidup berterusan (mandiri) dengan baik berbanding dengan kawalan. Kajian ini bertujuan untuk menilai keberkesanan inokulum AM dalam mempertingkatkan pertumbuhan dua jenis spesis anak benih hutan iaitu *Tectona grandis* dan *Gmelina arborea.* Keberkesanan inokulum AM ini dinilai menggunakan kaedah kuantitatif dan kualitatif.

Kajian ini melibatkan penggunaan dua jenis inokulum AM yang dilabelkan MM dan IM yang mana mempunyai asalusul yang berlainan. Kesan inokulum AM pada mulanya telah dinilai berasaskan kaedah piawaian pengiraan jumlah spora dan penilaian bioasai MPN. Selanjutnya keberkesanan 2 inokulum tersebut juga dinilai berdasarkan prestasi pertumbuhan anak benih untuk dua spesis pokok ladang hutan iaitu *T. grandis* dan *G. arborea*. Anak-anak benih tersebut ditumbuhkan dalam rumah kaca yang berpersekitaran terkawal selama 21 minggu dan pengukuran pertumbuhan direkod pada setiap 3 minggu. Kajian ini menggunakan rekabentuk faktorial rawak penuh (CRD) yang melibatkan tiga jenis media dan empat jenis inokulum mikoriza dengan enam replikasi setiap rawatan. Kajian isozim dan kolonisasi akar untuk penanda kecenderungan induk juga telah dijalankan untuk menyokong kajian ini.

Keputusan makmal dalam penilaian inokulum menunjukkan penghasilan jumlah spora yang rendah (24 spora setiap 10 g inokulum) dan bioasai MPN (87.5 propagul setiap g inokulum) pada MM. Inokulum MM terdiri daripada tiga jenis spora dari genera: *Glomus*, *Gigaspora* dan *Scutellospora*. Sementara itu, IM merekodkan jumlah spora yang lebih tinggi, 55 spora setiap 10 g dan bioasai MPN pada 325.0 propagul setiap g inokulum. Walaupun IM mengandungi tiga jenis spora seperti yang terdapat pada MM, tetapi hanya dua daripadanya adalah sama jenis, kecuali genus yang terakhir ialah *Acaulospora*.

Secara amnya, pertumbuhan ke atas kedua-dua jenis anak benih menunjukkan perbezaan bererti disebabkan oleh media, inokulum AM, dan interaksi antara rawatan. Didapati kedua jenis inokulum (MM dan IM) memberi tindakbalas yang sama (tidak menunjukkan perbezaan bererti) tetapi memberi kesan yang bererti ($P<0.05$) pada semua data pertumbuhan bila dibandingkan dengan kawalan. Semua anak benih yang telah diinokulasi kelihatan lebih subur berbanding kawalan mereka. IM didapati memberi kesan yang sangat bererti pada purata kadar fotosintesis (4.96 dan 4.72 mol/m^2/s) dan kandungan klorofil (358.33 dan 689.44 SPAD porometer) masing-masing pada *T. grandis* dan *G. arborea* berbanding dengan MM. Corak zimogram dari analisis isozim dengan alkaline phosphatase (ALP) menunjukkan kehadiran spora IM di dalam akar anak-anak benih tersebut. Kolonisasi akar daripada kecenderungan induk menunjukkan bahawa samada MM ataupun IM mempunyai kecenderungan yang sama luas. Daripada kajian ini kesimpulan dapat dibuat bahawa kulat AM mampu memperbaiki pertumbuhan dan ciri fisiologi anak benih.

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ABBREVIATIONS

µm	Micro meter
Al	Aluminium
ALP	Alkaline phosphatase
AM	Arbuscular mycorrhiza
ATP	Ammonium tri phosphate
BEG	Bank of European *Glomales*
C	Carbon
Ca	Calcium
CEC	Cation exchange capacity
Cl	Chloride
cl	Confident limit
CRD	Completely randomised design
Cu	Copper
DMRT	Duncan's Multiple Range Test
DNA	Deoxyribo nucleic acid
df	Degree of freedom
dw	Dry weight
E.C.	Enzyme commission
ECM	Ectomycorrhiza
ERM	Extraradical mycelium
EST	Esterase
FAO	Food and Agriculture Organization
Fe	Ferric or Iron
FPM	Fungal plasma membrane
FW	Fungal wall
g	Gramme
GOT	Glutamate oxaloacetate transaminase

HCl	Hydrochloric acid
IM	Arbuscular Mycorrhiza from Indonesia
IRM	Intraradical mycelium
KOH	Potasium peroxide
MDH	Malate dehydrogenase
Mg	Magnesium
mm	Mixed medium
MM	Arbuscular Mycorrhiza from Malaysia
MPN	Most Probable Number
MS	Means of square
MSIs	Mycorrhiza Specific Isozyme
Na	Sodium
oc	Organic compost
P	Phosphorus
PAM	Peri-arbuscular membrane
PCR	Polymerase chain reaction
pH	Potential hydrogen
Pi	Phosphate inorganic
Po	Phosphate organic
PPF	Plant plasma fungi
PPM	Plant plasma membrane
ppm	Parts per million
R/S	Root and shoot ratio
Rf	Relative front
rpm	Rotation per minutes
sd	Standard deviation
SEAD	Selective Enrichment of Amplified DNA
STE	Sucrose Triton Extraction
ts	Topsoil
Zn	Zinc

