Ganoderma Versus Mycorrhiza

Azizah Hashim*

ABSTRACT

Basal stem rot (BSR) disease, which has long been recognized as the most annihilating disease of field palms in Southeast Asia, still reigns as the number one killer of oil palms and serves a disastrous blow to palm oil production. With the identification of the causal agent, i.e. Ganoderma boninense, several control measures have been recommended and implemented. However till today, none of the methods practised has satisfactorily controlled or inhibited the spread or occurrence of this fatal disease. It is crucial to look for some other forms of control measures. The arbuscular mycorrhizal (AM) fungi offers a practical alternative that should be seriously considered and hopefully implemented.

What are AM fungi? The AM fungi are highly evolved, mutualistic associations between soil fungi and plant roots. Past and present AM research in Malaysia has concentrated on mineral nutrient acquisitions by these fungi from our highly weathered soils, hence designating AM’s role as a biofertilizer as well as a bioenhancer of plant growth. Recently, evidence obtained has proven further the fungal ability to secrete antibiotic substances and/or to produce secondary metabolites that could be utilized as a means of combating plant diseases, especially those of soil borne origin.

Several experiments were conducted to evaluate AM’s role as a biocontrol agent. The earliest work was on interaction of AM with one Ganoderma species, i.e. Ganoderma pseudoferum, which causes the red root disease of cocoa. Pre-inoculation of cocoa seedlings with AM significantly (p<0.05) reduced Ganoderma infection as compared to over 10% mortality of seedlings in the absence of this symbiosis. An infectivity study done on 6-week-old oil palm plantlets showed the appearance of necrosis on the leaf five weeks after inoculation with Ganoderma. A cross-section of the infected roots revealed breakdown of the cortical cells interspersed with the fungal hyphae, which penetrate right into the vascular regions. These symptoms were delayed in the mycorrhizal plants.

The promising results obtained prompted further research along this line. Two-leaf stage oil palm seedlings were subsequently inoculated with the AM fungi followed by inoculation with the Ganoderma after six weeks of establishment with AM. The symbiosis successfully lengthened the incubation period of the pathogen to bring about infection or kill the young palms. All non-mycorrhizal palms succumbed to the disease nine months after exposure to the pathogen. Only 20% of the mycorrhizal palms showed the disease symptoms after nine months, with only 10% mortality. Results from the pot trials were later confirmed in one preliminary field trial. A total of 120 palm seedlings, 60 mycorrhizal and 60 non-mycorrhizal, were planted in areas highly infested with Ganoderma. After 10 months in the field, five of the uninoculated palms had died, while only two mycorrhizal palms had succumbed to the disease.

The mechanisms of defense as a result of mycorrhizal symbiosis could probably be strong competition between AM fungi and pathogen for space and host photosynthates. Healthier mycorrhizal plants directly or indirectly produce more vigorous seedlings with higher and stronger internal resistance to ward off disease; higher density roots produced in the presence of mycorrhiza are able to compensate for loss of roots as a result of disease infections; significant Ca deposition in mycorrhizal cells, creates a physical barrier to advancement of the disease in the palm roots; higher deposition of secondary metabolites by mycorrhizal roots inhibits spread of the pathogen in the palm roots. This review paper aims to highlight these mechanisms and to determine how these benefits could be utilized or harnessed in the attempt to control this number one killer disease of oil palm, i.e. the BSR disease.

*Department of Land Management, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
ABSTRAK

Penyakit reput pangkal (BSR) yang telah lama diakui sebagai penyakit pemasnah sawit terpenting di Asia Tenggara, kekal sebagai pembunuh utama tanaman ini dan merupakan satu pakalan hebat terhadap penghasilan minyak sawit di Malaysia. Dengan pengenalpastian agen penyebabnya iaitu Ganoderma boninence, beberapa tindakan pengawalan telah disyorkan dan dilaksanakan. Sangguppun demikian, tiada satu kaedah pun yang diajak untuk berjaya memberi keputusan yang memuaskan sama ada untuk mengawal atau menghambat penyebaran atau kehadiran penyakit yang berbahaya ini. Berasaskan senario hara, penyakit ini adalah penting untuk mencari cara pengawalan yang lain. Kulat mikoriza arbuskul (MA) menawarkan satu-satunya penyelesaian alternatif yang praktikal dan seharusnya dipertimbangkan dengan serius semoga kegunaannya dapat diimplementasikan.

