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Establishment Of Mycorrhizas Of Arbuscular Mycorrhizal Spores From Oil Palm *in vitro* Using Petri-dish Observation Method

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Abstract

The aim of this project was to study the development of AMF isolated from oil palm mother trees and the interaction with the plant *in vitro* using petri-dish observation method. Once the plants (sorghum and maize seedlings) were inoculated with surface-sterilized spores of AMF, the germination of the spore and subsequent fungal development could be monitored easily and regularly without destroying any fungal structures. Within 2 weeks growth of extraradical mycelia and colonization by AMF on plant roots were occurred.

Keywords: arbuscular mycorrhizas, *Acaulospora*, *Glomus*, palm oil, spore

Introduction

Arbuscular mycorrhizal fungi (AMF) are root-inhabited soil fungi which form obligates symbiotic associations with over 80% of most terrestrial plant families (Harley & Smith 1983). They are abundant and ubiquitous in almost all plant communities in both natural and agricultural ecosystems. The potential use of AMF in agricultural production has been well documented for numerous plant species (Smith & Read 1997, Menge & Timmer 1982, Sylvia et al. 1993). Many methods had been used to study the development between AMF and the interaction with the plant (Hepper 1984). Mosse (1962) and Mosse & Phillips (1971) used test-tube culture in combination with various artificial supports for plant roots, for example filter papers and water agar. Other techniques involved the use of deep dishes (Allen et al. 1979). A "buried slide" technique was used by Hepper & Mosse (1975) to examine germ-tube development and colonization of roots by AMF. Setiadi (1995) used a test-tube culture with terragreen, an attapulgitic clay, as the supporting substrate for roots to produce

pure cultures of AMF from single spores. The present study attempts to examine the AMF-plant interaction using Petri dish observation chamber. Our primary goal was to test simply facilities that could be adopted by most laboratories.

Experimental and Methods

Spore extraction and surface sterilisation

AMF spores were extracted from 2 years old oil palm mother trees (Chiang Rai and Nan province) in pot-cultures by wet sieving and decanting technique (Gerderman & Nicolson 1963) using a 125 µm pore diameter sieve placed on top of a 45 µm. pore sieve. Spores were washed and placed in 1.5 ml. microcentrifuge tubes. One ml. of 0.005% sodium hypochlorite was pipetted into the tube and left for 3 min. The tube was then centrifuged to pellet the spores and the sterilant removed. Finally spores were washed three times with sterilized dH₂O before used.

Substrate preparation

Vermiculite and peat moss (ratio of 1:1) were used as substrate. Prior to autoclaved at 121°C for 20 min they were soaked with half strength Hoagland solution overnight. The Petri dishes (9 cm. diameter) were prepared by using a hot scalpel blade to cut a small hole (0.5 x 0.5 cm) into the side of the Petri dishes and their covers to insert plant seedlings. Each Petri dish were filled with substrate and were distributed. Petri dishes were kept in their sterile bags until needed.

Seed surface sterilisation and germination

Seed of sorghum (*Sorghum bicolor*) and maize (*Zea mays*) were surface sterilized by immersing the seed in sterilized dH₂O overnight then seeds were soaked in 30% sodium hypochlorite for 10-15 min. Finally seeds were rinsed three times with sterilized dH₂O. The seeds were germinated on fine autoclaved sand in container and kept in controlled conditions for 7-10 days.

Spore germination and plant inoculation

The sterilized single spore was inoculated onto the tap root of the 2 week-old germinated seedlings close to the root tip and seedlings were then planted in the Petri-dish through the hole. An individual seedling was gently placed into the slit of each Petri dish (10 seedlings per isolate) so that only the roots remain in aseptic conditions (Fig 1a). The cover was then replaced to match up with the bottom. After

placement of the Petri dish cover, autoclaved Lanolin (Sigma Aldrich) was used to seal the slit and hold the sealing in place. Each Petri dish was sealed with parafilm along the edge and then wrapped in aluminum foil, to reduce root or fungus exposure to light. Petri dishes holding seedlings were incubated upright in a plastic tray then transferred to a growth room 16:8 hour (day: night photoperiod). All seedlings were grown for 2 months and roots were examined for arbuscular mycorrhizal formation (Philips & Hayman 1970) every 2 weeks. After 2 months seedlings were removed from the Petri dishes and root segments were examined for infection.

