

Protein Patterns in Arbuscular Mycorrhizal Roots and Non-Mycorrhizal Roots of Oil Palm Seedling

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A comparison of the protein patterns in root extracts from none mycorrhizal and mycorrhizal oil palm roots has been made. The polypeptides were analyzed every three weeks up to 11 weeks. A factorial design of fungi species (no mycorrhizal, *Acaulospora tuberculata*, *Gigaspora margarita*) and with or without fertilizer was assessed. The result showed that specific polypeptides were detected in primary and secondary roots. In unfertilized oil palm root, a 60 kDa polypeptide was detected while it was absent in fertilized root. Inoculation of *A. tuberculata* with the addition of fertilizer application yielded a specific 26.7 kDa polypeptide in primary root on the 11th week after inoculation. A specific 64.2 kDa polypeptide of *G. margarita* was detected in unfertilized secondary root also on the 11th week.

Key words: polypeptide, *Elaeis guineensis*, *Acaulospora tuberculata*, *Gigaspora margarita*, fertilizer

Arbuscular mycorrhizal obligate biotrophic fungi that form mutualistic symbiosis with many of the important agricultural plants such as palm. The formation and function of arbuscular mycorrhiza (AM) induced considerable morphological, physiological, biochemical, and molecular changes for both the host and the symbiont (Samra *et al.* 1997). These changes are presumably the result of complex sequence of interactions between the fungus and the plant's roots.

As in the microbe and plant interactions, regulation of these interactions requires a continuous exchange of signals between both partners, which when perceived through the corresponding receptors would induce a cascade of events, leading to changes in the expression of certain genes (Benabdellah *et al.* 2000). Pacovsky (1989) reported that during the early infection there are specific polypeptides. The results showed that the quantity and quality of plant protein shifted in response to the physiological changes resulting from infection by N₂ fixing bacteria or by endomycorrhizal fungi. Moreover, Samra *et al.* (1997) showed that there were 12 polypeptides in the mycorrhiza compatible to pea genotypes which were never observed in root extract from the mycorrhiza resistant mutant. The induction and accumulation of these polypeptides seem to be more correlated to the establishment of the functional symbiosis than to the recognition stage and aporesium formation. Ferrol *et al.* (2002) showed that the expression of plasma membrane H⁺-ATPase genes was regulated differentially in leaves and root both in wild type and mycorrhiza defective tomato plants. Analysis of 2D-PAGE gels revealed that AM colonization induced changes in protein synthesis and the sequence of polypeptide showed a 75% identity with the N-terminal sequence of the 69 kDa catalytic subunit of the vacuolar type H⁺-ATPase of plants (Benabdellah *et al.* 2000).

Interaction between host and fungi in AM fungi symbiosis with oil palm has never been studied extensively. Understanding the mechanism of interaction is important and necessary in order to get more benefit from the symbiosis

mechanism. Therefore, it is a necessity to see the changes and gene expression involved in the infection process of the symbiont. The ability to quantify protein involved in specific mycorrhizal symbiosis could be used to examine the changes in roots which were colonized with AM fungi.

The effect of AM fungi symbiosis for the growth and nutrient absorption of oil palm has been reported before. However, the physiology of oil palm, especially the root's polypeptides during the early infection, was never been reported. Understanding the molecular and genetic basis of the plant-fungus interaction in a functional AM symbiotic system is required for the identification of gene and its gene products involved in this process. In this paper we used SDS PAGE to study the effect of AM colonization upon the oil palm polypeptide pattern. Two compatible AM fungi species were used in this work.

MATERIALS AND METHODS

Biological Material and Experimental Design. Plant material used were germinated oil palm of Dura X Pisifera from the Indonesian Oil Palm Research Institute. Acidic soil from Cikopomayak, West Java was used as planting medium (sterilized 3 x 8 hours intermitted overnight at 105 °C). Spores and infected root fragments were used as inoculant raised on *P. phaseoloides* plants grown in a glasshouse.

The experimental design used was a factorial complete blocked randomized design. Two treatments assessed were AM fungi species (*Acaulospora tuberculata* and *Gigaspora margarita*) and fertilizer (with and without fertilizer). These were used as the samples treatment. The dose of AM fungi inoculant followed the report Widiastuti *et al.* (2002), while the uninoculated treatment fertilizer as dose was recommended by Lubis (1992).

Growth Conditions. Oil palm seeds were germinated in sterilized sand until they were 3-months-old. At the same time, the preparation of fresh inoculant was done by inoculating *Pueraria phaseoloides* with AM fungi. *P. phaseoloides* was grown in 60 cm x 60 cm sized polybags

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(10 kg absolute dried soil) without a hole containing sterilized acidic soil from Cikopomayak. The oil palm was inoculated with AM fungi by transferring to polybag. The plants were watered with pre-boiled water. Plant harvesting was conducted at 3, 5, 8, and 11 weeks after inoculation. Roots were washed and observed for the AM infection, and analyzed for the polypeptide. A part of a root was sampled to determine the percentage of fungal colonization (Koske and Gemma 1989). The remaining root material was frozen at -20°C before extraction for protein analysis.

