

Preliminary study on biodiversity of arbuscular mycorrhizal fungi (AMF) in oil palm (*Elaeis guineensis* Jacq.) plantations in Thailand

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Abstract. Oil palm (*Elaeis guineensis* Jacq.) is one of the promising crop plants which has been used as raw material for producing daily products. In agricultural ecosystems, crop plants could develop a plant-fungal association with arbuscular mycorrhizal fungi (AMF). The objectives of this study were to determine the AMF biodiversity and mycorrhizal infection percentage (MIP) from field-collected soil samples of three oil palm plantations from Nong Khai, Surat Thani, and Chiang Rai provinces of Thailand. Soil characteristics (moisture content, pH, and available phosphorus) were also measured. Thirteen AMF species belonging to seven genera were identified from all soil samples, whereas *Glomus* spp. and *Acaulospora* spp. were most commonly found species. AMF biodiversity value from Chiang Rai was statistically different from other two provinces ($p < 0.05$). MIP value of soil samples from Surat Thani was statistically different as well. Furthermore, soil pH showed a positive correlation with AMF biodiversity. These results confirmed that AMF normally occurs in oil palm plantations, but at different levels of biodiversity possibly due to different environmental factors in each plantation. Nevertheless, this information could be useful for using AMF in plant growth promoter and pathogen resistance programs in order to achieve the agricultural sustainability, especially in oil palm plantations.

1. Introduction

Oil palm has become an economically important crop plant among Southeast Asian and African countries. For example, the Royal Thai government made a decision on massive land expansion for oil palm plantations during the last 20 years. Most terrestrial plants have a mutualistic association with arbuscular mycorrhizal fungi (AMF) where these fungi develop their structures within the cortical cells of plant roots and give beneficial values such as improving the nutrient content, especially for phosphorus and water absorption [1]. Obtaining the beneficial effects of AMF application could be an alternative solution for minimizing the chemical fertilizer and pesticide demand for oil palm expansion [2]. However, information on the biodiversity of AMF directly associated with oil palm is limited. In this study, we determined the biodiversity of AMF in field-collected soil samples from oil palm plantations of three provinces in Thailand (Nong Khai (NK), Surat Thani (ST) and Chiang Rai (CR)) by applying wet-sieving method and determined the mycorrhizal infection percentage (MIP) by using these soil samples as starting inocula and sorghum as host plants [3].



2. Materials and methods

2.1. Study site

Soil samples for this experiment were collected from three oil palm plantations in Thailand: 1) Rattanawapi Sub-district, Rattanawapi District, Nong Khai province; 2) Saisopa Sub-district, Phra Saeng District, Surat Thani province; and 3) Pa Tueng Sub-district, Mae Chan district, Chiang Rai province. The detailed information about these oil palm plantations is shown in table 1.

Table 1. Land use history of oil palm plantations.

Province	Coordinate	Land use before oil palm plantation	Fertilizer application	Herbicide application
NK	18°12'31.3"N 103°10'56.9"E	Rice field	• Inorganic fertilizers • Organic fertilizers (animal manure)	Yes
ST	8°35'5.9634"N 99°4'55.3116"E	Rubber plantation	• Inorganic fertilizers • Organic fertilizers (empty fruit bunch of oil palm)	Yes
CR	20°06'40.9"N 99°45'17.9"E	Rice field	• Inorganic fertilizers	No

NK = Nong Khai, ST = Surat Thani and CR = Chiang Rai.

2.2. Soil sampling

All soil samples were collected between late November 2014 and early March 2015. For each plantation, 5 samples were taken from 5 randomly placed one-square-meter plots that were separated by at least 5 m apart. Within each plot, 5 subsamples (approx. 300 cc) were randomly collected down to the soil depth of 15 cm before being mixed together to obtain a pooled sample for each plot. A total of 15 pooled soil samples of approx. 1.5 liter were collected. All soil samples were stored in plastic bags and kept at room temperature until used.

2.3. Soil characteristics analyses

Soil moisture content was calculated from each soil sample as percentage oven-dry weight of soil by drying at 105 °C for 48-72 h. Soil pH was measured in water (1:2, wt/vl). Available phosphorus (P) was extracted with Bray 2 solution [4].