GLOSSARY

Apoplast	:	An interconnected system in plants that consists of all the cell walls and the water that exists in the cell.
Appresorium	:	Hyphal swellings, which occur where the hypha penetrates into epidermal cell as a pre-penetrate structure.
Arbuscules	:	A repeated dichotomous branchings and reduction in hyphal width, starting from an initial trunk hyphae and ending in a fine branch hyphae. The function is to be the major site of symbiotic exchange of nutrients (C and P) with the host plant.
Aseptate hyphae	:	Hyphae that are normally without cross walls.
Autotroph	:	A living organism that can manufacture the substances it requires for its life and growth from inorganic compound which supplied with complex organic.
Auxiliary cells	:	External vesicles or accessory bodies, are clustered swellings on external hyphae. They ornamented by spines or knobs. They are only found in genera *Gigaspora* and *Scutellospora*.
Axenic	:	A pure culture of an organism usually applied to fungi and bacteria growing on artificial media under lab condition. There is only one species present.
Coarse roots	:	The distributive root system, which is responsible for mechanical support and the transport of substances between fine roots and the shoot.
Dichotomous branching	:	A symmetrical branching pattern which occurs when two branches arise simultaneously from the tip of a hyphae, plant or fungus organ and grow at the same rate.

Efflux	:	A flowing out of solution, fluid, or other materials.
Feeder/fine roots	:	The root that is responsible for nutrient and water uptake into the shoot. It forms as a major site for mycorrhiza formation.
Hartig net	:	Hyphae between cortical cells.
Heterotroph	:	An organism that obtains organic food molecules, such as carbohydrates and proteins, ready-made from other organisms. All animals and fungi are heterotrophs, as are many bacteria. Many heterotrophs are herbivores, feeding directly on plants (autotrophic organisms), which first manufacture the food by photosynthesis. Parasites and saprophytes are heterotrophs.
Hyphal mantel	:	Hyphae around the root.
Influx	:	An inflowing of solution, fluid, or other materials.
Intercellular hyphae	:	Hyphae which grow between the walls of adjacent root cells.
Interfacial	:	A connection point or linking where fungal cell and plant cell attach each other.
Internal hyphae	:	Intraradical hyphae. Aseptate hyphae, which grow within the cortex of a root to form a colony and later develop arbuscules and vesicles.
Intracellular hyphae	:	Extraradical hyphae. Hyphae which grow between the walls of root cells.
Lateral roots	:	Roots which grow from another root.
Lipids	:	Molecular containing fatty acid with a long hydrocarbon chain.

Loci/ locus	:	Specific location of position on a chromosome occupied by a particular gene, hence often used synonymously with gene.
Mannitol	:	A polyhydric alcohol, often found as a storage compound in ectotrophic mycorrhiza mantles.
Microcosm	:	A miniature representation.
Mycelium	:	A group of hyphae.
Root apex	:	The root tip which is covered by a root cap and secreted mucilage (water soluble polysaccharide).
Root hair	:	A thin hair, like cell extension of an epidermal cell on the root surface. They increase the roots contact area with the soil. Site of entry for *Rhizobium* symbiotic bacteria, but not AM fungi.
Sporocarps	:	An aggregation of spores into larger structures, which may contain specialised hyphae and can be encased in an outer layer (peridium).
Trehalose	:	A crystalline sugar ($C_{12}H_{22}O_{11}$), characteristically found in fungi.
Vesicles	:	Hyphal swelling on internal hyphae within the root cortex. They may be intercalary or terminal.

CHAPTER I

INTRODUCTION

Background

An estimated 3,870 million ha of forest area or 30 percent of the total world land area comprise 56 percent of tropical and subtropical forests, and 44 percent of temperate and boreal forests. Almost 95 percent of the land mass comes under natural forests while the rest are forest plantations. Unfortunately, tropical deforestation and degradation has negatively affected production of forest goods and services (Sauer and Andropogon Associates, 1998; Parrotta, 2000). From 1990 to 2000, the estimated net annual change in forest area was - 9.4 million ha, however the estimated rate of deforestation was 14.6 million ha and the increment rate in forest area was 5.2 million ha respectively (Food and Agriculture Organization, 2001a).

Major causes of deforestation vary from over harvesting of forest products to poor forest planning and management, over grazing, poor harvesting practices, air pollution, and the after effects from natural disasters e.g. fire, insect pests and diseases (Food and Agriculture Organization, 2001a). Land degradation due to vegetation removal or