EVALUATION OF THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN ENHANCING GROUND COVER PLANT RESPONSIVENESS, PROPAGATION AND GROWTH PERFORMANCE OF OKRA (*Abelmoschus esculentus* L.)

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
< >	More or less
ºC	Degree Celsius
nm	Nanometre
µm	Micrometre
mm	Millimetre
cm	Centimetre
m	Metre
µg	Microgram
mg	Milligram
g	Gram
kg	Kilogram
gsm	Grams per square metre
ml	Millilitre
L	Litre or length
mM	Micromolar
[P]	Concentration of phosphorus
AC	*Axonopus compressus*
ABS	Absorbance
AG	*Asystasia gangetica*
Al	Aluminium
AMF	Arbuscular mycorrhizal fungi
ATP	Adenosine triphosphate
ANOVA	Analysis of variance
APase	Acid phosphatase enzyme

Au	Auxin
BP	Before planting
C	Carbon or control (treatment)
Ca	Calcium
$CaCl_2$	Calcium chloride
$CaCl_2 \cdot 2H_2O$	Calcium chloride anhydrous
Chl.	Chlorophyll
CO_2	Carbon dioxide
COM	Commercial
DW	Distilled water
DF	Dilution factor
DNA	Deoxyribonucleic
DOA	Department of Agriculture (Malaysia)
DOSM	Department of Statistics Malaysia
et al.	et alia or and others
E	East
FAO	Food and Agriculture Organization (United Nations)
FDW	Fruit dry weight
FFW	Fruit fresh weight
Fe	Ferum
Fig.	Figure
FO	Frequency of occurrence
GA	Gibberellic acid
GCP	Ground cover plant
h	Hour
ha	Hectare
H	Height

H_2O_2	Hydrogen peroxide
$H_2PO_4^-$	Dihydrogen phosphate
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPO_4^{2-}	Hydrogen phosphate
K^+	Potassium cation
$K_2Sb_2(C_4H_2O_6)_2$	Potassium antimony tartrate
KH_2PO_4	Potassium dihydrogen phosphorus
KOH	Sodium hydroxide
L.	Linnaeus
mins	Minute
M	Molarity
Mn	Manganese
MP	*Mimosa pudica*
n	Sample size
N	Nitrogen or normality or north
NaOH	Sodium hydroxide
NH_4F	Ammonium flouride
$(NH_4)_6Mo_7O_{24}$	Ammonium molybdate
NH_4VO_3	Ammonium vanadate
NM	non-mycorrhizal
NRE	Natural Resources and Environment (Malaysia)
p-value	Significance of observational data
pH	Potential of hydrogen
pm	Post Meridium or after midday
P	Phosphorus

PALAPES	Pasukan Latihan Pegawai Simpanan
PGPR	Plant growth promoting rhizobacteria
Pi	Inorganic phosphorus
Po	Organic phosphorus
PO_4^{3-}	Phosphate
rpm	Revolutions per minute
RCBD	Randomized Complete Block Design
RDW	Root dry weight
RFW	Root fresh weight
RNA	Ribonucleic acid
sp.	Species
S	Sulfur
SDW	Shoot dry weight
SFW	Shoot fresh weight
SL	Strigolactones
SPSS	Statistical Package for Social Sciences
w	Weight
W	Width
v	Volume
VC	Vermicompost
Zn	Zinc
ZM	*Zea mays*

PENILAIAN PERANAN KULAT MIKORIZA ARBUSKULAR DALAM MEMPERTINGKATKAN DAYA BALAS DAN PROPAGASI TUMBUHAN PENUTUP TANAH, DAN PRESTASI PERTUMBUHAN OKRA (*Abelmoschus esculentus* L.)

ABSTRAK

Kulat arbuskular mikoriza (AMF) dikenali sebagai bio-baja kerana mampu menyokong penyerapan nutrien tumbuhan terutamanya P. Keserasian interaksi AMF-tumbuhan turut memberi manfaat kepada kelimpahan propagul AMF di dalam tanah. Namun, kajian terhadap tumbuhan di Malaysia untuk menentukan status AMF tumbuhan dan menilai tahap tindak balasnya terhadap AMF adalah tidak mencukupi. Justeru, kajian ini dijalankan untuk mengakses tahap tindak balas tumbuhan terhadap AMF menggunakan tumbuhan penutup tanah (GCP) dan menggunakan spesies GCP terpilih sebagai tumbuhan perangkap AMF untuk membiak propagul AMF di dalam tanah. Sampel tumbuhan dan tanah diambil daripada 24 jumlah kuadrat dari empat tapak persampelan. Dalam persekitaran rumah hijau, pembiakan inokulum AMF asal telah dijalankan menggunakan empat spesies tumbuhan perangkap; tiga spesies GCP terpilih (*Axonopus compressus*, *Asystasia gangetica* dan *Mimosa pudica*) dan satu tumbuhan perangkap rujukan (*Zea mays*). Tiga rawatan (kawalan, AMF dan AMF + 40% vermikompos) dengan lima replikat dikenakan ke atas setiap spesies tumbuhan perangkap, menjadikan sebanyak 60 tumbuhan yang diuji. Eksperimen rumah hijau kedua dilakukan untuk menguji kesan inokulum AMF yang dibiakkan daripada spesies tumbuhan perangkap yang berbeza terhadap pokok bendi (*Abelmoschus esculentus* L.). Terdapat dua rawatan tambahan iaitu kawalan dan rawatan AMF komersial. Kesemua enam rawatan dijalan dengan lima replikat, menjadikan jumlah pokok bendi yang diuji adalah 30. Semasa pensampelan lapangan, sebanyak 42

spesies GCP berjaya dikumpul dan mampu menjalinkan hubungan dengan AMF berdasarkan peratus jangkitan AMF pada akar tumbuhan dengan julat 2.86 - 80.57%. Enam spesis (*Borreria latifolia*, *Mimosa pudica*, *Asystasia gangetica*, *Desmodium triflorum*, *Ischaemum ciliare* dan *Axonopus compressus*) dianggap sebagai GCP dengan tahap tindak balas yang baik terhadap AMF. Bagi eksperimen rumah hijau, keputusan menunjukkan bahawa penambahan 40% vermikompos kepada inokulum AMF asal telah meningkatkan biojisim akar tumbuhan perangkap dengan ketara. Namun, peratus jangkitan dan kiraan spora AMF adalah lebih rendah berbanding rawatan AMF sahaja menunjukkan pertumbuhan AMF yang lemah. Penambahan vermikompos pada tahap optimumnya meningkatkan kandungan nutrien dalam tanah dan tidak meletakkan tekanan kekurangan nutrien terhadap tumbuhan perangkap. Dalam eksperimen rumah hijau berikutnya, tiada perbezaan ketara antara rawatan inokulasi AMF sama ada inokulum yang dibiakkan atau AMF komersial terhadap pertumbuhan pokok bendi. Namun, inokulum yang dibiakkan oleh *Axonopus compressuss* dicadangkan sebagai rawatan AMF terbaik kerana menunjukkan nilai tertinggi untuk beberapa parameter pertumbuhan (berat kering pucuk, berat kering buah dan berat kering per buah) dan kandungan nutiren dalam tumbuhan (P dan N). Kesimpulannya, GCP mempunyai potensi yang baik untuk berfungsi sebagai tumbuhan perangkap AMF dan mampu menyumbang kepada penghasilan inokulum AMF yang lebih kuat melalui kajian-kajian di masa hadapan.