Apakah kulat mikoriza arbuskul (MA)? Kulat MA adalah kulat berevolusi tinggi yang mengadakan hubungan simbiosis di antara kulat tanah dengan akar tumbuhan. Kajian yang dahulu dan kini di Malaysia, telah menekankan pengambilan nutrien yang berkesan oleh kulat, seterusnya mengesahkan peranan MA sebagai baja bio dan juga sebagai perangsang pertumbuhan tanaman. Baru-baru ini, maklumat yang diperoleh membuktikan keupayaan kulat untuk mengeluarkan bahan-bahan antibiotik serta menghasilkan metabolik sekunder yang dapat digunakan sebagai satu cara mengatasi penyakit tumbuhan, khususnya yang berpunca dari tanah.

Ekoran ini, beberapa kajian telah dijalankan untuk menilai peranan MA sebagai agen kawalan biologi. Kajian terawal adalah ke atas interaksi MA dengan satu spesies Ganoderma iaitu Ganoderma pseudoferum, yang menyebabkan penyakit akar pada tanaman koko. Inokulasi awal anak benih koko dengan MA mengurangkan dengan ketara (p<0.05) jangkitan Ganoderma berbanding kematian 10% anak benih yang tidak menerima simbiosis ini. Kajian jangkitan ke atas anak tumbuhan sawit menunjukkan kehadiran nekrosis pada daun lima menggugurkan inokulasi dengan Ganoderma, keratan rentas akar jangkitan memperlihatkan sel-sel korteks yang pecah disulami hifa kulat yang menembusi terus ke dalam bahagian vaskular. Simptom ini ditemui ke dalam tahapan awal penyakit.

Mekanisme pertahanan hasil bersimbiosis dengan mikoriza mungkin disebabkan oleh saingan kuat antara kulat MA dengan patogen untuk ruang dan fotosintat perumah; tanaman bermikoriza adalah lebih subur dan secara langsung atau tidak menghasilkan anak benih yang lebih sihat untuk ketahanan dalam yang lebih kukuh untuk menghindari penyakit; densiti akar yang pesat dengan kehadiran mikoriza berkemampuan mengatasi kehilangan akar ekoran jangkitan penyakit; kandungan Ca yang berarti dalam sel bermikoriza seterusnya menjadi penghalang fizikal kepada kemajuan penyakit dalam akar sawit; kemungkinan juga pemendakan tinggi metabolit sekunder oleh akar bermikoriza sekali gus menghindari merebaknya patogen dalam akar. Kertas kerja ini bertujuan untuk mengetahui mekanisme tersebut di samping menentukan bahan bimahkam faedah MA boleh dimanfaatkan atau digunakan sepenuhnya dalam usaha mengawal pembunuh utama tanaman sawit iaitu – penyakit BSR.

Keywords: basal stem rot, oil palm, Ganoderma, mycorrhiza, biocontrol agent.

INTRODUCTION

Basal stem rot (BSR) still reigns as the number one killer disease of field palms in spite of the intensive control measures adopted to combat the disease, from the time it was first reported.
by Turner (1981). BSR resulted in 46% yield difference between palm blocks with 10.9% BSR to that of 67.3% BSR (Gurmit, 1991). Khairuddin (1995) reported only 29% survival in 15-year-old diseased palms by the end of the second year.

To date, there is no report of an effective treatment for *Ganoderma* infection in an existing stand. Most control measures done have given variable results (Ho and Khairuddin, 1996). Recently, soil mounding has offered an attractive ameliorative alternative measure (George et al., 2000). However, the prospect of utilizing new root formation in the soil mounds for more effective fungicidal treatment (George et al., 2000) or for incorporation of antagonist soil microbes against *Ganoderma* (Sariah et al., 1998) needs further research. Application of selected agrochemicals into the environment has to be monitored as a result of the increasing public concern on environmental pollution. Application of agrochemicals into our agricultural soils calls for control measures which are environmentally friendly. The arbuscular mycorrhizal fungi offer a novel approach towards this direction (Azizah, 1999).

