Chitinase Activities of Oil Palm Root at Early Infection of Arbuscular Mycorrhizal Fungi

Happy Widiastuti

Indonesian Biotechnology Research Institute for Estate Crops
Jl Taman Kencana No. 1 Bogor, Indonesia 16151
Corresponding author: happywidiastuti@yahoo.com

Abstract

The activity of chitinase of oil palm root at the early of arbuscular mycorrhizal fungi (AMF) colonisation was studied. The results showed that early in colonisation (0-5 weeks), chitinase activity in roots increased nevertheless eventually suppressing occurs 5-8 weeks after inoculation. Inhibition of chitinase activity is influenced by AMF species and fertilization. Suppression of endochitinase activity of effective species is higher than the species that are less effective, especially in the fertilization treatment. The activity of exochitinase was depressed in the presence of AMF colonisation. In the treatment without fertilizer, suppression of exochitinase activity of inoculated roots with *G. margarita* is higher than those inoculated with *A. tuberculata*. Inhibition of chitinase activities on secondary roots that occurs at the inoculation of *G. margarita* is higher than those inoculated with *A. tuberculata* and vice versa in the primary root.

Keywords: chitinase activities, palm oil, early colonisation, arbuscular mycorrhizal fungi

Introduction

Arbuscular mycorrhizal fungi (AMF) is a fungus symbioses with the oil palm. Biochemical processes involved in plant symbiosis with fungi has been studied. Differences in proteins both quantitatively and qualitatively has been shown between AMF inoculated and uninoculated plants. AMF colonization seems to be in place for increasing the size of low protein expression as shown in tobacco (Dumas *et al*, 1996). The emphasis is more consistent in the chitinase activity which is also have a role in defense plants in the early stages of AMF colonisation. Penetration of AMF in root involves a series of changes in morphology and physiology in plants and fungi. However, as occurs in plant-pathogen interaction mechanisms of induction and suppression associated with plant defense is the key to the colonization of AMF and its compatibility with the host plant (Garcia-Garrido & Ocampo, 2002).

The effectiveness of plant defense responses associated with the speed of the process of introduction of specific signaling molecules called elisitor. Elisitor can be secreted by the microbes that infect or as a result of breakdown of plant cell walls. Regulatory mechanisms of defense responses through degradation of the molecule can be issued elisitor AMF for example by hydrolysis enzymes such as chitinase, glucanase B 1.3, kitosanase. Lambais and Mehdy (1996) observed a suppression of activity endochitinase up to nearly twice in the AMF inoculation treatment and the level of infection-suppressing this enzyme was higher in the effective strain AMF compared to strains that are less effective. This research aim was to study plant defense responses during early colonisation, especially AMF chitinase activity.

Materials and Methods

Planting materials used were germinated oil palm from IOPRI Medan. Germinated oil palm was grown in sterile sand until the age of 3 months. AMF inoculation on the palm was made by the

method of pre-nursery i.e by developing AMF colonization in *P phaseoloides* prior to colonisation to oil palm seedling. *P. phaseoloides* grown in polybags 50 x 50 cm in sized without holes containing 10 kg of sterilized Cikopomayak acid soils. Inoculum dose and optimum dose of fertilizer was based on the optimization experiment (Widiastuti *et al*, 2002). Plants were maintained in a greenhouse by watering with cooling boiled water. Harvesting was done at weeks 0, 3, 5, 8, and 11 after inoculation by washing the roots with water taps. Observations were conducted on fresh and dry weight of seedlings, chitinase activitieas (Lambais & Mehdy, 1996), and the percentage of AMF colonisation. Chitinase activity was determined by counting the hydrolyzed N acetyl glucosamine using BSA as protein standard. The two factors tested were AMF species (without AMF, *A. tuberculata*, and *G. margarita*) and fertilizing (without and with fertilizer). The inorganic fertilizer dose of control was based on Lubis (1992). Experimental design used was completely randomized group design with factorial pattern.