EVALUATION OF THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN ENHANCING GROUND COVER PLANT RESPONSIVENESS, PROPAGATION AND GROWTH PERFORMANCE OF OKRA (*Abelmoschus esculentus* L.)

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are known as bio-fertilizers because they are able to support plant nutrient uptake especially P. The compatibility of the AMF-plant interaction also benefits the abundance of AMF propagules in the soil. However, studies on plants in Malaysia to determine the plants AMF status and assess their level of responsiveness towards AMF are insufficient. Therefore, this study was conducted to assess the level of plant responsiveness towards AMF using ground cover plants (GCP) and employed the selected GCP species as AMF trap plants to propagate AMF propagules in the soil. Plant and soil samples were taken from 24 total quadrats from four sampling sites. In a greenhouse setting, propagation of indigenous AMF inoculum was carried out using four species of trap plants; three selected GCP species (*Axonopus compressus*, *Asystasia gangetica* and *Mimosa pudica*) and one reference trap plant (*Zea mays*). Three treatments (control, AMF and AMF + 40% vermicompost) with five replicates were applied to each trap plant species, making a total of 60 tested plants. The second greenhouse experiment was conducted to test the effects of propagated AMF inoculums from different trap plant species on okra (*Abelmoschus esculentus* L.). There were two additional treatments which are control and commercial AMF treatments. All six treatments were done in five replicates, making the total of tested okra plants is 30. During field sampling, a total of 42 GCP species were successfully collected and they were able to establish a relationship with AMF based on the percentage of AMF infection on plant roots with

a range of 2.86 - 80.57%. Six species (*Borreria latifolia*, *Mimosa pudica*, *Asystasia gangetica*, *Desmodium triflorum*, *Ischaemum ciliare* and *Axonopus compressus*) are considered as GCP with a good level of responsiveness towards AMF. In greenhouse experiment, the results showed that the addition of 40% vermicompost to indigenous AMF inoculum significantly increased the root biomass of the trap plants. However, the infection percentage and spore count of AMF were lower compared to the AMF only treatment indicating poor AMF growth. The addition of vermicompost at its optimum level increased the nutrient content in the soil and does not put nutrient deficiency stress on the trap plants. In the following greenhouse experiment, there was no significant difference between AMF inoculation treatments either propagated inoculum or commercial AMF on the growth of okra. However, propagated inoculum by *Axonopus compressuss* was suggested as the best AMF treatment because it showed the highest values for several growth parameters (shoot dry weight, fruit dry weight and dry weight per fruit) and nutrient content in the plant (P and N). In conclusion, GCP has good potential to be functioned as AMF trap plant and able to contribute for stronger AMF inoculum production through future studies.

CHAPTER 1

INTRODUCTION

The use of chemical fertilizer in the agricultural sector is a common practice worldwide to meet the increasing food demand (Iqbal *et al*., 2021). Although chemical fertilizer excellently improved plant growth and its productivity, heavily dependant on the fertilizer causes various detrimental effects on soil and environment (Pahalvi *et al*., 2021). Intensive input of chemical fertilizer had altered and decreased soil pH to become more acidic (Jisna *et al*., 2021) thus, causing unfavourable soil conditions and weak microbial activity (Gajda *et al*., 2020). The negative impacts would extend to other areas and ecosystems due to nutrient leaching particularly from nitrogen fertilizer in nitrate form (NO_3^-)and contaminated groundwater (Padilla *et al*., 2018). Further, this nutrient leaching lead to more serious problem on human health such as baby blue syndrome and carcinoma (Hashimi and Hashimi, 2020). The employment of bio-fertilizer is another alternative to reduce the reliance on the chemical fertilizer through the activities of beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF).

Based on the preliminary study (Hassan, 2015), the growth of okra treated with 100% level of chemical fertilizer was comparable with the growth of okra treated with combined use of 50% chemical fertilizer + AMF inoculum. Therefore, full dosage of chemical fertilizer was able to be reduced as much as 50% when the plant is inoculated with AMF. Meanwhile, the effect of AMF alone was also able to increase the growth of okra. The application of chemical fertilizer at the recommended dosage completely hindered the colonization activity of the fungi.

Whereas, half strength of chemical fertilizer from the recommended dosage caused significant decrease of the colonization.

The suppression on AMF colonization caused by chemical fertilizer was due to the alteration of soil environment especially soil acidity that increased along with the addition levels. However, AMF as ubiquitous soil microorganism in various soil conditions indicating that the fungi also have resilient ability towards acidic soil condition. According to Entry *et al*. (2002), the response of AMF towards the changes of the environmental conditions was influenced by species particularly indigenous species which naturally inhabited the respective areas. Moreover, species community of AMF that even stable in the acidic soil condition contributed to the better adaptation upon environmental changes (Wang *et al*., 2021).

Other factors influence the root colonization of AMF is plant species. A study by Dobo *et al*. (2018) showed that colonization levels of AMF were varied among different plant species. Functional group of plants also could affect that level of colonization where the grasses (Johnson *et al*., 2004) and forbs (Šmilauer *et al*., 2020) functional groups had recorded a considerably high colonization level. Aside from colonization, species and functional group of plants also lead to the difference in the community of AMF (Torrecillas *et al*., 2012).

AMF is the obligate symbiont that required host plant for living particularly as an organic carbon source (Bonfante and Desirò, 2014). In return, AMF will compensate the plant with nutrient support that is taken up through the proliferation of extraradical hyphae into the soil. The growth of AMF that reflected through colonization activity become a crucial indicator not only in evaluating the performance of plant growth but also important to evaluate the propagation of AMF

indigenous inoculum. Taking advantage of colonization variation caused by host plant species, a huge benefit can be acquired if certain plant species is spotted able to be colonized at the higher level consistently. Therefore, a strong propagated AMF inoculum can be provided for inoculation on the target plants.

Ground cover plants (GCP) have consisted of many species and several functional groups such as grasses, sedges and forbs. Generally, the common study areas in Malaysia related to the GCP were; 1) agricultural research where certain GCPs were evaluated to suppress the weeds in oil palm plantations (Samedani *et al*., 2015) and to improve soil fertility especially the leguminous species with N fixation ability (Othman *et al*., 2012), 2) environmental sciences where GCP is studying to control soil erosion (Ahmed and Teh, 2023) and water retention (Ismail *et al*., 2023) and 3) landscape architecture to implement the sustainability concept in landscape designing (Ramlee *et al*., 2023).