2.4. AMF spore extraction

AMF spores obtaining from each air-dry soil sample (50 g each) were extracted by wet sieving, decanting and a 60% sucrose gradient centrifugation [3,5]. The supernatant was rinsed with tap water for 2 min in a 38-µm sieve and transferred to 9 cm petri dishes for observation. Healthy spores were observed under a stereomicroscope (Olympus SZX-ILLB200) at 40-100X magnification. Then, 5-10 spores of each spore type collected from each soil sample were mounted on slides with polyvinyl alcohol-lactic acid-glycerol (PVLG) or PVLG mixed 1:1 (v/v) with Melzer's reagent and observed under a light microscope (Olympus CH2) at a magnification of up to 400X. Identification was based on the morphological descriptions provided by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, INVAM: <http://invam.caf.wvu.edu>.

The AMF biodiversity was calculated by Shannon-Wiener (H') index. Furthermore, it was calculated from the following equation (2), where pi is the proportion of individuals found in the i th species (pi is calculated from ni/N , [where ni is the number of individuals in the i th species and N is the total number of individuals]) [6].

$$H' = -\sum p_i (\ln p_i) \quad (1)$$

2.5. MIP test

The MIP test for each soil sample was conducted using field-collected soil samples as starting inocula and sorghum as the host plants. Sorghum seeds were grown into the growth substrate, consisting of rhizosphere soil (15 mL) from oil palm plantations and sterilized diluents (1:10, v/v). Sterilized diluents consisted of a mixture of soil and sand (1:2, v/v) (autoclaved for 2 h at 121 °C and repeated two times after a 24-h interval). Each soil sample was conducted in triplicate for a total of 45 MIP tests. The seeds of sorghum were previously surface-sterilized with 3% NaClO₃ for 2 min and used at 5-8 seeds for each pot. They were maintained with watering as needed for 28 days after shoots appearance. At the end of the test, plant shoots were discarded and roots were gently detached from the growth substrate and washed clean with water.

The mycorrhizal colonization of sorghum roots was assessed on 20 root pieces (approximately 1-1.5-cm segments) from each replication. The root segments were cleared in pre-boiled KOH (10%, w/v; 10 min), rinsed in tap water, acidified with HCl(1%, v/v; 30 min), stained in pre-boiled Trypan blue (0.02%, w/v; 15 min) and de-stained in acetic glycerin for at least 12 h. The stained roots were mounted on slides in PVLG with coverslips, oven-dried for 24 h at 60 °C, observed under a light microscope at 400X for quantification and scoring. Colonization percentage was scored based on the presence or absence of mycorrhizal structures (mycelium, vesicles, and arbuscules) of AMF observed in the stained roots. The colonization percentage by AMF was calculated using the following formula [7]:

$$MIP = \frac{\text{total number of root segments colonized}}{\text{total number of root segments studied}} \times 100 \quad (2)$$

2.6. Statistical analyses

All data in this experiment were reported as the mean value of three replications from each soil sample. The differences in soil samples from different plantations on the soil characteristics (soil moisture content, pH, and available P) and AMF values (AMF biodiversity and MIP) were calculated by univariate ANOVA tests. The significance of differences between the samples from different plantations in soil characteristics and AMF values were tested using Least Significant Difference test (LSD) at P<0.05. The relationships between soil characteristics and AMF biodiversity and between AMF values (AMF biodiversity and MIP) were calculated through Pearson correlation coefficients. Statistical procedures were conducted with the software package SPSS 16.0 for Windows.

3. Results and discussions

3.1. Soil characteristics

No significant difference was found among moisture contents (%) and pH(s) of soil samples from all provinces (figure 1a and 1b). However, available phosphorus values were significantly different between soil samples from the three provinces ($p < 0.05$) (figure 1c).