### WHAT IS ARBUSCULAR MYCORRHIZAL (AM) FUNGI?

The AM fungi are highly evolved, mutualistic associations between soil fungi and plant roots. In Malaysia, past and current AM research has been geared towards mineral nutrient acquisitions from highly weathered soils (Masri, 1997; Azizah, 1999), hence designating AM’s role as a biofertilizer. Of late, other beneficial roles of the AM fungi have been suggested (Sieverding, 1991; Smith, 1995). Recent evidence has proven that the endomycorrhiza fungi have the ability to secrete antibiotic substances (Azcon-Aguilar and Barea, 1996) and/or produce secondary metabolites such as phytoalexins (Harrison and Dixon, 1993) and phenolic compounds (Grandmaison et al., 1993), hence giving yet another significant contribution of the AM fungi as a biocontrol agent in combating plant diseases, especially those that are soil-borne.

One of the earliest preliminary experiments done on AM as a biocontrol agent in Malaysia was on *Ganoderma pseudoferum* that causes the red root disease of cocoa (Azizah et al., 1990). Pre-inoculation of two-week-old cocoa seedlings with the AM fungi successfully reduced the disease incidence in these plants by 50% as compared to 100% mortality in uninoculated seedlings, five months after exposure to the pathogen.

Subsequently, further experiments were established by Rini (2001) with the following objectives:

- to evaluate AM’s role in the production of primary, secondary and tertiary roots of oil palm seedlings and total phenolic content in these roots; and

- to determine AM’s potential as a biocontrol agent against *Ganoderma boninense*.

### Experiment 1

A pot experiment was set up in the glasshouse of Universiti Putera Malaysia (UPM), where uniform two leaf stage DxP oil palm seedlings were inoculated with 40 g mycorrhiza per seedling. These seedlings were then sampled at two, three, four and five months respectively. Primary, secondary and tertiary roots were then measured accordingly.

### Experiment 2

Another pot experiment was set up with the following treatments: a) pre-inoculation with mycorrhiza for three months followed by inoculation with *Ganoderma* (+M+G); b) inoculation with *Ganoderma* only (-M+G); c) inoculation with mycorrhiza only (+M-G) and d) without any inoculation (-M-G). A total of 20 palms were used per treatment. The number of seedlings that were infected by *G. boninense* and those that died as a result of the disease were recorded at three, six and nine months after *Ganoderma* inoculation (GI). The lignin and calcium contents were also determined in the roots of these seedlings at the respective sampling times.

### RESULTS

#### Experiment 1

There was a significant increase in root length as a result of symbiosis with the mycorrhizal fungi. The length of the primary roots was significantly increased (P<0.05) in the mycorrhiza treated plants resulting in increased branching of the secondary and tertiary roots, hence increasing the overall total root length (Figure 1).
Within the fifth month of sampling, the length of the primary roots was increased by 45%, secondary roots by 38% and tertiary roots by 88% when compared to the controls. The increase in root length had a direct impact on both the vegetative and reproductive growth of oil palm seedlings (Table 1).

Mycorrhizal palms had the following root lengths: secondary roots (2384.9 cm) > tertiary roots (701.1 cm) > primary roots (379.5 cm) in contrast to non-mycorrhizal roots which had the following: secondary roots (1730.9 cm) > tertiary roots (372.9 cm) > primary roots (260.6 cm), all at the fifth month of sampling. Positive alterations to the oil palm root system were subsequently accompanied by tremendous and significant increase in nutrient uptake, especially P in the inoculated seedlings (data not shown).