Results and Discussions

In total 291 and 329 spores/50 g⁻¹ soil were obtained in each oil palm mother trees where originated from Chiang -Rai and Nan province respectively. Two morphospecies of AMF were identified using spore characteristics and molecular techniques as *Acaulospora tuberculata* (94% similarity) and *Paraglomus occultum* (100% similarity) (Fig. 1b-c). Oil palm root samples were highly colonized by AMF. (Fig. 1d-f).

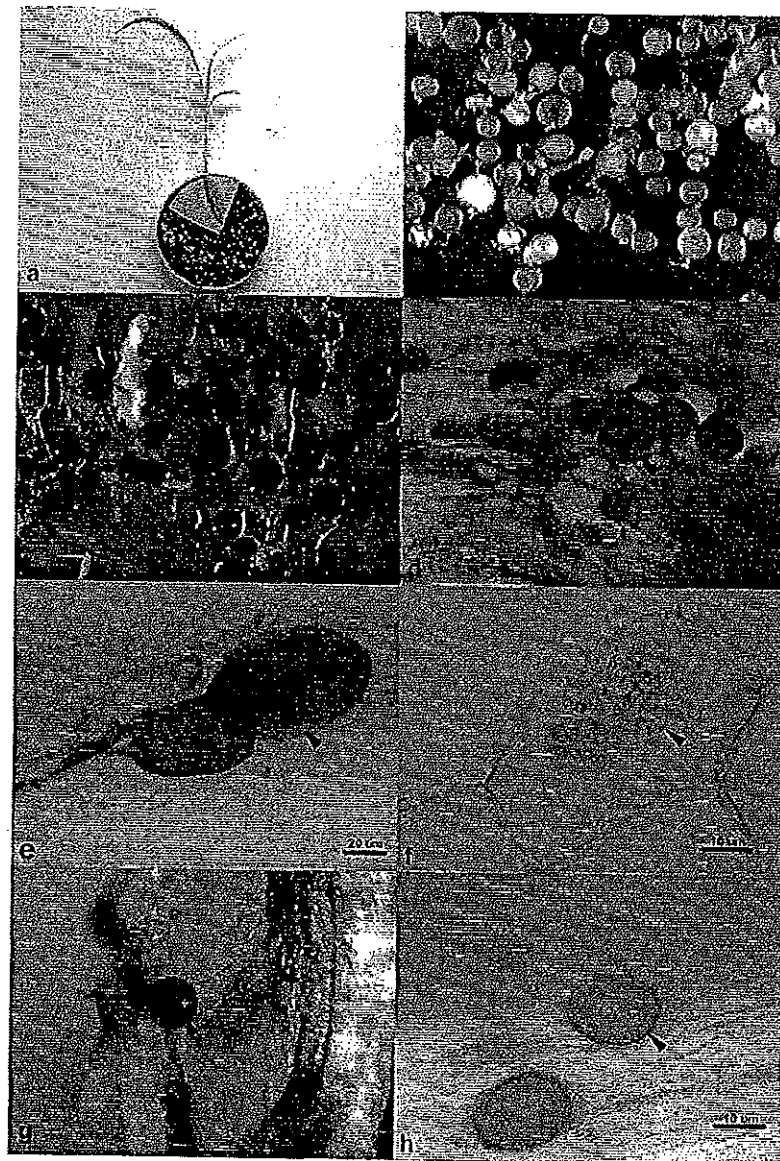


Fig.1 Establishment of mycorrhizas *in vitro* and microscopic photos demonstrated arbuscular mycorrhizas; (a) Petri dish observation chamber, (b) *Acaulospora* spores extracted from oil palm mother trees under stereomicroscope, (c) *Paraglomus* spores extracted from oil palm mother trees under stereomicroscope, (d-e) fine roots of oil palm showing stained roots with vesicles (arrowhead), (f) fine roots of oil palm showing stained roots with arbuscule (arrowhead), (g) germination of *Acaulospora* spore (arrow) produced hyphal branching after inoculated with sorghum seedling (arrowhead) and (h) fine roots of oil palm showing stained roots with vesicles (arrowhead) in sorghum seedling.

