Effect of Non-Host Plant on the Community of Arbuscular Mycorrhizal Fungi

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Abstract

Arbuscular mycorrhizal (AM) fungi are widespread across lands managed for production as well as conservation. In general, AM communities become less diverse as land becomes intensively farmed, and the benefit of AM fungi may differ with the crop. In Thailand, there is little knowledge of AM community. This study aimed to determine the AM community under two crop regimes; maize (*Zea mays* L.) as AM host plant and cabbage (*Brassica oleracea* L. cv. cabitata) as non-AM host plant, grown on Pak Chong soil series (Ultisol). The pot experiment was undertaken with field soil containing 12 AM fungal species across 4 genera: 2 species of *Acaulospora*, 2 species of *Entrophospora*, 7 species of *Glomus* and 1 species of *Scutellospora*. The maize and cabbage were grown for 120 days. One month after harvest, the number of AM spores of each morphospecies was determined. The spore number of 6 AM species (*Entrophospora* sp. 2, and *Glomus* spp. 1, 4, 5, 6 and 7), were significantly increased under maize. By contrast, cabbage crop had both positive and negative effect on the AM community. There were 3 species (*Entrophospora* sp. 2, *Glomus* spp. 1 and 7) increasing in spore number but spore number of 4 species (*Acaulospora* sp. 1, *Entrophospora* sp. 1, *Glomus* spp. 2 and 3) were decreased. Therefore, the results indicate that the AM community was altered by the plant crop. The non-AM host plant decreased the abundance of some AM species in the community.

Keywords: arbuscular mycorrhizal fungi community; non-host plant; cabbage

1. Introduction

AM fungi are ecologically obligate symbionts of an enormously wide variety of host plants. Almost 80 % of terrestrial plant including most herbaceous legumes and *Gramineae* are formed AM symbioses (Smith and Read, 1997). Some plant families including the *Brasicaceae*, *Chenopodiaceae*, *Cyperaceae*, *Juncaceae* and *Cryophyllaceae*, are rarely mycorrhizal symbiosis (Newman and Reddell, 1987). The AM symbiotic association is characterized by bi-directional movement of

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nutrients. The host plant supplies the AM fungus with compounds of carbon while the AM fungus helps the plant in the uptake of nutrients in soil. Increasing growth and nutrient uptake of plants by AM colonization have been documented. Researchers commonly report that the effects of increased uptake of mineral nutrients on the growth of plants are the direct result of fungal colonization (Marschner and Dell, 1994). Particularly in phosphorus (P) which is the main nutrient transporting by AM fungi to plants (Merryweather and Filler, 1995a, 1995b). This symbiotic association also increase host plant tolerance against biotic (Hol and Cook, 2005) and abiotic stresses (Sudova *et al*., 2007).

The AM host plant species has been shown to have a positive impact on AM fungi community; diversity and infectivity. In low-input agronomy, soils under wheat-rye-grass rotation had a greatly enhanced capacity to initiate AM symbiosis (Mäder *et al*., 2000). In large-scale field crop production, indirect methods such as crop rotation with AM host plants may offer a beneficial AM community that is more practical approach than directs manipulation by inoculation (Thompson, 1994). The study of Galvez and co-worker observed increasing in AM spore density following AM host cover cropping (Galves *et al*., 2005). Furthermore, including AM host plant in crop rotation can maintain beneficial AM fungi community, high soil quality and increase yields of subsequent crops (Vestberg *et al*., 2005).

By contrast, many studies have been reported that previous *Brassica* crop reduced AM root colonization and spore number in subsequent crop. *Brassica* rotation crop in India was reported by Harinkumar and Bagyaraj that previous crop with Indian mustard reduced AM spore number in the subsequent AM-host crop (Harinkumar and Bagyaraj, 1998). In wheat belt regime of southern NSW, Australia, which was studied by Ryan (2001), reported that previous *Brassica* (canola; *B. napus* L. and Indian mustard) crop reduced AM root colonization in the subsequent wheat crop. Similarly, pot experiment in Japan also reported decreasing in AM spore number and root colonization of sunflower and maize that grew after white mustard (*B. alba* L.) (Karasawa *et al*., 2001) Furthermore, a short reason of rotation crop could substantially alter the inherent AMF potential of soil to significantly influence the mycorrhizal status in the subsequent crop due to changing in the mycorrhizal root mass, and spore number under rotation crop (Panja and Chaudhuri, 2004). Therefore, including non-mycorrhizal plants in the rotation may reduce the AM spore number and the AM colonization in following crops.

