Proceedings of International Symposium on Innovative Crop Protection for Sustainable Agriculture 2018

Date: March 7-8, 2018

Venue: 6th Floor, UGSAS Building, Gifu University, Japan

The United Graduate School of Agricultural Science, Gifu University

Organizing Committee

Gifu University

Prof. Masateru SENGE	(Dean of UGSAS-GU)
Prof. Kohei NAKANO	(Vice Dean of UGSAS-GU)
Prof. Ken HIRAMATSU	(Faculty of Applied Biological Sciences)
Assoc. Prof. Masafumi SHIMIZU	(Faculty of Applied Biological Sciences)
Assoc. Prof. Haruhisa SUGA	(Life Science Research Center)
Prof. Koji KAGEYAMA	(River Basin Research Center)
Mr. Akiharu NAKAMINE	(Section Chief, Administrative Section of UGSAS-GU)
Ms. Ayano IMAI	(Administrative Section of UGSAS-GU)
Ms. Satoko YOSHIDA	(Administrative Section of UGSAS-GU)
Ms. Tomoko KASAI	(Administrative Section of UGSAS-GU)

＊ This symposium is supported by IC-GU12.

Mycorrhizal oil palms seedlings response to different sources of *Ganoderma boninense* as the causal agent of basal stem rot disease

Maria Viva RINI, Syaifudin Nur HASAN, Kuswanta Futas HIDAYAT, Titik Nur AENY

(Faculty of Agriculture, The University of Lampung)

SUMMARY

Basal stem rot (BSR) caused by *Ganoderma boninense* is the important disease of oil palm in Indonesia and Malaysia. BSR is characterized by a decay of roots and bole, production of aerial symptoms such as multiple spears and production of fruit bodies on the base of the trunk. These studies were aimed to evaluate the ability of arbuscular mycorrhiza fungi in controlling the BSR. Two different experiments were carried out. In the first experiment, rubber wood blocks of size 3 x 3 x 6 cm were used to grow the *G. boninense* inoculum for 1 month. The blocks were then inoculated to one primary root of 5 months old mycorrhizal and control oil palm seedlings. In the second experiment, the soil collected from rhizosphere of Infected palm by *G. boninense* was used as media to grow the three months old mycorrhizal and control oil palm seedlings. The first experiment showed that both mycorrhizal and control seedlings were infected by *G. boninense*. However, in the control seedling, the length of primary root that rot by the pathogen was longer than that of mycorrhizal. In the second experiment, no infection of *G. boninense* were observed in mycorrhizal and control seedlings.

Introduction

Basal stem rot (BSR) caused by *Ganoderma* species is the most serious disease of oil palm. Infection by the fungi causes significant loss in yield, often resulting in the palm's death as the disease progressed. The BSR affects the root and basal stem portion of the palm. Infection by the fungus begins in the roots and move into the stem causing a dry rot, which eventually lead to the death of the palm. Infection of living palm occurs through contact of healthy palm root with the infected root mass or bole tissue which serve as the inoculum source (Turner, 1981, Paterson, 2007). Generally, the first visible symptom of infected field palm is the presence of excessive spear leaves, while the foliage appears pale green when compare to that of healthy palm. Progressive yellowing, desiccation and mottling of the lower fronds, followed by necrosis is the characteristic feature of the disease of young palms. In older palms, the typical symptoms are skirting of the lower fronds, production of multiple unopened spears and overall paleness of the canopy (Fee, 2011; Gurmit, 1991).

Mycorrhizal fungi are ubiquitous and form symbiotic relationship with the roots of majority terrestrial plants including oil palm (Sieverding, 1991; Smith and Read, 2008). The mutual symbiosis benefits both the host and the fungus. The largest group, which predominantly associated with agricultural crops is the arbuscular mycorrhiza fungi (AMF). Infection by AMF has been shown to stimulate the growth of many plant species (Smith and Smith, 2011; Zhang *et al*., 2010), increase nutrient uptake especially phosphorus (Rini, 2004; Smith *et al*., 2011), improve the soil structure (through external hyphae that extends into the soil) for better aeration and water percolation, and improve plant physiological processes such as photosynthesis rate and water relation (Lu *et al*., 2007; Rini *et al*., 2000; Ruiz-Lozano and Azcon, 2010). Arbuscular mycorrhiza also has been proposed as an alternative for the management of soil borne pathogen. AM fungi has been proven to impair the development of soil borne pest and pathogens and consequently inhibit or reduce disease severity (Amer and Abou-El-Seoud, 2008; Jung et al., 2012; Tsvetkov *et al*., 2014). Therefore, this study was conducted to evaluate the ability of arbuscular mycorrhiza fungi in controlling the BSR.