Experiment 2

It is clearly evident that all the 20 seedlings without mycorrhiza but inoculated with G. boninense showed signs of the disease as early as three months after inoculation (Table 2). In contrast, only six out of the 20 +M+G palms were infected at three months after exposure to Ganoderma. The number of palms infected increased progressively but slowly with increase in sampling time. In the -M+G treatment, three out of the 20 control seedlings died within the

### Table 1. Effect of Arbuscular Mycorrhizal (AM) Inoculation on the Respective Root Lengths (cm) of Oil Palm Seedlings

<table>
<thead>
<tr>
<th>Root length (cm)</th>
<th>Mycorrhiza treatment</th>
<th>Time of sampling (months after AM inoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Primary root</td>
<td>+ M</td>
<td>103.6&quot;</td>
</tr>
<tr>
<td></td>
<td>- M</td>
<td>79.1</td>
</tr>
<tr>
<td>Secondary root</td>
<td>+ M</td>
<td>495.6’</td>
</tr>
<tr>
<td></td>
<td>- M</td>
<td>348.9</td>
</tr>
<tr>
<td>Tertiary root</td>
<td>+ M</td>
<td>84.9’</td>
</tr>
<tr>
<td></td>
<td>- M</td>
<td>47.8</td>
</tr>
</tbody>
</table>

Note: *Data within a parameter of a column are significantly different at P=0.05 of t-test.
first three months of infection. The remaining 17 seedlings died three months later, i.e., at six months after inoculation (Table 3). In contrast, mycorrhiza-inoculated seedlings showed stronger resistance to the disease. At nine months after \( G. \) boninense inoculation, only six out of eight seedlings with positive \( Ganoderma \) symptoms had died (Figure 2).

Table 4 gives the lignin and calcium contents in oil palm roots as a result of the different treatments given. Maximum lignin contents of 42.9%, 40.42% and 36.82% were all recorded in the \(+M+G\) palm roots at three, six and nine months of sampling respectively. In the \(-M+G\) treatment, lignin content of 35.72% was recorded at the third month of sampling, after which no lignin could be analysed as all the palms had died. A similar trend was seen in the calcium (Ca) content. The \(+M+G\) palms recorded high Ca content, in contrast to the other treatments.

**DISCUSSION**

The accelerated plant growth as a result of a mutualistic symbiotic association between plant roots and soil-borne fungi, namely, the endomycorrhiza fungi, has been well reviewed and documented (Sieverding, 1991; Linderman, 1994; Azizah, 1999). The main contribution of the mycorrhizal fungi in enhancing plant growth has often been increase in plant rooting density and hence, increase in nutrient uptake, mainly of the less mobile elements such as P (Masri, 1998; Azizah, 1999). The data reported here give clear evidence of AM’s role in increasing the rooting density of oil palm seedling roots.

The degree of root colonization by the AM fungi and hence, the effects of the symbiosis may vary, depending on the tripartite interactions between the host, the symbiont and the environment. Overall in most instances, mycorrhizal plants performed and developed better than the non-mycorrhizal ones when both were exposed to soil pathogen interactions. All the plants were found to have greater tolerance towards fungal pathogens than their non-mycorrhizal counterparts (Linderman, 1994).

An increase in the plant nutrient status, especially P makes plants more tolerant or resistant towards attack by soil-borne pathogens (Cordier et al., 1996). Most studies done on AM – pathogen interactions suggest several defense mechanisms exhibited by mycorrhizal plants. The possible defense mechanisms for curbing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sampling time (months after ( G. ) boninense inoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>With mycorrhiza + with ( Ganoderma )</td>
<td>(+M+G)</td>
</tr>
<tr>
<td>Without mycorrhiza + with ( Ganoderma )</td>
<td>(-M+G)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sampling time (months after ( G. ) boninense inoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>With mycorrhiza + with ( Ganoderma )</td>
<td>(+M+G)</td>
</tr>
<tr>
<td>Without mycorrhiza + with ( Ganoderma )</td>
<td>(-M+G)</td>
</tr>
</tbody>
</table>
a. Oil palm infected with Ganoderma (sporophore) - drying of leaves and stunted growth.

b. Healthy growth of seedling as a result of mycorrhizal symbiosis.