In Thailand, there is little knowledge of AM community under different crops. It might be possible that AM community would be changed by plant crop. Therefore, this study aimed to examine AM community under two different crop regimes; maize and cabbage crop in Pak Chong soil series.

2. Materials and Methods

The soil sample belonged to the Pak Chong soil series: clay-loam, kaolinitic,

isohyperthermic, Typic Paleustults (Soil survey staff, 1998). The soil chemical properties were pH 7.0 (1:1, soil : H_2O) (Peech, 1965), and available P 10 mg kg^{-1} (Bray II) (Olsen and Dean, 1995). Five kg coarse washed river sand was placed into glazed clay pots (32 cm diameter at the top, 25 cm diameter at the bottom and 30 cm in height) to enhance drainage and then 7 kg subsoil and 13 kg topsoil were placed, respectively. The pots were left uncovered and exposed to rain during rainy season. Vertical plastic sheets (1.5 m high) were placed between pots to prevent rain-splash.

Preliminary experiment was set up before starting the pot experiment. This was undertaken to produce a uniform population of AM fungi in 8 pots which were planted with maize for 120 days. After that, pot experiment was conducted with maize and cabbage in 4 replications. One plant was grown per pot. Nitrogen fertilizer was applied two times at the rate of 1.12 g urea per pot (equivalent to 50 kgN ha^{-1}), equally split at 14 days after planting (D) and D40. P fertilizer was applied as TSP at the rate of 1.12 g TSP per pot (equivalent to 21.8 kg P ha^{-1}) by surface banding on one side of the plant at D14 and Zn fertilizer was applied at the rate of 0.38 g of Zn per pot (equivalent to 30.4 kg Zn ha^{-1}) at D40. These fertilizer rates did not eliminate AM fungi in soil (Na Bhadalung, 2005).

Plant shoot was harvested at maturity, D120. One month after harvest, soil samples were collected at the depth of 0-20 cm using a 2 cm diameter steel auger, 3 augers per pot. The soil sample was left to air-dry for determining AM spore number and AM infectivity. The AM spore number was separated from soil by wet sieving and decanting method (Gerdemann and Nicolson, 1963) followed by sucrose centrifugation (Daniels and Skipper, 1982).

3. Results

In the preliminary experiment, AM community was composed of 12 species in 4 genera: 2 species of *Acaulospora* (A1-2), 2 species of *Entrophosphora* (E1-2), 7 species of *Glomus* (G1-7) and 1 species of *Scutellospora* (S). The spore morphological characteristics are presented in Table 1 and features of each AM fungal species are shown in Figure 1. The total spore number was 1282 ± 40 spores 100 g^{-1} soil. *Glomus* spp. was dominant. The spore number of 7 *Glomus* species contributed 69 % of the total spore number. The spore number of 2 species of *Acaulospora,* 2 species of *Entrophosphora* and 1 species of *Scutellospora* were 20, 11 and 1 % of the total spore number, respectively.

In the pot experiment, the spore number of AM fungi at the start of crop was compared to those at the end of crop under maize crop (Fig. 2a). *Glomus* was more responded to maize than other genus. The spore number of 5 species in total 7 species of *Glomus* was increased. *Glomus* sp. 1, 4, 5, 6 and 7 were increased in spore number by 57, 48, 39, 28 and 72 % of spore number at the start of crop.

There was spore number of *Entrophospora* sp. 2 increasing (P=0.001) by 50 % when compared to spore number at the start, whereas spore number of *Entrophospora* sp. 1 did not change. However, spore number of 2 species of *Acualospora* and 1 species of *Scutellospora* did not alter.

Table 1 The characteristics of the AM fungal taxa found in the soil and their codes