Nevertheless, the investigation aimed at clarifying the arbuscular mycorrhizal fungi (AMF) status for a variety of prevalent ground cover plants, including *Axonopus compressus* (*Rumput Parit*) and *Chrysopogon aciculatus* (*Kemuncup*) in Malaysia, remains sparse. Furthermore, the inherent attribute of AMF, characterized by low specificity towards particular plant species, may reveal potential advantages of ground cover plants (GCP) in mycorrhizal research. Consequently, this could enhance the value attributed to these plants, which are frequently regarded as mere weeds in numerous instances. Therefore, a pertinent research endeavor is proposed, which should encompass: 1) the identification of GCP species, 2) the mycorrhizal status of these GCP species (whether mycorrhizal or non-mycorrhizal), 3) the documented percentage of colonization levels for the GCP species, and 4) the evaluation of suitable GCP species that may function effectively as AMF trap plants.

1.1 Objectives of Study

Therefore, the main objectives of the present study are as follows:

i. i. To access the potential GCP species through the assessment of plant responsiveness levels towards AMF and choose the most suitable GCP species as AMF trap plant.

ii. To evaluate the effect of vermicompost in propagating the indigenous AMF inoculum.

iii. To determine the effect of propagated AMF inoculums by using different trap plants species on the growth of okra.

CHAPTER 2

LITERATURE REVIEW

2.1 Mycorrhiza

Kingdom of Fungi consisting of nine phylum which are Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Glomeromycota, Mucoromycota, Neocallimastigomycota, Opisthosporidia and Zoopagomycota (Naranjo-Ortiz and Gabaldón, 2019) with estimated 1.5 - 5 million species (Choi and Kim, 2017). However, the described species of fungi only represented 20% from the total species (Kendrick, 2017). Despite 148,000 fungi species has been recognized from the estimated range of 2.2 - 3.8 million total species, over 90% species remain unknown (Cheek *et al*., 2020). One of the common functions of fungi is as decomposer of organic waste materials (Yuvaraj and Ramasamy, 2020).

Ascomycota is the most dominant phylum in soil. Several studies have recorded high percentage domination of the phylum for example 39.82% in monoculture fruit tree soil (Wieczorek *et al*., 2024) and 43.5 - 75.6 % in slow filtration sand (Rosenqvist et al., 2025). According to Egidi *et al.* (2019), the dominance of Ascomycota might be due to its better performance in colonizing a wide range of environments. The species from the Ascomycota and Basidiomycota phyla have also been described the most in the Fungi kingdom (Amses *et al.*, 2022).

Meanwhile, phylum Glomeromycota is not the dominant phylum and was the latest to be included in the five true phyla of fungi along with Chytridiomycota, Zygomycota Ascomycota and Basidiomycota. The glomeromycotan has a close association with the root of plants and majority of the fungi are arbuscular mycorrhizal (AMF) type compare to other mycorrhiza types (Kosal, 2023).

Glomeromycotan species were recorded in all seven continents, 87 countries, 11 biogeographical realms, and 14 biomes (Stürmer *et al*., 2018). Meanwhile, the biomes with the largest number of AMF species were tropical and subtropical moist forests (Stürmer and Kemmelmeier, 2021).

A mycorrhiza is a symbiotic association formed between fungi and plants. The term mycorrhiza was originated from the Greek words; 'myco' means fungus and 'rhiza' means root that reflected the association established between the two organisms. Aside from symbiotic, the interaction between fungi and plant can also be pathogenic (Priyashantha *et al*., 2023). Mycorrhiza fungi are symbiont in mutualistic symbiosis with host plant by benefiting the plant growth through nutrient acquisition from the soil and transfer it into the plant. According to Brundrett (2006), three characteristics were required to define fungi-plant association as mycorrhizal; 1) the presence of localized interface of specialized hyphae, 2) synchronization of fungi-plant development and 3) plant benefits from nutrient transfer.

Most terrestrial plant species associated with mycorrhizal fungi for nutrient support and protection from stress and pathogens. Around 50,000 fungal species originated from Ascomycota, Basidiomycota, Glomeromycota and Mucoromycota were able to form mycorrhizal associations with 250,000 - 340,000 plant species (Bonfante and Anca, 2009; van der Heijden *et al*., 2015; Genre *et al*., 2020). There are seven types of mycorrhizal and four main types including arbuscular mycorrhizal (AM), ectomycorrhiza (EcM), ericoid mycorrhiza (ErM) and orchid mycorrhiza (OrM) (Brundrett and Tedersoo, 2018). The remaining types are ectendomycorrhiza, arbutoid mycorrhizal and monotropoid mycorrhizal (Smith and Read, 2008).

AM and EcM are the dominant types of mycorrhiza which colonized on numerous plant families. Whereas, ErM and OrM are particularly presence within families Orchidaceae and Ericaceae respectively (Brundrett, 2017). A study by Brundrett and Tedersoo (2018) recorded majority of vascular plants were mycorrhizal plant and the dominance types can be arranged from AM (71%), OrM (10%), EcM (2%), ErM (1.4%), nonmycorrhiza (NM, 8%) and inconsistent nonmycorrhiza-arbuscular mycorrhiza associations (NM-AM, 7%). Meanwhile, according to the global online database, the most common mycorrhiza types is AM (70%), NM (17%), EcM (8%), EcM-AM (2%), ErM (2%) and OrM (1%) (Soudzilovskaia *et al*., 2020).

A huge percentage gap can be observed between AM and the other types of mycorrhiza indicating its dominance. Besides, the data also show the presence of NM, inconsistent NM-AM and dual-mycorrhizal type of associations. The presence of NM is due to the loss of many symbiotic genes in certain plant families such as Brassicaceae (Delaux *et al*., 2014). Meanwhile, inconsistent NM-AM might due to poor growth of AMF with low formation or arbuscule representing rudimentary AM phenotype. Fig. 2.1 shows the difference in AMF growth on different host plant species resulting in AM, NM and inconsistent NM-AM type of associations. The dual-mycorrhizal association is due to ability of plants to be associated with AM and EcM. Thus, forming their respected characteristics which are arbuscule or coil structures for AM and Hartig net or similar structures for EcM (Teste *et al*., 2020).