Figure 2.
TABLE 4. EFFECT OF \textit{Ganoderma boninense} INOCULATION ON LIGNIN (%) AND CALCIUM (%) CONTENTS IN ROOTS OF OIL PALM SEEDLINGS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sampling time (month after G.I)</th>
<th>Treatment</th>
<th>+M+G</th>
<th>+M-G</th>
<th>-M+G</th>
<th>-M-G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin (%)</td>
<td>3</td>
<td>36.82a</td>
<td>36.50a</td>
<td>35.72a</td>
<td>34.78a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>40.42a</td>
<td>39.00a</td>
<td>-</td>
<td>36.66b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>42.90a</td>
<td>41.04a</td>
<td>-</td>
<td>38.54b</td>
<td></td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>3</td>
<td>1.44a</td>
<td>0.98b</td>
<td>0.90b</td>
<td>0.85b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.71**</td>
<td>1.70**</td>
<td>-</td>
<td>1.66**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.71**</td>
<td>1.69**</td>
<td>-</td>
<td>1.65**</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Data followed by the same letter in a row are not significantly different (P< 0.05) by DMRT.
*All plants died as a result of \textit{Ganoderma} inoculation (G.I.).

BSR disease are as follows:

- a three month pre-establishment of the AM fungi in the roots of oil palm seedlings prior to exposure to \textit{G. boninense} propagules allows the fungi a head start over the pathogens in terms of competition for space, food and host photosynthates. This is critical since both symbiont and pathogen fight for the same \textit{infection site} on the rhizoplane and rhizosphere of the oil palm seedlings (Jalali and Jalali, 1991).

- an increase in the nutrient content, especially P, in mycorrhizal shoot and roots of the palm, makes the plant more tolerant or resistant towards pathogen attack (Cordier \textit{et al.}, 1996). Significant P content in mycorrhizal plant tissues has been shown to be closely associated with a reduction in fungal or viral diseases in vegetables (Finck, 1992).

- morphological and anatomical changes in mycorrhizal palm roots (significantly higher production of the secondary and tertiary roots) further strengthen the plant’s resistance to \textit{Ganoderma}. This is evident from the results reported here, where only 30% of the mycorrhizal palms died compared to 100% death of non-mycorrhizal palms within nine months of exposure to the pathogens. This is indeed a very significant contribution by the endomycorrhizal fungi (Rini, 2001). Similar results were reported by Tuck (1998). The massive roots produced and the longer survival period of these roots (Masri, 1998) probably helped in reducing BSR disease incidence and enabled better survival of the moderately infected palms. The mechanism involved was root compensation.

- activation of the plant’s defense mechanisms. This comes in the form of increased production of secondary metabolites (such as flavonoids, cell-wall bound phenolic acids) or even through significant Ca deposition in the cell walls, hence restricting the spread of the pathogen within the host’s cells (Azizah \textit{et al.}, 1990). Benhamou \textit{et al.} (1994) reported that enhanced defence mechanisms by mycorrhizal plants to pathogen infections could also be due to increased secretions of β-1,3 glucanases and peroxidase activity by these plants.

As such, it is really appropriate that the endomycorrhizal fungi be fully utilized as one of the tools in combating soil-borne plant diseases. The role of the AM fungi is very holistic since they can act as biofertilizers, bioregulators, bioprotectors. Recently, it has been shown that they can also act as biocleansers of environments heavily polluted with carbon dioxide.

**CONCLUSION**

AM fungi still remain as one of the best biological tools and non-chemical alternative that should be seriously considered in the future overall management of plant root diseases. Although, more field trials need to be conducted.
so as to ascertain how best to utilize these microorganisms, we should not deny the fact that the presence of these arbuscular mycorrhizal fungi in our plant system would be more of an asset than a liability. In fact, this paper strongly supports the suggestion that the AM fungi are the universal compensators needed to accomplish the monitoring of sustainable agriculture.

ACKNOWLEDGEMENT

The funding of this project by IRPA, project code: 01-02-04-0248, is highly appreciated.

REFERENCES