AM fungal species	Spore characteristic
Acaulospora sp. 1 (A1)	Globose shape, 76-112.5 µm, white to orange, soporiferous succule (80-100 µm). Two layers of spore wall, combined thickness 3-7 µm.
Acaulospora sp. 2 (A2)	Globose shape, 100-120 µm, yellow to orange, soporiferous succule (120-130 µm) and fine spines present. Two layers of spore wall, combined thickness 5-10 µm.
Entrophospora sp. 1 (E1)	Globose shape, 165-208 µm, orange to dark orange, hyaline outer layer, and hyaline, subglobose soporiferous succule (180-220 µm) and thick spines present.
Entrophospora sp. 2 (E2)	Globose shape, 60-80 µm, hyaline colour and hyaline subglobose soporiferous succule (60-80 µm). Three layers of spore wall, combined thickness 2-5 µm.
Glomus sp. 1 (G1)	Globose shape, 80-125 µm, white to cream colour, single chlamydospore. Four to five layers of spore wall, combined thickness 4-8 µm.
Glomus sp. 2 (G2)	Sporocarp formation without peridium (200-1500 x 200-2000 µm), globose (80-140 µm) or subglobose (80-96 x 120-140 µm) chlamydospore, pale yellow, forms spores in roots.
Glomus sp. 3 (G3)	Sporocarp formation without peridium (200-800 x 200-1000 µm), globose (60-120 µm) or subglobose (40-112 x 96-188 µm) chlamydospore, pale yellow to yellow, forms spores in roots. Two to three layers of spore wall.
Glomus sp. 4 (G4)	Sporocarp formation without peridium (1-3 mm), globose (80-100 µm) or subglobose (60-100 x 90-140 µm) chlamydospore, yellow to orange. Three layers of spore wall, combined thickness 3-5 µm.
Glomus sp. 5 (G5)	Sporocarp without peridium, globose chlamydospore (92-112 µm), white to cream. Three layers of spore wall, combined thickness 5-7 µm.
Glomus sp. 6 (G6)	Globose shape, 120-300 µm, pale yellow to yellow, recurved septum. Two layers of spore wall, combined thickness 4-5 µm.
Glomus sp. 7 (G7)	Globose shape, 124-180 µm, yellow-brown to dark orange-brown, shiny and containing lipid content. Two to three layers of spore wall.
Scutellospora sp. (S)	Globose shape, 168-240 µm, white to cream colour, germination shield and auxillary cell. Five layers of spore wall, combined thickness 4-8 µm.

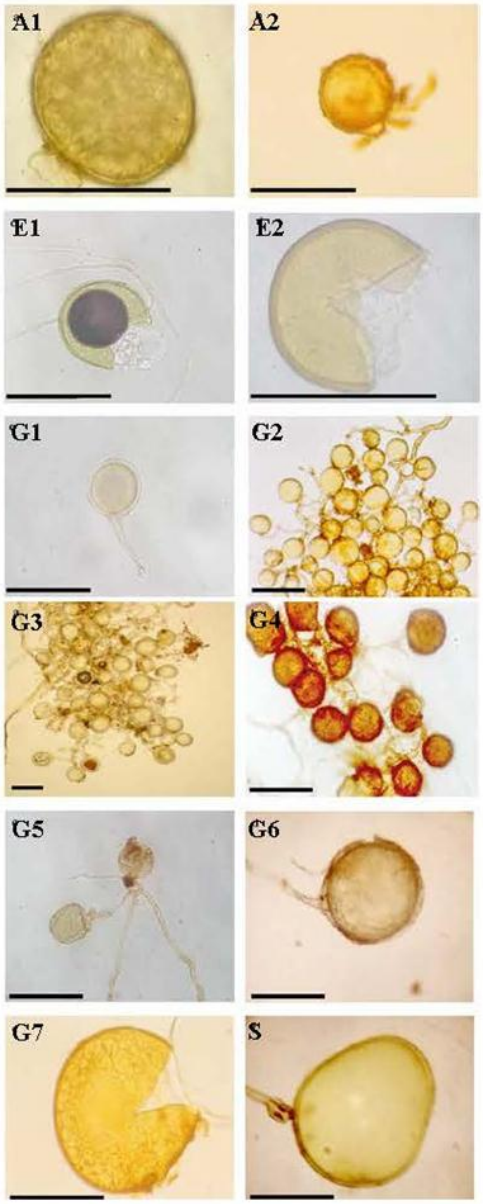

Figure 1 Spore features of *Acaulospora* sp. 1, 2 (A1, A2), *Entrophospora* sp. 1, 2 (E1, E2), *Glomus* sp. 1-7 (G1-G7) and *Scutellospora* sp. (S). Bar=100 µm.

The AM fungal species responded variously to *Brassica* as a non-AM host crop. There were increased in spore number of *Entrophospora* sp. 2 and *Glomus* sp. 1 and 7 by 61, 33 and 40 % of spore number at the start of crop, respectively (Fig. 2b). Whereas, there were decreased in spore number of *Acaulospora* sp. 1, *Entrophospora* sp. 1 and *Glomus* sp. 2 and 3 by 35, 47, 33 and 42 % of spore number at the start of crop, respectively (Fig. 2b). However, spore number of *Acualospora* sp. 1, *Glomus* sp. 4, 5, 6 and *Scutellospora* sp. did not change.