Results and Discussion

AM fungi colonisation in oil palm root

Chemical analysis showed that the soil was very acid (pH H_2O 3.8), the content of C, N, P_2O_5, K_2O, CaO, MgO were very low at 1.59; 0.097; 0.013; 0.01; 0.066; 0.054% and 16.23 Aldd me 100 g^{-1} respectively. Colonisation of AMF began weeks after inoculation in both the primary and secondary roots. However, colonisation in secondary roots was higher than those in primary roots. Moreover, colonisation of AM fungi in no fertilization treatment was higher than those both *A. tuberculata* and *G. margarita* (Fig 1).

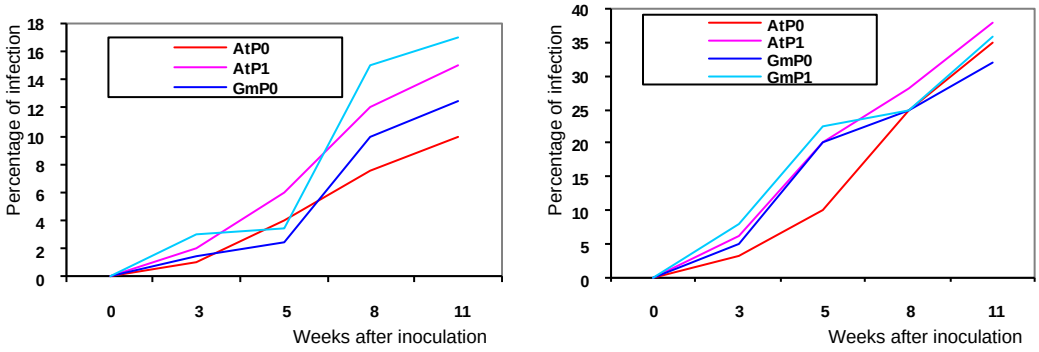

Figure 1. Percentage of AMF colonisation in primary (left) and secondary (right) oil palm roots.

Chitinase activity in the primary root

The analysis showed that in the primary roots which were not inoculated with AMF, the activity of exochitinase was higher compared to those of the inoculated one. Exochitinase activity increased until the fifth week after inoculation and subsequent declined at week eight and very low at week 11. These results indicated that the exochitinase activity was temporary and there is emphasis of exochitinase activities particularly began 8 weeks after AMF inoculation. The effect of fertilization showed a trend that in palm roots without AMF inoculation and inoculated with *G. margarita*, fertilization causes the higher activity of exochitinase compared to those not fertilized. However, these results differed with exochitinase activity of palm roots inoculated with *A. tuberculata.* The exochitinase activities of the root that are not fertilized, is higher compare to those fertilized. Emphasis of the exochitinase activity in the inoculated roots of palm inoculated with *A. tuberculata* both at without fertilization and fertilized was higher than the activity exochitinase palm roots inoculated with *G. margarita*.These results indicate that in the primary root, the activity of exochitinase influenced by AMF species and the presence of fertilization.

Endochitinase activity was lower in primary roots compared to exochitinase activity. Endochitinase activity in primary roots that not inoculated with AMF was higher compared to those inoculated. Unlike to the exochitinase activity, the peak of endochitinase acitivity occurred at week 5 and declined until week 8. Activity of endochitinase of oil palm root unfertilized inoculated with *A. tuberculata* is higher than those of inoculated with *G. margarita*. This result was in line with exochitinase activity (Fig. 2). With the application of fertilizer, the activity endochitinase of primary palm root inoculated with *G. margarita* was higher than those of the inoculated with *A. tuberculata*. These results indicated that the dose of fertilizer and type of AMF affect endochitinase activity on secondary roots.

The data of chitinase activity in general, showed that in the primary root chitinase activity of oil palm uninoculated with AMF was higher than those of inoculated with AMF. This trend happened in the oil palm root inoculated with *G. margarita,* but the opposite occurred in oil palm roots inoculated with *A. tuberculata*.