Material and Method

The first experiment. A single factor experiment arranged in a completely randomized design was used with ten replications per treatment. The treatment was inoculated with (+M) and without (-M) AM fungi. The *Ganoderma boninense* inoculum was prepared on rubber wood blocks measuring 3 x 3 x 6 cm by inoculating the block with five 1 cm^2 plugs from 7-10 days old *G. boninense* culture grown on malt extract agar with one plug on each side of the block (done in the air laminar flow). The blocks were incubated at room temperature 27 ± 1 °C for ten weeks. A small hole (± 3 cm depth) was made on the top of the blocks using an electric drill. Five month old mycorrhizal (inoculated with inoculum containing mix species of *Glomus mosseae*, *Scutellospora callospora*, and *Acaulospora laevis*) and nonmycorrhizal seedlings were inoculated with these blocks using single root inoculation technique. One of the primary root of the seedling was washed with tap water, the root tip was excised and the cut end of the root was inserted into the hole of the inoculum block. The inoculated seedlings were then put inside a polybag and filled up with the soil (mineral soil:sand = 2 : 1) and water daily. Six month after inoculation, the seedlings were removed from the polybags. The inoculated primary roots were then carefully separated from the bole of the seedlings. The length of inoculated root that rot due to G. *boninense* and AMF root infection were measured. Total phenolic content in the roots was analyzed following the method of Anderson and Ingram (1993). Data obtained were subjected to t-test analysis.

The second experiment. The treatment design used was a factorial design 4 x 2 with 5 replication arranged in completely randomized design. The first factor was application of AMF i.e. without AMF (control, m_0), inoculation with AMF *Glomus* sp. (m_1), *Entrophospora* sp. (m_2) and mixture of *Glomus* sp. and *Entrophospora* sp. (m_3). The second factor was *Ganoderma* i.e. without *Ganoderma* (planting media was sterile soil) and with *Ganoderma* (planting media was rhizosphere soil collected from *Ganoderma* infected palm + fruiting body or sporophore of *Ganoderma*). The one month old oil palm seedlings were inoculated with AMF according to treatment and the seedling were kept in green house for 2 months after which the seedlings were then transferred to bigger polybag according to *Ganoderma* treatments. The seedlings were then kept for another 5 months in green house. At the end of experiment, data on fresh and dry weight of shoot and root and *Ganoderma* infection were recorded.

Result and Discussions

The first experiment. Percentage of AMF colonization 6 month after *Ganoderma* inoculation was in range 42.7—49.0%. Presence of AMF in the root of oil palm significantly reduce the length of the inoculated primary root that rot due to *G. boninense* (Table 1). The length of primary root that rot in nonmycorrhizal seedlings was 11.1 cm compared to only 7.4 cm in mycorrhizal seedling. Length of primary root that rot as a result *Ganoderma* infection was significantly reduced when root was earlier precolonized by AMF. This indicate that the spread of *Ganoderma* infection within primary root of mycorrhizal seedlings was slower compared to that of nonmycorrhizal control. In the present study, the length of rotten root can be estimated 1.23 cm and 1.68 cm per month. The spread of *Ganoderma* infection in the present study was faster compared to Arifin and Idris (1990) who found only 1 cm/month, especially for nonmycorrhizal seedlings (1,68 cm/month). This faster speed could be due to the different size and substrates used to grow *Ganoderma* inoculum. Idris (1999) showed that utilization of different subtrates as source of *Ganoderma* inoculum resulted in different growth rate of the *Ganoderma* mycelia within the primary roots of oil palm.

Table 1. Length of primary roots infected by *G.boninense* after 6 months of inoculation

	+Mycorrhiza	Non-mycorrhiza
Length (cm)	7.4	11.1
P value		<0.05

Total phenolic content in the roots was also significantly higher in mycorrhizal seedlings. The values were 24.43% and 21.75% in mycorrhizal and nonmycorrhizal seedlings respectively (Table 2.)

Table 2. Total phenolic content in mycorrhizal and non mycorrhizal root after 6 months of *G. boninense* inoculation

	+Mycorrhiza	Non-mycorrhiza
Total phenolic in the roots (%)	24.43	21.75
P value		<0.05

Plant phenolic are the most widespread classes of secondary metabolites and are known to be involved in plant microbe interactions. One of the biological functions of phenol is its antimicrobial activity which play an important role in the plant defence mechanism (Morandi, 1996). Result from this study show that total phenolic content in the roots of mycorrhizal seedlings is higher that that of nonmycorrhizal ones, suggesting that phenolic compounds could be implicated in disease resistance, resulting in slower rate of *Ganoderma* spread within the primary root of seedlings. Devi and Reddy (2002) reported that AMF significantly increase the quantity of phenolics compound in roots and shoots of groundnut. In (1998), Rabie believed that a significant increase in free and total phenolic contents in preinoculation of *G. mossea*e in faba bean contributed to increased resistance of the plant to chocolate spot disease.