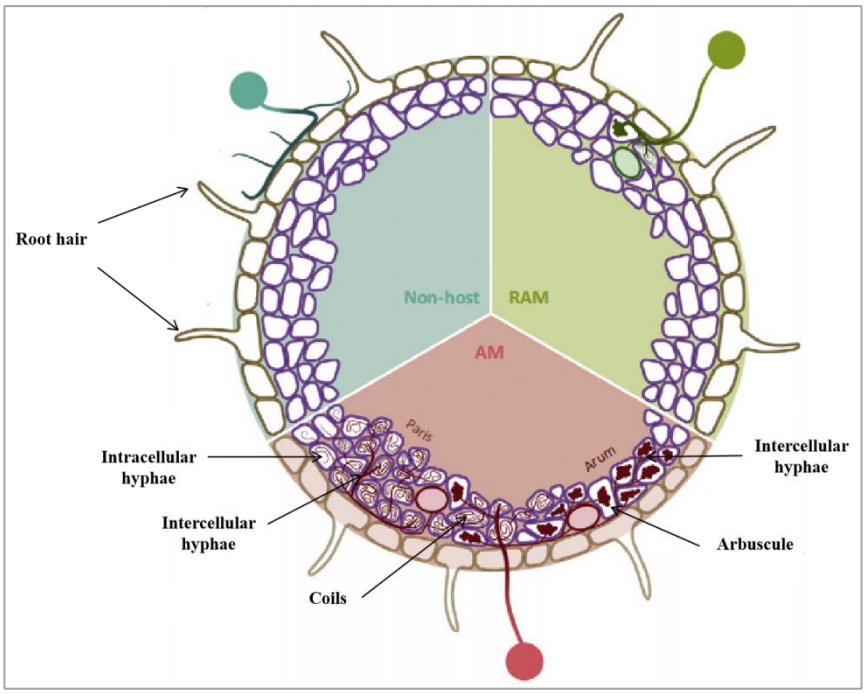

Figure 2.1: A root horizontal cross-section illustrates different host phenotypes concluded from species of the Brassicaceae plant family; the AM host phenotype shows the Arum, Paris types and intermediate of morphological colonization; the NM host phenotype in which colonization never occurs; and the rudimentary AM (RAM) host that do not form prominent AM phenotype and shows poor colonization but with only few symbiotic fungal structures (Source: Cosme *et al*., 2018).

2.2 Arbuscular Mycorrhizal Fungi

2.2.1 Characteristics

AMF was once known as vesicular-arbuscular mycorrhizal (VAM). The lack of agreement upon the name to represent the fungal was caused by the inconsistent appearance of vesicle and arbuscule structures. For example, in several recent studies, a few species of *Scutellospora* and *Gigaspora* genera were incapable to produce vesicle including Paraglomerales group (Błaszkowski *et al*., 2017) and no arbuscule presence in the senescent roots (Brundrett, 2004; Brundrett 2008).

Arbuscular mycorrhiza is a symbiotic relationship that caused by fungi belonging to the phylum Glomeromycota (Schüßler *et al*., 2001). As mentioned above, AM is the most common fungi colonizing the plant roots. AMF able to colonize more than 80% angiosperm species (Harrison, 2005) and 92% of plant

families (Smith and Read, 2008). The remaining percentage was NM plants with estimated of 29% vascular plant species (Cosme *et al*., 2018). Example of NM plant families are included Amaranthaceae, Brassicaceae, Cyperaceae, Caryophyllaceae and Polygonaceae (Brundrett, 2002; Yamato, 2004; Massenssini et al., 2014).

Symbiotic interaction established between AMF and plants is mutualism. The fungi received carbon (C) source from the plant which essential to complete its life cycle and in return, AMF support the nutrient uptake from soil to plant for growth (Harrison, 1997). Plants allocated 4 - 20% of photoassimilate to AMF (Bago *et al*., 2000). However, some C is lost into the soil as CO_2 through respiration process of extraradical hyphae and indirectly contributed to C cycle between biosphere and atmosphere (Zhu and Miller, 2003; Parihar *et al*., 2020).

AMF is well known in taking up the least mobile macronutrients P from soil to plant (Bucher, 2007). The uptake of P by AMF can be more dominant than plants root and lead to the decrease of P uptake from roots (Smith *et al*., 2003; Smith *et al*., 2011). Efficacy of AMF in exploiting the soil P can be increased through its species diversity (van der Heijden *et al*., 1998; Frew, 2019). The fungi also able to improve the uptake of other macronutrients nitrogen (N) and potassium (K) (Chandrasekaran, 2020), and immobile micronutrients such as zinc (Zn) (Bhantana *et al*., 2021), cuprum (Cu) and ferum (Fe) (Chen *et al*., 2017; Ingraffia *et al*., 2019).

AMF is obligate biotroph that need plants to complete its life cycle. Apart from receiving C source, the influence of plants towards AMF begin at pre-symbiotic stage. Root exudates containing; 1) flavonoids which are able to enhance spore germination, hyphal growth, hyphal branching, secondary spore formation and increase the arbuscule formation (Salloum *et al*., 2018; Pei *et al*., 2020), 2)

strigolactones which are able to intensify hyphal branching of germinated spores (Akiyama *et al*., 2005; Requena *et al*., 2007; Parniske, 2008), required for hyphopodium formation and penetration into the roots (Kobae *et al*., 2018) and 3) lipophilic compound known as branching factor contributed in triggering the development of AMF (Buee *et al*., 2000).

Both flavonoids (Lidoy *et al*., 2023) and strigolactones (López-Ráez *et al*., 2008) are signaling compounds in AM symbioses. Strigolactones have been found to be involved in mitochondria metabolism (Mitra *et al*., 2021b), stimulate mitochondrial respiration (Kowalczyk and Hrynkiewicz, 2018) and caused mitochondria density to be increased. Hence, generating more energy in the fungal cells (Besserer *et al*., 2006). Meanwhile, AMF can produce a specific diffusible factor which was unable to be produced by other fungal soil and stimulate a response from the host plants (Kosuta *et al*., 2003).

There was scanty information regarding the production of these signaling compounds in NM plants. Even AMF somehow succeed in colonizing the root of NM plants in this case Brassicaceae, the colonization itself trigger production of another compound (glucosinolate) that inhibit AMF development (Sharma *et al*., 2023). In short, the interaction of AMF-plants occurred prior physical contact established between the symbionts. However, the sprouting hyphae from germinated spore will arrest its development and retract into the spore to be in the dormant state again if the fungi fail to meet with the host plant (Bonfante and Genre, 2010). The process occurred within 8 - 20 days (Giovannetti *et al*., 2010).

Therefore, production of spores is impossible without host plants. In early days, there were attempts to germinate the spores and grow the fungi without host

plant. For example, spore of *Gigaspora rosea* was germinated *in vivo* using a sterile media under axenic conditions. However, the hyphae was autolysed (Bago *et al*., 1998). Over years, the attempts to grow AMF under laboratory condition had shown several achievements due to various modifications such as *Glomus intraradices* able to complete its life cycle from spore germination until reproduction of new spores through nutrient alteration of the media culture (St-Arnaud *et al*., 1996) and co-cultured of AMF with bacteria *Paenibacillus validus* (Hildebrandt *et al*., 2002; Kameoka *et al*., 2019). Co-cultured of AMF with bacteria *Pseudomonas fluorescens* caused hyphal elongation (Pivato *et al*., 2009) and the presence of strigolactones and jasmonate phytohormones induced the number and size of secondary spore (Tanaka *et al*., 2022).