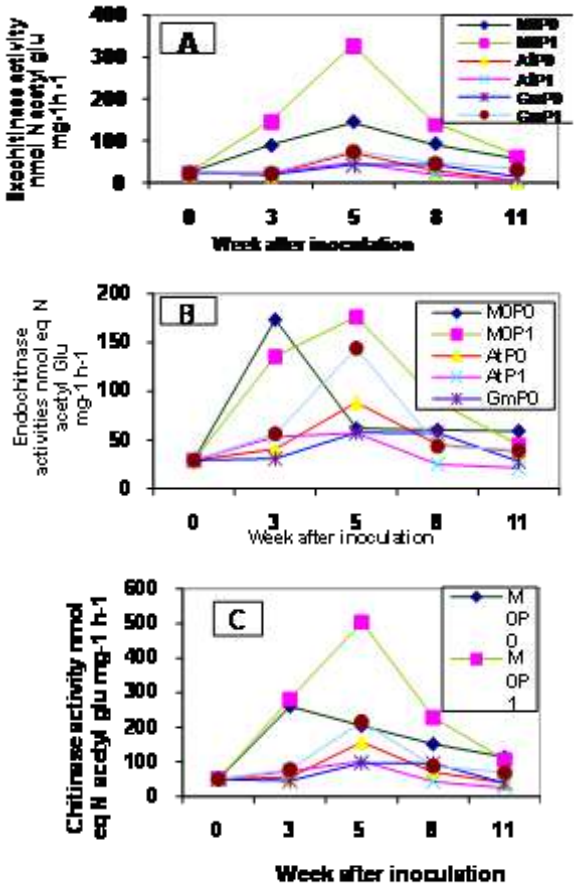

Figure 2. Exochitinase activity (A), endochitinase (B), and total chitinase (C) of primary oil palm roots in response to treatment.

Chitinase activity in the secondary roots

Exochitinase activity on the secondary roots of palm oil was much higher compared with exochitinase activity in the primary root. This difference reached 10 times. Endochitinase activity in oil palm roots without AMF inoculation was higher than those of inoculated with AMF. In the secondary roots inoculated with *A. tuberculata*, endochitinase activity without fertilization was higher than those fertilized root. These results are similar to those in primary roots. But for oil palm roots

inoculated with *G. margarita*, there was no difference of exochitinase activity between a fertilized and unfertilized. In addition, it was shown that in fertilization oil palm root, the emphasis of chitinase activity was slower compared to those of without fertilized.

Activity of endochitinase of secondary roots of palm was lower than exochitinase activity on the same root. In addition, endochitinase activities of palm root that were not inoculated was higher compared to those inoculated with the AMF. In secondary oil palm roots inoculated with *A. tuberculata* but unfertilized, endochitinase had a higher activity compared with those of fertilized, but in contrary to the roots of oil palm inoculated with *G. margarita*. These results were similar to those observed in primary roots. At the root of the fertilized palm, the activity of the endochitinase the activity of root inoculated with *G. margarita* was higher compared to those inoculated with *A. tuberculata,* whereas the opposite occured in the treatment without fertilization. These results were in line with that occured in the primary root. The high of endochitinase activity in roots inoculated with *A. tuberculata* followed by a higher emphasis anyway.

General description for chitinase activity in the secondary roots of palm oil showed that the activity of chitinase in the palm oil root that were not inoculated with AMF was higher than that inoculated with AMF (Fig 3.). In oil palm inoculated with *A. tuberculata*, fertilization actually decreased the activity of chitinase in secondary roots. While palm oil inoculated with *G. margarita*, there was no difference between a fertilized and unfertilized.