Harvesting Procedure and Sampling. Three plants from each treatment were taken and the roots were washed carefully with tap water and weighed for shoot and root of the plant. Roots were pooled to make the root samples. Primary and secondary roots were separately analyzed.

Protein Extraction. Freshly harvested oil palm roots were carefully cleansed in deionized water, weighed, and ground into fine powder in liquid nitrogen. Subsequently the proteins were extracted. The protein content was determined by the Bradford assay (Bradford 1976) and bovine serum albumin (BSA) was used as the standard (Samra *et al.* 1997).

Gel Electrophoresis and Staining. Sodium-dodecyl-sulfate-polyacrilamide-gel-electrophoresis (SDS PAGE) was performed as described Laemmli (1970). As much as 30 μM protein was injected into the gel electrophoresis. Gels were stained using the silver staining method (Oakley *et al.* 1980). Molecular weight of proteins were determined by using a commercial molecular weight marker kit (Bio-Red, Munich Germany).

RESULTS

Development of AM Fungal Infection. Observation showed that the AM fungi was absent in the uninoculated treatment while it was present in the inoculated plant. In the unfertilized treatment, AM fungi infection was smaller compared to fertilized oil palm seedlings both in *A. tuberculata* and *G. margarita* (Figure 1). In addition, there were differences of infection pattern between *A. tuberculata* and *G. margarita*. *A. tuberculata* infection was slower compared to *G. margarita* (Widiastuti 2004). In addition, the results showed that AM fungi infection in secondary root was higher compared to those in primary root (Figure 1 and 2). Generally, the enhancement of AM fungi infection in secondary roots was higher after three weeks inoculation, while in primary roots the higher infection was found five weeks after inoculation, especially in *G. margarita*. However, the results showed that fertilizer enhanced AM fungi infection.

Polypeptide Pattern. The polypeptide pattern in inoculated root was slightly different compared to the uninoculated one. The same result was found between the fertilized and unfertilized oil palm roots. Fertilizer also affected the root polypeptide pattern of inoculated seedling as well as uninoculated seedling.

In general, polypeptide patterns of oil palm root in 3, 5, 8, and 11th week after inoculation were slightly different between treatment both in primary and secondary roots. However, there were only some polypeptides which appeared to be specific. SDS PAGE analysis of primary root in 11th

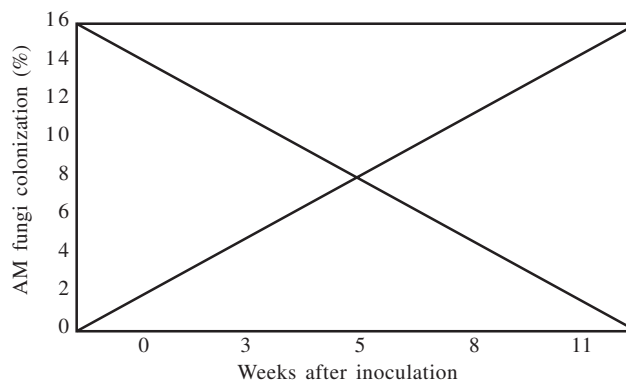






Figure 1 AM fungi colonization in primary root of oil palm.  *A. tuberculata* without fertilizer,  *A. tuberculata* with fertilizer,  *G. margarita* without fertilizer,  *G. margarita* with fertilizer).

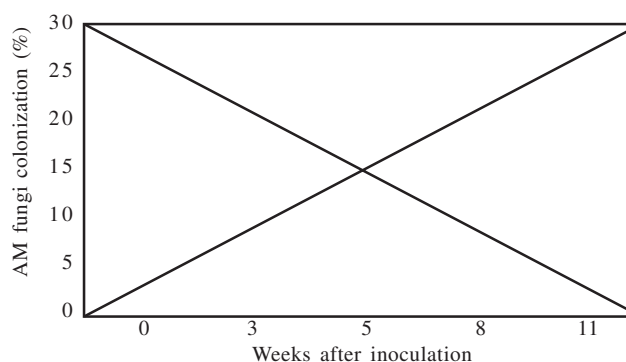






Figure 2 AM fungi colonization in secondary root of oil palm.  *A. tuberculata* without fertilizer,  *A. tuberculata* with fertilizer,  *G. margarita* without fertilizer,  *G. margarita* with fertilizer).

weeks after inoculation is shown on Figure 3. A polypeptide with a molecular weight of 60 kDa was present in unfertilized plant roots, but this polypeptide was not detected in fertilized plants (Table 1). In addition, in the primary root of oil palm root inoculated with *A. tuberculata* and fertilized has a 26.7 kDa polypeptide. This polypeptide was not found in plant root inoculated with fertilizer for *A. tuberculata*. This result showed that probably the polypeptide was specific for *A. tuberculata* colonies in fertilized roots (Table 1).