AMF have been long considered to be reproduced asexually and evolved for more than 500 million years without the presence of sexual reproduction. However, the advancement of genetic and molecular studies unravelled several traits of sexual reproduction exist in AMF (Corradi and Bonfente, 2012). For example, the existence of nuclei recombination and genetic exchange (Chen *et al*., 2018; Sperschneider *et al*., 2023), has high number of lineage-specific genes through the presence of interstrain diversity (Reinhardt *et al*., 2021) and similarity of a putative mating-type locus of AMF with mating-type locus with some Dikarya (Ropars *et al*., 2016). According to Yildirir *et al*. (2020) generation of nuclear diversity in AMF could be contributed by its parasexual and sexual evolution.

The phylum Glomeromycota of AMF is an ancient group with estimated origin of 600 - 620 million years ago (Redecker, 2008). A fossil with arbuscule structure was discovered at an Early Devonian plant (*Aglaophyton major*), providing the evidence for AMF establishment already existed for more than 400 million years

ago (Remy *et al*., 1994; Taylor *et al*., 1995). Another discovery was spore and hyphae fossil in 460 million years old rock from the Ordovician and it is among the oldest recognized fossil of fungal (Redecker *et al*., 2000a).

2.2.2 Taxonomy

AMF belong to the phylum Zygomycota under genus *Endogone*. In 1974, the fungi were classed to four genera (*Acaulospora*, *Gigaspora*, *Glomus* and *Sclerocystis*) under the order Endogonales by Gerdeman and Trappe. In 1990, new order Glomales was created by Morton and Benny (Morton and Benny, 1990). The order was later renamed to Glomerales. The fungi were organized to three families (Acaulosporaceae, Gigasporaceae and Glomeraceae), six genera (*Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*) under order Glomerales of phylum Zygomycota (Koide and Mosse, 2004).

Rapid development in technology of molecular genetics had left a significant remark on AMF taxonomy studies. The first molecular study was carried out by Simon *et al*. (1993) to investigate the origin and clarify the phylogenetic relationship of AMF through the sequence of rDNA genes from 12 AMF species. A genetic study by Redecker *et al*. (2000b) revealed the inaccuracy of at least five AMF species from their previous defined families. Remarkably, a study by Schüßler *et al*. (2001) caused huge reformation on AMF taxonomic history where all AMF species had been moved from Zygomacota to the new phylum Glomeromycota. Therefore, AMF was belong to the phylum Glomeromycota with one class (Glomeromycetes), four orders (Archaeosporales, Diversisporales, Glomerales and Paraglomerales), seven families (Acaulosporaceae, Archaeosporaceae, Diversisporaceae, Geosiphonaceae, Gigasporaceae, Glomeraceae and Paraglomeraceae) and ten genera (*Acaulospora*,

Archaeospora, *Diversispora*, *Entrophospora*, *Geosiphon*, *Gigaspora*, *Glomus* A, *Glomus* B, *Paraglomus* and *Scutellospora*).

Taxonomic inaccuracy was caused by traditional classification system that relied heavily on morphological structures. For example, the earlier classification of Glomeromycotan under phylum Zygomycota was influenced by characteristic of hyphae which both Zygomycota and Glomeromycota have coenocytic aseptate hyphae (White *et al*., 2006). Therefore, the species of Glomeromycota had classified under Zygomycota.

Spores that used as the main structure to identify AMF at species level were bounded to the inaccuracy when the spores had poor quality (Redecker *et al*., 2003) and was limited with terminology in describing the species. Proposal for new classification has been evoked because of several reasons including phylogenetic of the Glomeromycotan was hypothesized to be monophyletic rather than paraphyletic Zygomycota, formation of mutual symbiosis with plant roots and the presence of arbuscules (Stürmer, 2012). Removal of Glomeromycotan from Zygomycota was also due to the absence of zygospores or any sexual spore (Naranjo-Ortiz and Gabaldón, 2019) since Glomeromycota is not reproduce sexually (Kosal, 2023).

Molecular technique for the AMF genetic caused the fungi taxonomic studies becoming precise and systematic (Liu and Feng, 2010) particularly in the taxonomic revision and reclassification (da Silva *et al*., 2006; Morton and Msiska, 2010). Within the span of 10 years (2001 - 2010), there was addition of new six families (Ambisporaceae, Dentiscutataceae, Entrophosporaceae, Pacisporaceae, Racocetraceae and Scutellosporaceae) and ten genera (*Ambispora*, *Cetraspora*, *Dentiscutata*, *Fuscutata*, *Intraspora*, *Kuklospora*, *Otospora*, *Pacispora*, *Quatunica*

and *Racocetra*) (Schüßler *et al*., 2001; Schüßler and Walker, 2010). Meanwhile, a study by Oelh *et al*. (2011) shown the addition of one new order (Gigasporales), one family (Sacculosporaceae) and ten genera (*Alpahypha*, *Claroideoglomus*, *Funneliformis*, *Orbispora*, *Redeckera*, *Sacculospora*, *Septoglomus*, *Simiglomus*, *Tricispora* and *Viscospora*). Therefore, AMF belonging to the phylum Glomeromycota were classified into 1 class, 5 orders, 15 families and 30 genera.

Certainly, the numbers will keep increasing with new discoveries. The growth of Glomeromycota taxonomic to this recent year has brought to the several formations. For example, formation of new order Entrophosporales with three new Entrophospora species (Błaszkowski *et al*., 2022) and new genus of Glomeraceae named *Blaszkowskia* (da Silva *et al*., 2023). There were also discoveries of new taxas under Glomeraceae (Błaszkowski *et al*., 2021a) and Polonosporaceae (Błaszkowski *et al*., 2021b), two new species under Glomeraceae (Błaszkowski *et al*., 2021c), one species of Diversisporaceae and two species of Scutellosporaceae (Niezgoda *et al*., 2024). As summary, the phylum Glomeromycota was distributed into 3 classes (Archaeosporomycetes, Glomeromycetes and Paraglomeromycetes), 5 orders (Archaeosporales, Diversisporales, Gigasporales, Glomerales and Paraglomerales), 16 families and 50 genera (Wijayawardene *et al*., 2020).

A review by Stürmer (2012) stated that there were 126 described species of AMF had been listed by Schenck and Perez in year 1988. Through the first molecular technique conducted by Simon *et al*. (1993), 130 species had been identified. There were 150 AMF species when the new phylum Glomeromycota had erected (Schüßler *et al*., 2001). The number increased to 200 species (Redecker and Raab, 2006), 214 species (Schüßler and walker, 2010) and 230 species (Krüger *et al*., 2012). By far,

the number of AMF species had increased to 326 (Wijayawardene *et al*., 2020) and 330 AMF species (Błaszkowski *et al*., 2021a).

2.2.3 Benefits

The symbiosis interaction formed between AMF and plants is mutualism that benefits both symbionts. The fungi received carbon source from plants and used to complete their life cycle. Meanwhile, AMF giving out by helping the plants taking up the nutrient and water from the soil with several other functions benefit for plant growth.