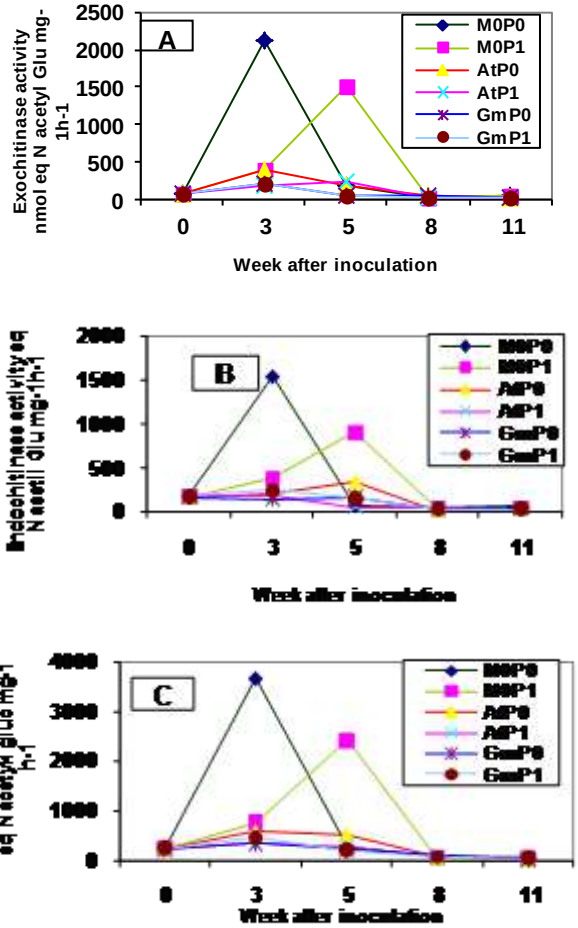

Figure 3. Activity of exochitinase (A), endochitinase (B), and total chitinase (C) of secondary oil palm roots in response to treatment.

Possible differences in plant response to AMF colonisation in particular is evident from the activity of chitinase can be a bookmark for the selection of the effectiveness of AMF. In the present study demonstrated that inoculation suppressed the activity of chitinase. Suppression level was influenced by the AMF species and doseage of fertilizer. Emphasis on treatment without fertilization was highest in week 3 after inoculation, while the emphasis of chitinase activity on fertilization treatment was observed 5 weeks after inoculation. Fertilization appears to slow the emphasis of chitinase activity.

The same is true of the exochitinase activity are fertilized palm that inoculated with *G. margarita*. Despite this emphasis on endochitinase activity of *G. margarita* inoculation lower compared to those inoculated with *A. tuberculata*. Total chitinase activity in the secondary roots either by fertilization or without fertilization showed that the inoculation of *G. margarita* more suppresse than thoses the inoculation with *A. tuberculata*.

Changes in isoenzyme patterns and biochemical properties of several enzymes associated with plant defense as chitinase has been shown for tomato root colonization by AMF (Pozo *et al*., 1996) with the induction of new isoforms. These hydrolytic enzymes act as a defense against pathogen attack because of its potential hydrolyze fungal cell wall polysaccharides (Pozo *et al*., 1996). Induction of this enzyme activity in AMF symbiosis may also be involved in the protection of plants against pathogenic fungi (Dumas-Gaudot *et al*., 1996).

The present study indicated that there was an emphasis on the chitinase activity which was influenced by inoculation with AMF, AMF species, and fertilization. Fertilization slow emphasis chitinase activity. In addition, the data showed generally lower total chitinase activity in the presence of fertilization compared with those not fertilized, especially in the inoculation with *A. tuberculata*. While at the inoculation with *G. margarita* fertilization increased the chitinase activity. The same thing also happened in controls. The results in this study seemed in line with Blee & Anderson (1996) suggested that P can regulate plant defense responses. The results suggested that fertilization decreased 1.3 glucanase (occurs with β mRNA). The delay of suppression of chitinase activity supposed to be caused by the slow of AMF colonisation in the presence of fertilization.