The observation of secondary roots on the 8th week after inoculation showed that there was a 60 kDa polypeptide found in uninoculated oil palm root given no fertilizer (Figure 4, Table 2). The polypeptide was not found in the plant treated with fertilizer. However, there were no differences the polypeptide pattern of secondary and primary roots between inoculation with *A. tuberculata* and *G. margarita*. The same result was found in treatment with fertilizer in the two inoculated plants.

SDS PAGE analysis of secondary oil palm root at the 11th week after inoculation showed that there were no differences in polypeptide pattern of the uninoculated root between fertilized and non-fertilized plants. A 35 kDa polypeptide was present in palm root inoculated with *A. tuberculata* that was treated with fertilizer while it is absent in the plant that were not treated with fertilizer. In addition, a 64.2 kDa polypeptide present in secondary roots that were

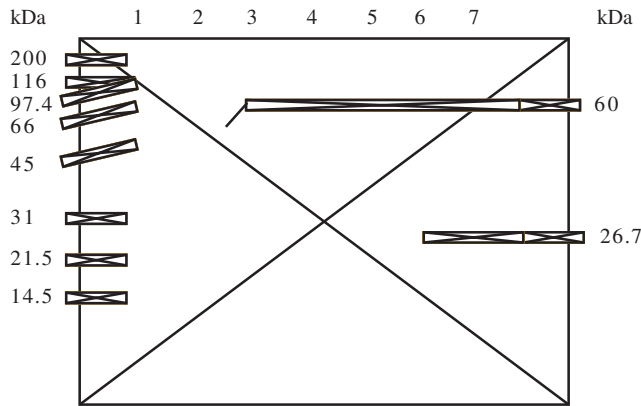


Figure 3 Silver stained SDS PAGE of primary palm root extracts 11 week after inoculation. Lane 1: Marker, 2: uninoculated without fertilizer, 3: uninoculated with fertilizer, 4: inoculated with *A. tuberculata* without fertilizer, 5: inoculated with *A. tuberculata* with fertilizer, 6: inoculated with *G. margarita* without fertilizer, 7: inoculated with *G. margarita* with fertilizer.

Table 1 Specific polypeptide developed at 11 week of primary oil palm root

Treatment	Size of specific polypeptide (kDa)
Control: uninoculated without fertilization	60
<i>A. tuberculata</i> without fertilizer	60
<i>G. margarita</i> without fertilizer	60
<i>A. tuberculata</i> with fertilizer	26.7

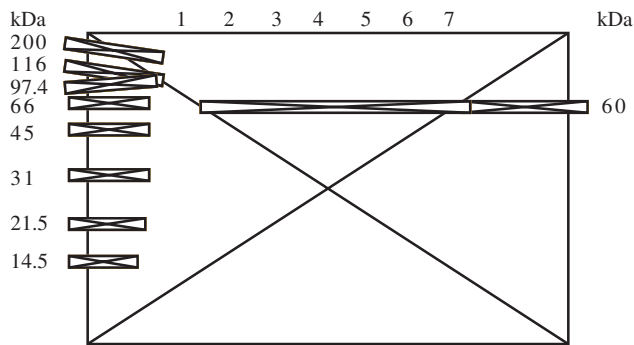


Figure 4 Silver stained SDS PAGE of secondary palm root extracts 8 week after inoculation. Lane 1: Marker, 2: uninoculated without fertilizer, 3: uninoculated with fertilizer, 4: inoculated with *A. tuberculata* without fertilizer, 5: inoculated with *A. tuberculata* with fertilizer, 6: inoculated *G. margarita* without fertilizer, 7: inoculated *G. margarita* with fertilizer.

Table 2 Specific polypeptide developed at eight weeks of secondary oil palm root

Treatment	Size of specific polypeptide (kDa)
Control: uninoculated without fertilizer	60
<i>A. tuberculata</i> without fertilizer	60
<i>G. margarita</i> without fertilizer	60

inoculated with *G. margarita* although the plant was treated with fertilizer.