2.2.3(a) Phosphorus and Water Uptake

The signature function of AMF is the ability to increase phosphorus (P) uptake from the soil to plant (Smith *et al*., 2011). P is an important element required for the formation of adenosine triphosphate (ATP), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). P is placed second after N as the most required nutrient by plant for growth and productivity. Therefore, it has been classified as macronutrients alongside N and K. According to Abbas *et al*. (2021), macronutrients are the nutrients required and consumed in large quantities ranging from 0.2 - 4.0% of plant tissues dry matter weight. P is able to stimulate the fundamental processes such as photosynthesis, N fixation, flowering, fruiting that include seed production, and plant maturity (Brady and Weil, 2008). Insufficient P element in plants is shown by the formation of anthocyanosis on a nonchlorotic background of the oldest leaves (de Bang *et al*., 2021).

However, adequate P supply for plants faces several problems such as 1) the amount of total P content in the soil is low which ranges between 200 - 2000 kg/ha measured at 0 - 15 cm soil surface layer, 2) P is commonly found in highly insoluble

compounds binding with calcium (Ca), iron (Fe), aluminum (Al) and manganese (Mn) which is unavailable to be taken up by the plants and 3) low accessibility of P in its available forms such as dihydrogen phosphate ($H_2PO_4^-$), hydrogen phosphate (HPO_4^{2-}) and phosphate (PO_4^{3-}). This is because available P anions are easily to be fixed to insoluble complex of compounds and again become unavailable for plants (Brady and Weil, 2008; Ikhajiagbe *et al*., 2020). Therefore, phosphorus has been characterized as immobile element.

High demand of P by the plants to maintain its high [P] requirement (5 - 20 mM) and low [P] in the soil (2 µM) restricted P uptake by plant roots in the continuous adequate amount. This contributed to the formation of P depletion zone in the rhizosphere (Bieleski, 1973; Schachtman *et al*., 1998). Moreover, P depletion zone around the roots was also generated when P absorption by roots is higher than P diffusion rate in the soil solution (Ferrol *et al*., 2019). The zone can be formed at 0.2 - 1.0 mm from the root surface (Mikkelsen and Director, 2005) and can be extended up to 2 mm (Hummel *et al*., 2021), 3 mm (York *et al*., 2016) and 4 mm (Ye e*t al*., 2018).

There are several responses by the plants itself to enhance P uptake and alleviate P deficiency such as modification of root architecture to the formation of lateral roots, root hairs and cluster roots. Length of the root hairs can be equal to the distance of P depletion zone from the root surface. Hence, increasing P uptake by twofold than hairless root (Gahoonia and Nielsen, 2003). Other studies showed that root hairs were accounted for 50% of total P uptake (Ruiz *et al*., 2020) and high nutrient absorption up to 80% (Jungk, 2001). Meanwhile, plant families such as Cyperaceae, Fabaceae and Proteaceae able to develop special type of root called cluster roots or proteoid in response of P deficiency (Li *et al*., 2016).

Another plant response is secretion of root exudates. The presence of root exudates had increased availability of P in the soil and improve the P uptake by plants (Zhang *et al*., 2016). In general, root exudates consist of a complex mixture which have direct or indirect effects on the nutrient acquisition. Carboxylates compound capable to displace both inorganic P (Pi) or organic P (Po) from Al, Fe and Ca complexes (Hinsinger, 2001). Carboxylates also are P acquisition strategy for cluster root forming species where it has been secreted in a large amount compared to non-cluster root plants (Chai and Schachtman, 2022). Secretion of carboxylates can be associated with proton release resulting in rhizosphere acidification thus enhance soil P availability (Poirier *et al*., 2022). Root exudates also consisted of enzymes such as acid phosphatase (APase) where the enzymes can hydrolyze the Po and release the phosphate group to be available for the plants (Vance *et al*., 2003; Nannipieri *et al*., 2011). Moreover, rhizosphere acidification indirectly benefits the APase as the enzymes work best under acidic condition (Hurley *et al*., 2010; Bilyera *et al*., 2022). Mechanism of soil P availability enhancement by the compounds and particle that mentioned above are illustrated in the Fig. 2.2 (Lambers *et al*., 2018).

Finally, root exudates also stimulate soil microorganisms including AMF for nutrient support. By far, it is known that root exudate compounds such as flavonoids and strigolactones had influenced AMF development since pre-symbiotic phase. Whereas, colonization of the fungi on plant roots able to increase P uptake. Despite the plant itself undergoes root modification and exudate secretion, colonized plant roots are more efficient in P uptake as the AMF can increase root absorbing surfaces 100 - 1000 times through its extraradical hyphae network (Gupta, 2020). A study by Mai *et al*. (2019) showed that 3 cm hyphal growth from the root surface increased P absorption by 15 fold. Thus, this validate the dependency of plants is higher towards

mycorrhizal than root hairs (Ma *et al*., 2021). According to Smith *et al*. (2003), the efficiency of AMF in P absorption may hinder P uptake by root hairs completely.

There are several factors that contribute to the AMF efficiency in increasing P absorption such as ability of the extraradical mycelium to explore a wider soil space as far as 120 - 130 cm from the root surface exceeding far beyond the P depletion zone (Friese and Allen, 1991). The fungal hyphae length density is higher (2 - 40 m/g) than root length density (<0.10 m/g) (Allen *et al*., 2003; Smith *et al*., 2010; Camenzind and Rillig, 2013) and thus provide more surface for P absorption. Extraradical hyphae are also have been modified into several structures with different diameters such as spore germination tubes (2 - 5 μm), infectious hyphae (2 - 3 μm), runner hyphae (10 - 15 μm) and absorptive hyphae (Friese and Allen, 1991; Bago *et al*., 1998). This allows the penetration of the fungal hyphae into the soil pores that cannot be passed by plant roots. A study by Lai *et al*. (2011) showed that the diameter of fine roots included fibrous root and lateral roots is ≤1000 μm. Although the fine root diameter can be less than 300 μm (Xiao *et al*., 2020), the least diameter of the fine roots is still significantly bigger than AMF extraradical hyphae diameters.

AMF also has an affinity for P ions and P absorption is feasible below the threshold level which is the minimum level of [P] that plants can absorb (Bolan, 1991). Similar to plant roots, extraradical hyphae of AMF able to secrete exudates to enhance the soil P availability such as APase (Joner *et al*., 2000; Sato *et al*., 2015). A study by Chandra *et al*. (2022) showed the presence of APase was greater in AMF inoculation treatment. The fungi also released a significant amount of carboxylic organic acids (Plassard and Dell, 2010; Andrino *et al*., 2021a). Overview of P uptake via root hairs and AMF extraradical hyphae with the presence of P depletion zone is illustrated in the Fig. 2.3.