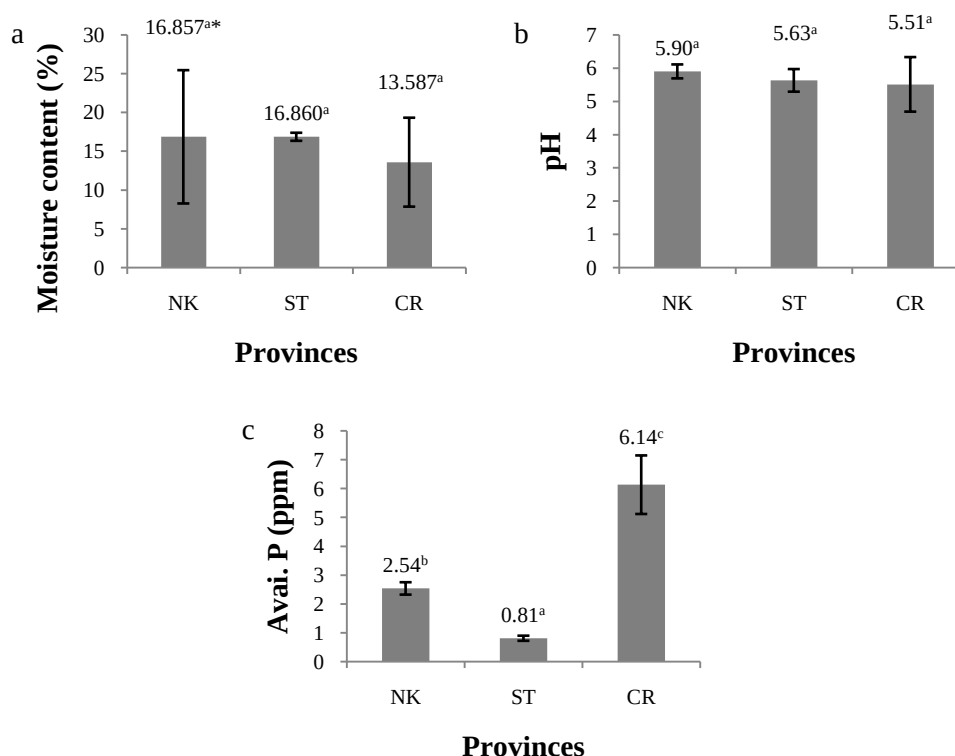


Figure 1. Mean values of (a) moisture content (%); (b) soil pH; (c) available phosphorus from all provinces. *Mean value followed by the same letters are different significantly ($p < 0.05$). NK = Nong Khai, ST = Surat Thani and CR = Chiang Rai. Standard deviation (moisture content and pH) and standard error (available P) of each province are indicated by error bars.

Soil moisture contents might vary in the range of a few percent in drier soils to excess than 100% in higher-plasticity clays [8]. In this study, the soil moisture contents were low (13.587 – 16.860%) (figure 1a). Soil pH(s) in arable land is commonly within the range of 5.0 to 6.2 [9]. Soil pH(s) in this study were within this range (figure 1b). Moreover, available P concentrations in soils might vary from less than 11 to more than 25 ppm [10]. Thus, the available P concentrations in this study were very low and ranged from 0.81 to 6.14 ppm (figure 1c).

3.2. AMF biodiversity and root colonization by AMF

We identified 13 species belonging to 7 genera from three different oil palm plantations. The mycorrhizal genera were *Gigaspora*, *Funneliformis*, *Glomus* (3 species), *Acaulospora* (3 species), *Dentiscutata*, *Scutellospora* and *Rhizophagus* (3 species) (figure 2). The number of species found in this study was slightly low when compared with other studies: twenty five AMF species from soil samples of two date palm plantations and its surrounding areas [11]; thirty four AMF species from rhizospheric samples of physic nut in Thailand [12]; sixteen AMF species from soil samples in dune and twenty four AMF species from soil samples in salt marsh of Mediterranean ecosystems [13].