The mean percentage of oil palm root infection ranged from 90-100. It appears that oil palms are strongly mycorrhizal dependent. The Petri dish systems was effective, using this technique mycorrhizal formation could be established. With the techniques spore germination and root colonization could be observed. The germination hyphae grew in random direction, often toward to the roots and subsequently produced branches that formed a hyphal bridge that connected adjacent roots and then colonized them (Fig. 1g). Generally the colour of the hyphae was hyaline. Colonization of the roots was detected within two weeks by *P. occultum* whilst colonization for *A. tuberculata* was not detected until 4 weeks both in sorghum and maize seedlings respectively. Successful colonization resulted in forming of vesicle in sorghum root segments by *A. tuberculata* and *P. occultum* in maize (Fig. 1h). The mean percentage of root infection for sorghum and maize by *P. occultum* were 10% and 20% respectively at two weeks. At four weeks, in contrast, the mean percentage of root infection for sorghum and maize by *P. occultum* were exceeded 60% whilst those of *A. tuberculata* were 50-100% respectively. At two months higher infection rate were detected in both host plants and AMF species (Fig. 2).

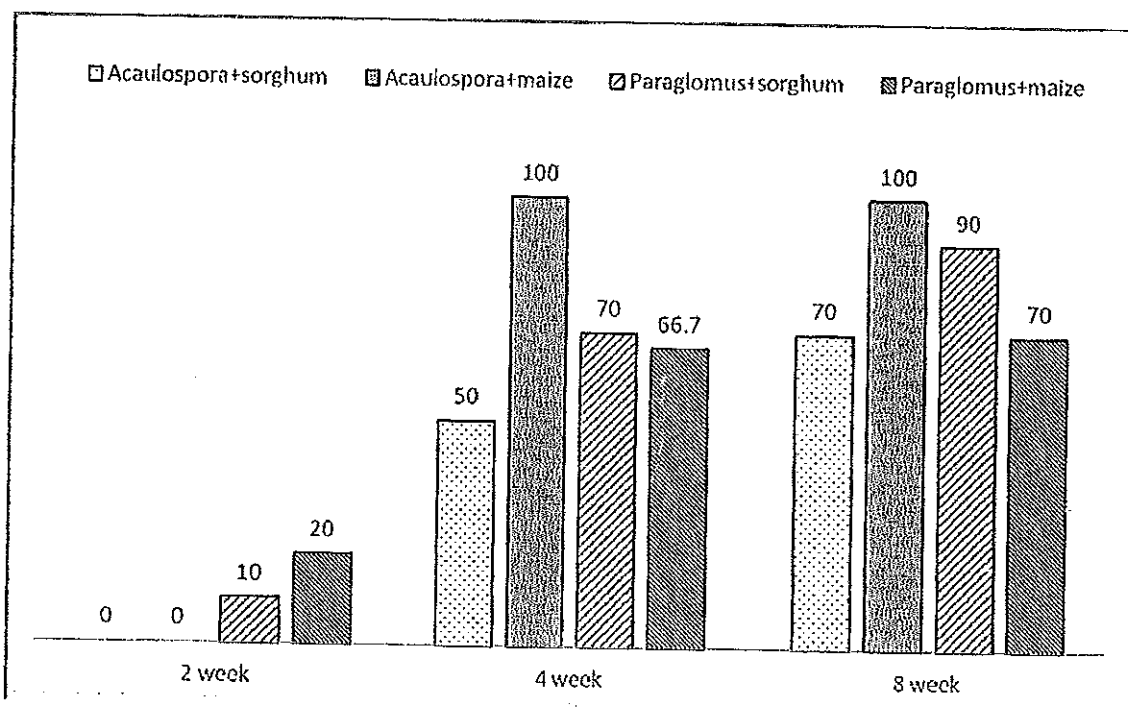


Fig.2 Percentage of roots infection in maize and sorghum seedlings using Petri-dish observation method.

Conclusions

In the present study we found that oil palm roots are usually heavily colonized by AMF and they are probably functionally dependent on mycobiont. Therefore AMF may have potential use for sustainable oil palm plantation. Within 2 weeks root colonization by AMF were detected. Well- established of selected AMF from oil palm mother trees and plant hosts could be set up using Petri-dish chamber method with simpler facilities which could be adopted by most laboratories.

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