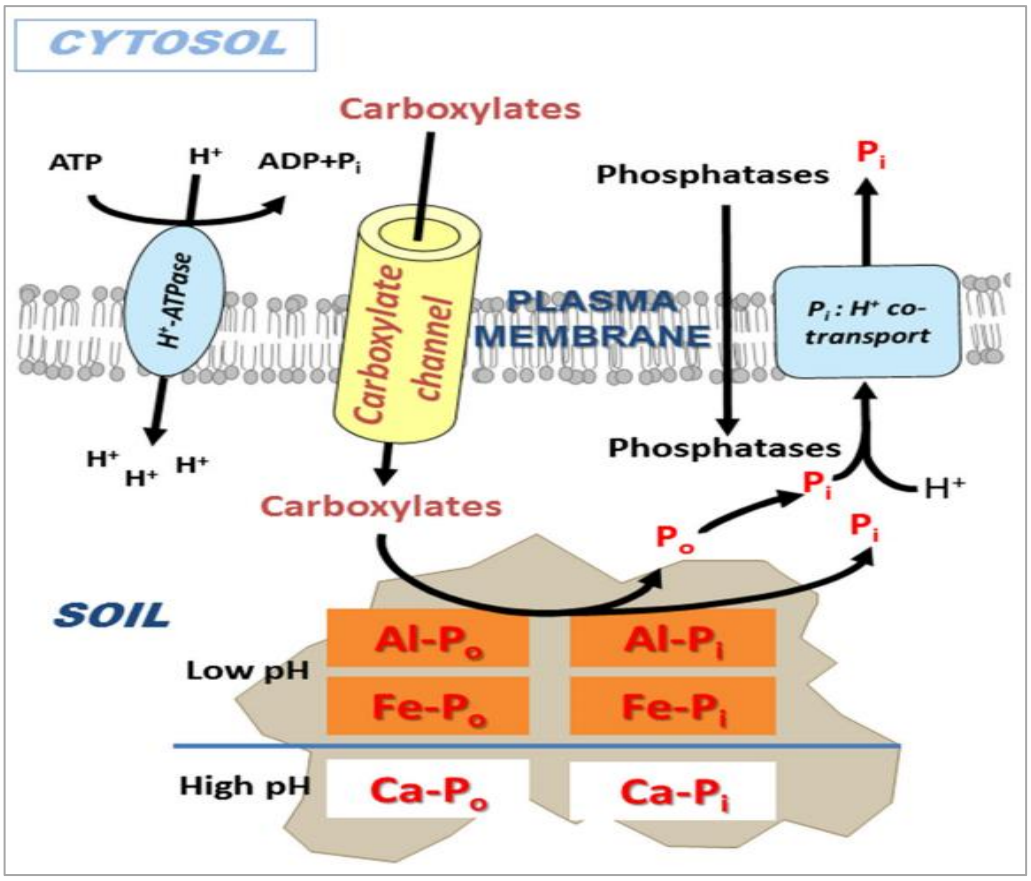

Figure 2.2: Effects of carboxylates, phosphatase enzyme and other exudates on mobilization of phosphorus (P) in the soil (Source: Lambers *et al*., 2018).

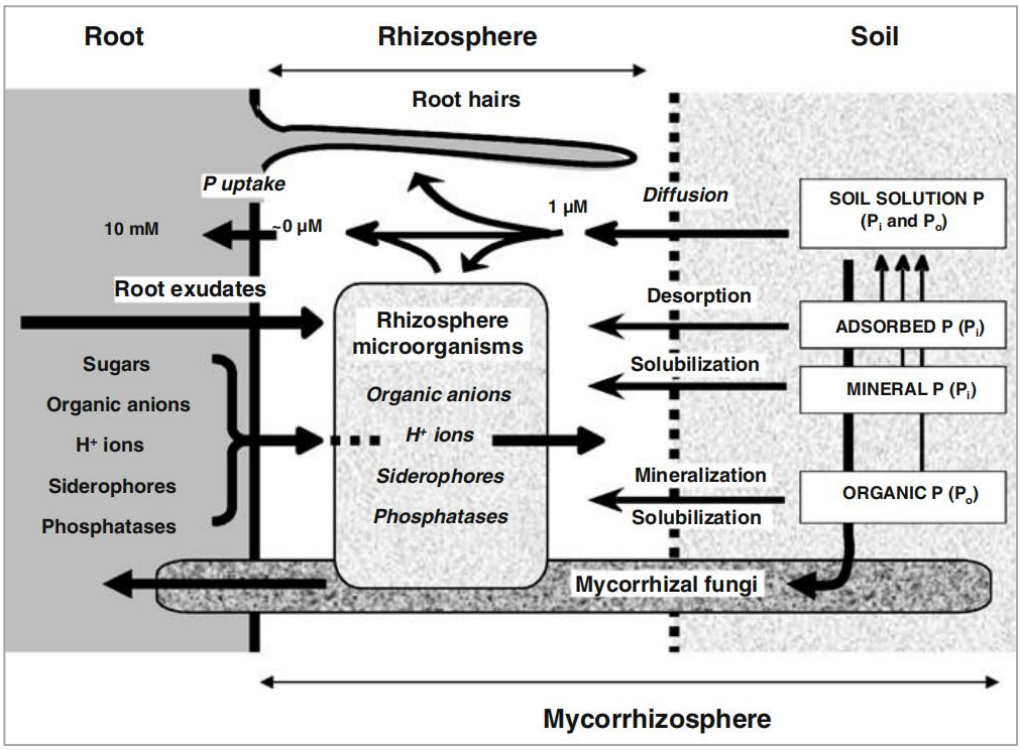

Figure 2.3: Physiological and chemical processes that influence phosphorus (P) availability and its transformation in the rhizosphere with the presence of P depletion zone (Source: Richardson *et al*., 2009).

Extraradical hyphae capable in exploring a wider soil volume and penetrating inaccessible soil pores had also benefits the plants under water deficit especially during drought season (Fernández-Lizarazo and Moreno-Fonseca, 2016). A study by Khalvati *et al*. (2005) showed that AMF help to increase the water uptake at least 4% and is expected to be increased with high density of hyphae and colonization level (Marulanda *et al*., 2003). Interestingly, the drought stress had stimulated AMF hyphal growth and colonization activity (Wu *et al*., 2008). Although the effect of the drought stress was not necessarily improving AMF physical growth yet the drought stress had increased hyphal water absorption rate 2 - 7 times higher than well-water condition (Zhang *et al*., 2018a) and maintained high relative water content and water potential than plants without AMF inoculation (Meddich *et al*., 2015).

Water is the most basic source needed by living things. Water deficit of drought stress was due to temperature dynamic, light intensity, salinity and low rainfall (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). It causes severe effects on plant morphology, physiology, biochemistry and molecular attributes (Seleiman *et al*., 2021). Plants under drought stress experience continual dehydration, wilting and eventually die out of dryness (Ruiz-Lozano, 2003; Lisar *et al*., 2012). The absorption of water via the extraradical mycelium of arbuscular mycorrhizal fungi (AMF) within the soil facilitates the growth of plant systems by maintaining hydration in the plants. Consequently, the mitigation of water stress becomes feasible through the enhancement of water uptake efficiency. Thus, the improvement of nutrient absorption, particularly phosphorus (P), chlorophyll concentration (Hazzoumi *et al*., 2015), biomass accumulation, and overall crop yield is achievable (Amiri *et al*., 2015; Sharma *et al.*, 2015; Leventis *et al*., 2021).