4. Discussion

The plant species altered the composition of AM population in the community. The results of this study indicated that AM community had differentially responded to AM host plant (maize) and non-AM host plant (cabbage). This might be due to the community of AM fungi is closely related to plant species and soil environments (Smith and Read, 1997). It is possible that a short season of rotation crop could substantially alter the inherent AM community due to changing in the mycorrhizal root mass, and spore number under rotation crop (Panja and Chaudhuri, 2004). Therefore, plant species have influenced on the AM population in the community. For example, the study of Johnson and co-worker which indicated that AM fungi are individualistic in their responses to cropping history (Johnson *et al*., 1991). They observed that *G. aggregatum*, *G. leptotochum* and *G. microcarpum* were more abundant in soil with maize monocrop history. Menéndez and co-

worker observed that *G. mosseae* is dominant in continuous red clover crop while *Scutellospora pellucida* is dominant in continuous barley crop (Menéndez *et al*., 2001).

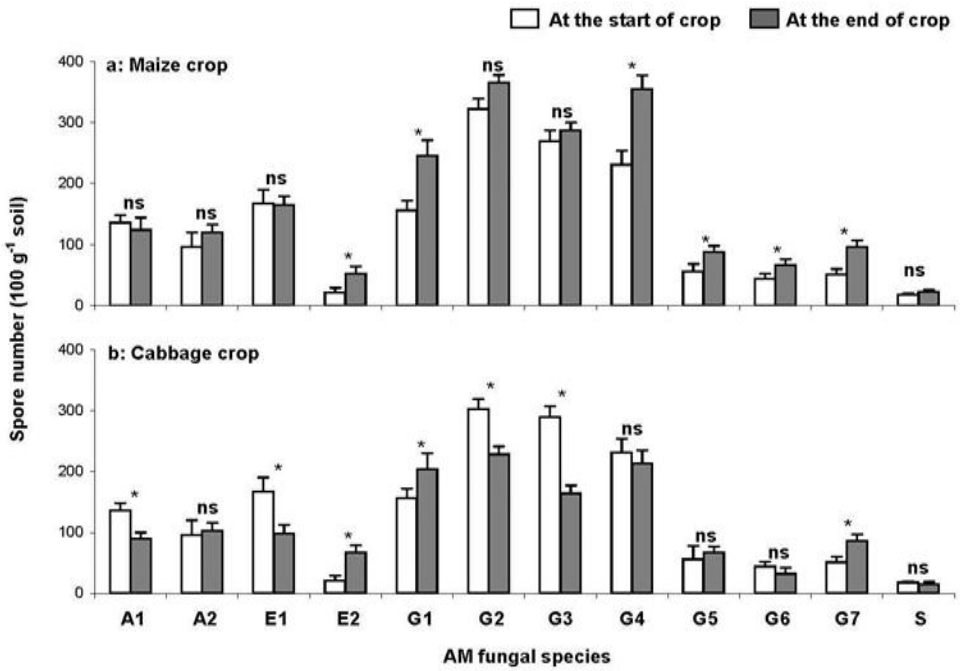

Figure 2 The spore number of 12 AM species under maize (a) and cabbage (b) at the start compared to the end of crop. Bars are means ± SE (n=4). Non- significant differences at $P < 0.05$ by t test are shown by "ns".

However, in this study, cabbage cropping is likely to be a negative effect on some AM fungal species. Generally, brassicas are considered to be the non-AM host plants because their roots contain glucosinolates (GSLs) that are released into the soil in the form of isothiocyanates (ITCs) when roots are disturbed or after the shoots has been harvested. The ITCs are known to have broad biocidal activity including phytotoxic effects (Petersen *et al*., 2001), nematicidal (Henderson *et al*., 2009) and fungicidal (Dunne, 2003) effect. According to *Brassica* green manures, rotation crops or seed meal amendments have been reported to suppress pest and disease organisms when grown or incorporated in the soil (Gavito and Miller, 1998). ITCs are usually considered to be the most active compounds (Brown and Morra, 1997). In field and pot trials, the number of AM spores was strongly depressed by white mustard (*B. alba* L.) (Karasawa *et al*., 2002). Furthermore, many previous studies have been reported that including *Brassica* plant in crop rotation reduced the rate of AM colonization in following crop (Ryan *et al*., 2002).

5. Conclusion

This study suggested that AM community is altered by species of plant. *Brassicaceae*, non-AM host plant, seems to be a negative effect on some AM species, while, the spore number of many AM fungal species have increased under maize, AM host plant. Further work is needed to consider crop rotation with different plant species might be a tool for management a beneficial AM community in sustainable agriculture.

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7. References

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