The second experiment.

Data obtained from analysis of variance showed that there were no interaction between AMF factor with *Ganoderma* factor for all data recorded. Moreover, results showed that seedlings growth were significantly enhanced by AMF treatment. All AMF inoculated seedlings had better shoot and root fresh weight and dry weight compared to control one. However, no differences were observed within AMF treatment. All AMF treated seedlings whether single (*Glomus* sp. or *Enthrophospora* sp.) or their combination statistically had the same shoot and root fresh weight and dry weight. For *Ganoderma* treatment, no effect were detected in shoot fresh and dry weight. Contrary to shoot, *Ganoderma* treatment increase root fresh and dry weight. Oil palm seedling planted in *Ganoderma* infected soil had higher root fresh weight and root dry weight (Table 3 and Table 4).

Table 3. Fresh weight of shoot and root of 8 months old oil palm seedling treated with AMF and *Ganoderma*.

Treatment	Fresh Weight (g)	
	Shoot	Root
Control	43.3 b	09.7 b
G*lomus* sp. (G)	66.4 a	13.1 a
Entrophospora sp. (E)	64.0 a	13.9 a
G + E	66.7 a	13.5 a
LSD 5%	9.3	2.8
Sterile Soil	56.2 a	10.0 b
Ganoderma Infected Soil	64.0 a	15.0 a
LSD 5%	6.5	2.0

Table 4. Dry weight of shoot and root of 8 months old oil palm seedling treated with AMF and *Ganoderma*.

Treatment	Dry Weight (g)	
	Shoot	Root
Control	13.5 b	3.3 a
G*lomus* sp. (G)	19.3 a	4.2 a
Entrophospora sp. (E)	17.6 a	3.8 a
G + E	19.0 a	4.0 a
LSD 5%	3.5	0.9
Sterile Soil	16.7 a	3.2 b
Ganoderma Infected Soil	18.1 a	4.4 a
LSD 5%	2.5	0.6

In this study, AMF gave the beneficial effects on oil palm seedling growth as indicated by fresh and dry weight of shoot and root. The enhancing in growth could be due to the increase in uptake of nutrient especially phosphorus as mycorrhiza hyphae that developed in the soil can absorb nutrients directly from the soil matrix (Neumann and George, 2010; Rini, 2005) and improve in plant water relation such as increase in water uptake and photosynthesis rate (Ruiz-Lozano and Azcon, 2010; Doubkova *et al*., 2013).

In this study, AMF treatment gave a better impact on plant growth. However, its significance in reducing or control *Ganoderma* infection cannot be examined. All seedling planted in *Ganoderma* infected soil mix with its fruiting body had no *Ganoderma* infection in their root (Table 5).

Table 5. Root infection by AMF and Ganoderma as a result of AMF and Ganoderma treatments

Treatment	Root infection (%) by *Ganoderma*
Control	0
G*lomus* sp. (G)	0
Entrophospora sp. (E)	0
G + E	0
LSD 5%	-
Sterile Soil	0
Ganoderma Infected Soil	0
LSD 5%	-

Contrary to the first study, *Ganoderma* inoculum prepared in rubber wood block successfully infect both mycorrhizal and nonmycorrhizal seedling. Base on this result, it can be suggested that type of inoculum affect the success of *Ganoderma* to infect the root of oil palm seedling. Using rubber wood block to grow the *Ganoderma* inoculum confirmed the statement of Turner (1981) that *Ganoderma* pathogen is a facultative parasite. It is capable of living saprophytically on rotting stumps and roots. When a suitable host like oil palm root becomes available, the pathogen will colonize it and establishes a parasitic relationship. Using infected soil mix with the *Ganoderma* fruiting bodies as *Ganoderma* inoculum failed to cause infection. This result suggest that *Ganoderma* spores that exist in the soil and spores within fruiting body, within the constraints of this study, is not capable in infecting oil palm seedling root.

Conclusion

Base on the results from the study, the following conclusions could be made: (1) arbuscular mycorrhiza fungi improved the oil palm seedling growth and increase the seedling tolerance to *Ganoderma* infection (2) Spores of Ganoderma from the infected soil and *Ganoderma* fruiting bodies were failed to caused disease infection, contrary to the inoculum prepared on rubber wood block that successfully infect the seedling root.

Acknowledgement

The author would like to thank to staff of Labortorium Produksi Perkebunan, Faculty of Agriculture, The University of Lampung for their help in conducting this research.

Reference

1)Amer MA and Abou-El-Seoud II (2008) Mycorrhizal fungi and *Trichoderma harzianum* as biocontrol agents for suppression of *Rhizoctonia solani* damping-off disease of tomato. Commun. Agric. Appl. Bio. Sci.,73 (2):217-232.