2.2.3(b) Salt Stress Mitigation

The ability of AMF in relieving water stress effect may indirectly reduce the salt stress on plants. Salinity stress constitutes a significant limitation to vegetative growth and agricultural yield (Parihar *et al*., 2015), predominantly attributable to irrigation practices (Ahmad *et al*., 2013). The plants under salt stress might suffer from osmotic stress, ion toxicity and nutritional imbalance (Abbasi *et al*., 2016). According to Serrano and Rodriguez-Navarro (2001), salt stress occurred due to imbalance ions of high Na^+ and low K^+ ions. Salt stress inhibits plant growth via two phases; 1) high salt concentration forming osmotic pressure in the soil and cause water removal from plant tissue and 2) excessive salt in the plant cells result in premature aging of the plants (Munns, 2002).

Plants colonized by AMF may alleviate the salt stress by balancing the Na^+ and K^+ ions in plant (Giri *et al*., 2007), improve water permeability of membranes, stimulate water transport (Cheng *et al*., 2021), increase relative water content, water use efficiency, gas exchange capacity and lower the concentration of salt ions (Wu *et al*., 2015). According to a comprehensive meta-analytic investigation, in the context of salinity stress conditions, plants colonized by arbuscular mycorrhizal fungi (AMF) exhibited a beneficial effect on water stress and gas exchange processes (Chandrasekaran *et al*., 2019), thereby ameliorating the physiological systems of the plants (Sheng *et al*., 2008).

2.2.3(c) Soil Aggregations

The fungi also play a role in soil aggregation. Aggregates were formed when particles of sand, silt and clay are bound together by inorganic and organic materials into various sizes where <20 µm as small micro-aggregates, 20 - 250 µm as large micro-aggregates and >250 µm as macro-aggregates (Totsche *et al*., 2018). Soil

aggregation contributes to the soil structure stability where aggregates and pores able to remain intact when subjected to stress (Tisdall, 2020). Therefore, it is crucial in controlling topsoil hydrology, crustability and erodibility which related to surface runoff and soil erosion (De Ploey and Poesen, 2020). Soil aggregation also has influence on organic C regulation and N storage (Mustafa *et al*., 2020).

As the aggregates were bound by organic materials, soil biota gives a positive impact on the soil aggregations where the effect of bacteria and fungi were more significant than soil animals and the fungi have stronger effect on macro-aggregates (Lehmann *et al*., 2017b). Meanwhile, the aggregates support the distinct soil microbial habitats according to the aggregates different size classes (Trivedi *et al*., 2017).

AMF positively influenced soil aggregation particularly by forming more macro-aggregates (Morris *et al*., 2019) through its extensive extraradical hyphae (Lehmann *et al*., 2017a) and glomalin production. Glomalin is a glycoprotein capable in binding soil particles (Singh *et al*., 2013) and it was produced since the emergence of new hyphae (Driver *et al*., 2005). Concentration of glomalin can be influenced by the fungal biomass (Holátko *et al*., 2021) and species (Saidi *et al*., 2015). Moreover, appropriate amount of N addition was able to stimulate AMF for glomalin secretion (Zhang *et al*., 2023). As the glomalin resist heat, strong acids and bases, it can also enhance the plant growth under abiotic stress (Irving *et al*., 2021). In general, the growth of the fungal extraradical hyphae in soil helps in soil aggregation and serve multifaceted benefits for the soil and plants.

2.2.3(d) Interaction with Other Soil Microorganisms

Similar to plant roots, AMF also has the ability to secrete its own exudates through hyphae. A review by Zhang *et al*. (2022) had listed a diverse compound from amino acids, amines, carbohydrates, polyols, carboxylates, nucleic acids, proteins, peptides and potential signals presence in the hyphal exudes that carry their distinct functions. For example, fructose is able to stimulate the phosphatase gene expression in a phosphate solubilizing bacterium (PSB) *Rahnella aquatilis* and caused the increment of APase secretion rate and promoting the mineralization of organic phosphorus (Po) to inorganic phosphorus (Pi) (Zhang *et al*., 2018b). Alternatively, extraradical hyphae may function as passage for PSB in reaching Po patches in the soil and hyphal exudate (glucose) serve as energy enhancing the activity of the bacterium in Po mineralization (Jiang *et al*., 2021).

The effect of hyphal exudates also influence in shaping soil microbial community (Qin *et al*., 2016). Different microbial community was observed between attached and non-attached hyphae. A study by Scheublin *et al*. (2010) indicated certain bacteria of Oxalobacteraceae, a family which comprised with plant growth promoting members (Gaspar *et al*., 2016; Yu *et al*., 2021) were highly abundant on the hyphae. Interaction of AMF with other soil microorganisms that closely attach to the hyphae and influenced by the hyphae exudates feasible the benefits beyond AMF capability. For example, production of alkaline phosphatase through the stimulation of certain bacteria (Zhang *et al*., 2018c) able to hydrolyze Po optimally at high pH. It is known that AMF is able to produce acid phosphatase into the hyposphere (region around fungal hyphae and influence by hyphal exudates) for Po mineralization (Sato *et al*., 2015) but hyphal exudates intensify the process by triggering a particular soil bacteria with the same ability.

AMF also has been interacted with N fixing bacteria in legume plants where the presence of the bacteria increased AMF abundance (Ji *et al*., 2024). Application of AMF and N fixing bacterium creating a synergy effect that promote shoot biomass and N content in *Mimosa scrabrella* (Primieri *et al*., 2021). Similar to PSB translocation, extraradical hyphae served as a passage for N fixing bacterium (*Bradyrhizobium diazoefficiens*) and caused nodulation on roots (de Novais *et al*., 2020). Despite the benefits provided from the symbiosis, interaction of AMF and N fixing bacteria can be changed according to vegetation succession stage. A study by Liang *et al*. (2023) showed that cooperation and mutual interactions have been observed during the early and middle succession stages but becoming competitive during later succession stage. The synergy effect of the dual symbioses has not limit for nutrient stress alleviation but also towards pathogen attacks and metal stress alleviation (Soumare *et al*., 2015).

The presence of diverse soil microorganisms also influenced the activity of AMF in supporting plant growth. For example, N fixing bacteria of gram-positive bacteria *Bacillus* increased AMF abundance (Ji *et al*., 2024), *Paenibacillus validus* stimulated the germination of AMF spore (Hildebrandt *et al*., 2002) and exudates secreted by *Rhodotorula mucilaginosa* enhanced AMF growth and colonization (Fracchia *et al*., 2003). Moreover, association of other beneficial bacteria in AMF-plants symbiosis able to improve AMF efficiency by stimulating spore germination, mycelial growth, root colonization, sporulation and reducing stress (Lies *et al*., 2018). These considered 'mycorrhiza helper bacteria' have been classified into two groups; gram-negative bacteria that consisted Proteobacteria (*Agrobacterium*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bradyrhizobium*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, and *Rhizobium*) and gram-positive bacteria that consisted Actinobacteria