DISCUSSION

Infection of AM fungi in secondary roots was higher compared to primary root (Figure 1 and 2). The same result has been reported by Widiastuti *et al.* (2003). Based on the

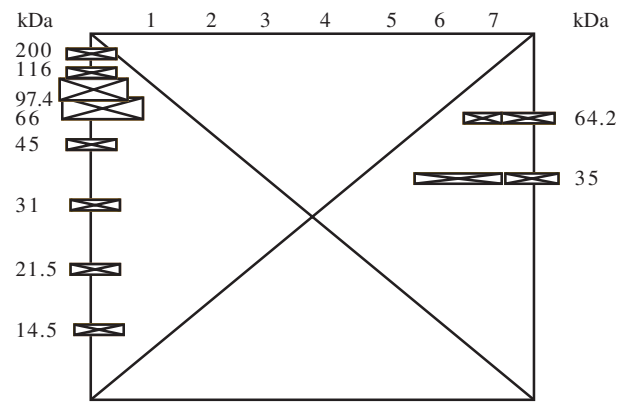


Figure 5 Silver stained SDS PAGE of secondary palm root extracts at 11th week after inoculation. Lane 1: Marker, 2: uninoculated without fertilizer, 3: uninoculated with fertilizer, 4: inoculated with *A. tuberculata* without fertilizer, 5: inoculated with *A. tuberculata* with fertilizer, 6: inoculated with *G. margarita* without fertilizer, 7: inoculated with *G. margarita* with fertilizer.

Table 3 Specific polypeptide developed at 11 week of secondary oil palm root

Treatment	Size of specific polypeptide (kDa)
<i>A. tuberculata</i> with fertilizer	35
<i>G. margarita</i> without fertilizer	64.2

data obtained, was conclude that infection of AM fungi first took place in secondary roots followed by infection in primary roots.

Comparasion of polypeptide pattern between primary and secondary roots showed that a 60 kDa polypeptide was detected from primary roots of uninoculated plants without treatment with fertilizer at week 11. However, it does not in the plant that were treated with fertilizer. Similar results were found in observations at week 8 after inoculation with either *A. tuberculata* or *G. margarita* in which the 60 kDa polypeptide was absent. The results suggested that there was a dynamic of pattern of polypeptide synthesis within oil palm roots. We suggest that the polypeptide was expressed in secondary roots in the first infection and followed by expression in primary roots. That's the infection process was took place in secondary roots first, followed by infection in primary roots.

SDS-PAGE analysis of primary roots harvested at the 11th week after inoculation with *G. margarita* that was not treated with fertilizer showed a specific polypeptide (64.2 kDa). This polypeptide was also detected in secondary roots inoculated with *G. margarita* at week 11. This result is interesting since the same size of polypeptide was detected in two different root types within the same period. The result suggested that the response of oil palm to *G. margarita* inoculation both in primary and secondary root was typically the same. Detection of similar polypeptide in both primary and secondary root showed that both root types have similar protein expression.

Pacovsky (1989) showed that in soybean inoculated with *Glomus fasciculatum* has specific protein with the size of 16, 17, 18, 22, and 30 kDa. Samra *et al.* (1997) revealed that there were 12 polypeptides expressed in compatible peas. Five polypeptides were detected in the early stage of symbiosis,

while others were observed later. It was thought that the induction and accumulation of these polypeptides seem to be more correlated to the establishment of the functional symbiosis rather than recognition stage and appressorium formation. Benabdellah *et al.* (2000) showed that a specific polypeptide with a size of 69 kDa in the plasma membrane of tomato root extract inoculated with AM fungi. However, the role of the specific polypeptide detected in this research is not known. In addition, the origin of the polypeptide detected in this research is not known. It could be of fungal or plant. However, it was correlated with chitinase analysis and formation of AM fungi organs especially the arbuscule (Widiastuti 2004) it could be that the polypeptide detected in this research correlated with the final stage of recognition of symbion or early functional symbiosis. However, there was a different size of polypeptide detected in this study compared with the previously study. In this study the polypeptide was detected over a longer time (11 weeks) compared to the previously study. It seems likely that the different period of the appearance of specific polypeptide was correlated with the process of AM fungi infection. However, the differences of AM fungi species and host plant species suggested different characteristics of polypeptide and also the time of their detection.

This research also showed that the expression of several polypeptides could be detected in same period. These are shown in Figure 1, 2, and 3 both in fertilized and unfertilized oil palm and inoculated and uninoculated palm. But, from our study it is shown that the selected polypeptide was detected only in a certain period. This result in the same result as the result of Garcia-Garrido and Ocampo (2002).

In general, it has been shown that AM fungi penetration inside roots and their development in roots involved a series of morphological and physiology differentiation in both the plant and AM fungi. Comparison of polypeptide pattern isolated from mycorrhizal and non-mycorrhizal oil palm root showed that in all fractions AM colonization induced three major changes in oil palm root gene expression: up regulation and down regulation of some constitutive polypeptides already present in oil palm root and induction of some new polypeptides. This result was also shown in tomato as reported by Benabdellah *et al.* (2000). However, at this point in time we do not know the role of the differentially expressed polypeptides. In addition, detection of polypeptide using SDS-PAGE in one dimension is not very sensitive.

Furthermore, the polypeptide induced in the early infection was very low and differentially expressed.

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