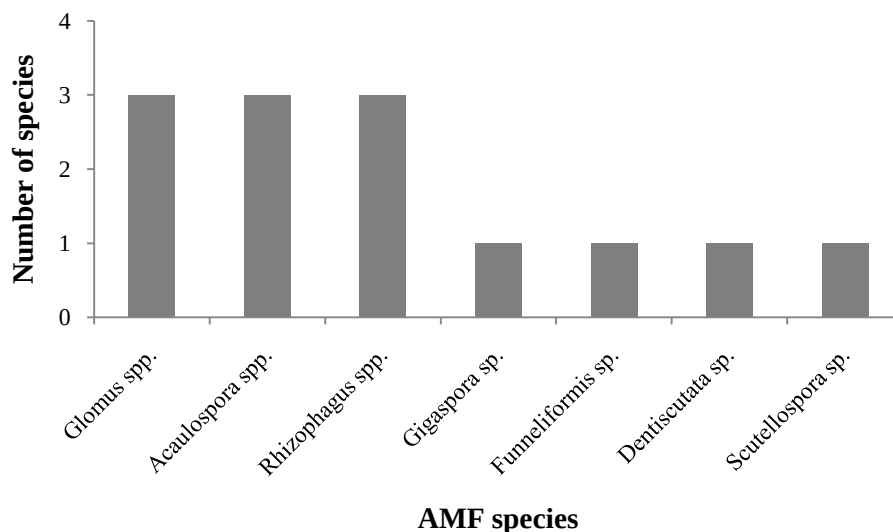


Figure 2. A number of mycorrhizal species observed from oil palm plantations, Thailand.

Glomus and *Acaulospora* were commonly found mycorrhizal genera from soil samples in this study. The remaining genera (*Rhizophagus*, *Gigaspora*, *Funneliformis*, *Dentiscutata*, and *Scutellospora*) were also present in this study, but in low frequency. Similar to our results, *Scutellospora*, *Acaulospora*, *Gigaspora*, and *Glomus* were mycorrhizal genera which could be found in disturbed and natural habitats in tropical Australia [14]; from the hot-dry valley of the Jinsha River, southwest China [6]; in mined soil samples [15].

AMF biodiversity of Chiang Rai was significantly different when compared to the other two provinces ($p < 0.05$) (figure 3a). These values of soil samples from three different oil palm plantations varied from low (0.81) to slightly moderate (1.33 – 1.51) (figure 3a). Other studies found AMF biodiversity in various numbers: AMF biodiversity within four shrub plants in a semi-arid Mediterranean ecosystem after revegetation was 0.78 [16]; from soil samples of physic nut in Thailand was 0.28 - 0.83 [12]; from soil samples in five host plants of rhododendron species were 1.75 - 2.40 [7]. The AMF biodiversity value could be determined from a mutual selection between mycorrhizal fungal communities and environmental factors [17]. For example, the two areas of Yungas forest have various values of AMF biodiversity where these values were significantly affected by seasonal changes. The higher values of AMF biodiversity were obtained in spring (wet season) [18]. Moreover, the highest value of AMF biodiversity from mountain grassland, Cordoba, Argentina was obtained in wet seasons (summer and spring) [19]. In this study, the relatively low value of mean AMF biodiversity values could be because these samples were collected in a dry season.

Moreover, mycorrhizal infection percentage (MIP) value of soil samples from Surat Thani was significantly different as well (figure 3b). MIP values give information about the activity of mycorrhizal community in certain ecosystems [20] and information on whether certain plants are mycorrhizal dependent or not [21]. Overall, the MIP values of soil samples from oil palm plantations were low for each province (3.00 – 9.63%) (figure 3b). Moreover, MIP values might vary within seasonal changes. The MIP values in soil samples from conventional and organic apple orchards in Brazil ranged from 43.70 – 59.70 (dry season) and 17.50 – 24.60 (wet season). In this study, we conducted MIP test in rainy period (wet season). In addition, the low number of MIP values might be due to mycorrhizal spores in soil samples have less adaptation level to different soil and climate conditions [22].