Emphasis on fertilization treatment to endochitinase activity in roots inoculated with *A. tuberculata* is higher than those on inoculated roots of *G. margarita* and vice versa in the treatment without fertilizer. In a previous study demonstrated that *A. tuberculata* is more effective in improving growth and P uptake of oil palm seedlings in comparison with *G. margarita* (Widiastuti *et al*, 2002). In addition Widiastuti *et al* (2005) showed that the optimum dose of inorganic fertilizer of oil palm inoculated with *G. margarita* was higer compared to those inoculatred with *A. tuberculata*. These results suggest that suppression of effective species to endochitinase activity is higher than those to species that are less effective, especially in the fertilization treatment. Similar to Lambais & Mehdy (1996) the effective strain is more pressing AMF endochitinase activity compared to those that are less effective. However this only occurs at an optimal symbiosis is the application of fertilizer, while the treatment without fertilization occurs the opposite of the less effective strain is more pressing endochitinase activity compared to effective strain.

In this study exochitinase depressed activity in the presence of AMF colonisation, however, there were differences in emphasis between the root level of the inoculated *A. tuberculata* and *G. margarita*. In the treatment without fertilizer, suppression activity of inoculated roots exochitinase of *G. margarita* was higher than *A. tuberculata*. While the result of fertilizer there was a difference in primary and secondary roots. The emphasis on secondary roots that occurs at the inoculation *G. margarita* was higher than *A. tuberculata* and vice versa in the primary root. These results indicate that in the less effective strain, the emphasis on exochitinase activity higher compared to the effective strains, especially on secondary roots. These results were different from those expressed Lambais and Mehdy (1996) who reported increased activity exochitinase up to 3 times the effective strain AMF. In this study exochitinase suppression activities occured on the effective strain is lower than those that are less effective. The results of this study indicated that

plants respond differently to AMF colonisation compared to pathogen infection. The mycorrhizal plant has a defense mechanism that temporally and very weak in contrast to pathogen.

References

Blee KA, AJ Anderson. 1996. Defence-related transcript accumulation in *Phaseolus vulgaris* L. colonized by the arbuscular mycorrhizal fungus *Glomus intraradices*, Schenck & Smith. *Plant Physiol. 110, 675-688.*

Dumas-Gaudot, ES Slezack, B Dassi, MJ Pozo, V Gianinazzi-Pearson, and S Gianinazzi. 1996. Plant hydrolytic enzymes (chitinases and ß-1,3-glucanases) in root reactions to pathogenic and symbiotic microorganisms. *Plant and Soil. 185, 211-221*.

Garcia-Garrido, JM, and JA Ocampo. 2002. Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. *J. Exp. Bot. 53, 1377-1386.*

Lambais MR, and MC Mehdy. 1996. Soybean roots infected by *Glomus intraradices* strains differing in infectivity exhibit differential chitinase and β-1,3-glucanase expression. *New Phytol. 134, 531-538.*

Lubis AU. 1992. Kelapa Sawit (*Elaeis guineensis* Jacq) di Indonesia. Pusat Penelitian Perkebunan Marihat Bandar Kuala. Pematang Siantar. Sumatera Utara.

Pozo, MJ, E Dumas-Gaudot, S Slezack, C Cordier, A. Asselin, V Gianinazzi-Pearson, C Azcon-Aguilar and JM Barea. 1996. Detection of new chitinase isoforms in arbuscular mycorrhizal tomato roots: possible implications in protection against *Phytophthora nicotianae* var. *parasitica*. *Agronomie, 16, 689-697.*

Widiastuti, H. E. Guhardja, N. Soekarno, L. K. Darusman, D. H. Goenadi and S. Smith. 2002. Optimasi simbiosis cendawan mikoriza arbuskula *Acaulospora tuberculata* dan *Gigaspora margarita* pada bibit kelapa sawit di tanah masam. *Menara Perkebunan 70, 54-62*.

-- back to Table of Content --