2)Anderson JM and Ingram JSI (1993) Tropical soil biology and fertility. A handbook of methods. Wallingford:Cab International.

3)Ariffin D and Idris AS (1990) Artificial inoculation of oil palm seedlings with *Ganoderma boninense*. Paper presented at Joint meeting agronomy/Breeding/ Pathology, pp.3, Palm Oil Research Institute of Malaysia.

4)Devi MC and Reddy MN (2002) Phenolics acid metabolism of groundnut (*Arachis hypogaea* L.) plants inoculated with VAM fungus and Rhizobium. Plant Growth Regulation,87:151-156.

5)Doubkova P, Vlasakova E and Sukova R (2013). Arbuscular mycorrhizal symbiosis alleviates drought stress imposed on *Knautia arvensis* plants in serpentine soil. Plant and Soil,370:149-161.

6)Fee C G (2011) Management of Ganoderma diseases in oil palm plantations. The Planter,87(1022):325-339.

7)Gurmit S (1991) Ganoderma: The scourge of oil palms in the coastal areas. The Planter,67:421-444.

8)Idris AS (1999) Basal stem rot (BSR) of oil palm (*Elaeis guineensis* Jacq.) in Malaysia:factors associated with variation in disease severity. Ph.D. Thesis, Wye College, University of London.

9)Jung SC, Martinez-Medina A, Lopez-Raez JA and Pozo MJ (2012) Mycorrhizal-induced resistance and priming of plant defenses. J. Chem. Ecol.,38: 651-664.

10)Lu J, Liu M, Mao Y and Shen L (2007) Effects of vesicular-arbuscular mycorrhi-zae on the drought resistance of wild jujube (*Zizyphys spinosus* Hu) seedlings. Front. Agric. China,1(4):468-471.

11)Morandi D (1996) Occurrence of phytoalexins and phenolic compounds in endomycorrhizal interactions, and their potential role in biological control. Plant and Soil,185:241-251.

12)Neumann E and George E (2010). Nutrient uptake: the arbuscular mycorrhiza fungal symbiosis as a plant nutrient acquisition strategy. *In Arbuscular Mycorrhizas: Physiology and Function*. Edited by H Koltai and Y Kapulnik, pp.137-167. New York, Springer.

13)Paterson RRM (2007) Ganoderma disease of oil palm – A white rot perspective necessary for intergrated control. Crop Protection,26:1369-1376.

14)Rabie GH (1998) Induction of fungal disease resistance in *Vica faba* by dual inoculation with *Rhizobium leguminosorum* and vesicular arbuscular mycorrhiza fungi. Mycopathologia,141:159-166.

15)Rini MV (2004) Influence of arbuscular mycorrhiza infection on growth, P uptake, and root morphology of oil palm seedlings (*Elaeis guineensis* Jacq.). Jurnal Tanah Tropika, 18:145—154.

16)Rini MV (2005) Effect of arbuscular mycorrhiza inoculum density on growth, root infection, and nutrient uptake of oil palm seedlings. Jurnal Tanah Tropika (journal of Tropical Soils),10(1):39-44.

17)Rini MV, Jamal T, Idris ZA and Azizah H (2000) Effect of arbuscular mycorrhiza fungi colonization on growth and physiological responses of grafted cocoa under field condition. Malaysian Journal of Soil Science, 4: 67—78.

18)Ruiz-Lozano JM and Aroca R (2010) Host responses to osmotic stresses: stomatal behavior and water use efficiency of arbuscular mycorrhizal plants. *In Arbuscular Mycorrhizas: Physiology and Function*. Edited by H Koltai and Y Kapulnik, pp 239-256. New York, Springer.

19)Sieverding E (1991) *Vesicular Arbuscular Mycorrhizae Management in Tropical Agroecosystem*. Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) Gmbh, Eschborn.

20)Smith SE and Read DJ (2008) *Mycorrhizal Symbiosis*, 3rd edition. Elsevier, New York.

21)Smith FA and Smith SE (2011) What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? Plant Soil,348: 63—79.

22)Smith SE, Jakobsen I, Gronlund M and Smith F A (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol,156:1050–1057.

23)Tsvetkov I, Dzhambazova T, Kondakova V and Batcharova R (2014) Mycorrhizal fungi *Glomus* spp. and *Trichoderma* spp. in viticulture (Review). Bulgarian J. of Agric. Sci.,20(4):849-855.

24)Turner PD (1981) Oil palm diseases and disorder. Kuala Lumpur, Oxford University Press.

25)Zhang Y, Zhong CL, Chen Y, Jiang QB, Wu C and Pinyopusarerk K (2010) Improving drought tolerance of *Casuarina equisetifolia* seedlings by arbuscular mycorrhizas under glasshouse conditions. New Forests, 40: 261-271.