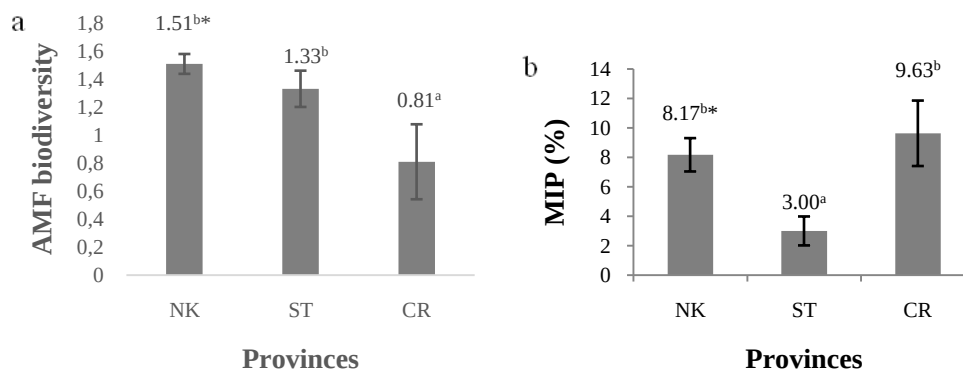


Figure 3. Mean values of (a) AMF biodiversity and (b) MIP from all provinces. *Mean value followed by the same letters are different significantly ($p < 0.05$). NK = Nong Khai, ST = Surat Thani and CR = Chiang Rai. Standard errors of each province are indicated by error bars.

3.3. Relationships between soil characteristics, AMF biodiversity, and MIP values

There is a significant negative correlation between soil moisture content (%) and available phosphorus values from all samples ($p < 0.05$) (figure 4a). Furthermore, soil pH(s) have positive correlation with AMF biodiversity ($p < 0.05$) (figure 4b).

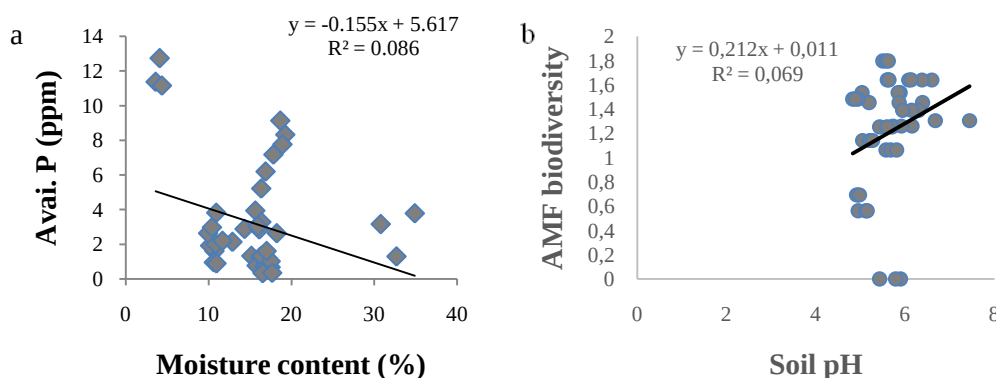


Figure 4. Correlation between (a) soil moisture contents (%) and available P and (b) soil pH and AMF biodiversity values from all samples.

Soil characteristics could be significantly affecting the arbuscular mycorrhizal fungal communities in diverse habitats [14]. Furthermore, the relationship between soil characteristics and AMF values might be affected by land use history such as fertilizer and herbicide applications in plantations. The non-significant correlation between AMF biodiversity and MIP values was comparable with several studies which showed no significant correlation between AMF biodiversity found in the samples and MIP of host plants: from rhizospheric soil samples of rhododendron species in Kumaun region of the Indian Central Himalaya [7] and from both transects in arid grassland [23].

4. Conclusion

Thirteen AMF species were found from the rhizosphere of oil palms. They were placed in seven genera: *Gigaspora*, *Funneliformis*, *Glomus* (3 species), *Acaulospora* (3 species), *Dentiscutata*, *Scutellospora* and *Rhizophagus* (3 species). *Glomus* spp. and *Acaulospora* spp. were the predominant species because they were found in all soil samples. This result indicates that both mycorrhizal genera (*Glomus* and *Acaulospora*) might have significant values such as improving the plant nutrient uptake

and protecting oil palms from pathogens. This study also showed low to slightly moderate AMF biodiversity in the rhizosphere of oil palms from three different plantations with the highest AMF biodiversity from Nong Khai province. The root colonization of AMF was highest from Chiang Rai soil samples; however, it was not significantly different from Nong Khai soil samples. Moreover, this study found a positive correlation between AMF biodiversity and soil pH and this correlation might be affected by land use history (fertilizer and herbicide applications) in oil palm plantations. Indeed, the difference in soil characteristics may determine the difference in AMF communities. Nevertheless, the results from this study support the potential for exploiting the AMF species in plant growth promoter and pathogen resistance programs in order to achieve the sustainability, especially in oil palm plantations.

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