TECHNIQUE FOR INOCULATION OF OIL PALM GEMINATED SEEDS WITH *Ganoderma*

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MPOB INFORMATION SERIES • ISSN 1511-7871 • JUNE 2006 MPOB TT No. 314

Basal stem rot (BSR), caused by the fungus *Ganoderma*, is the most serious disease of oil palm in Malaysia and Indonesia. Previously, research on BSR was hampered by the failure to artificially infect oil palm with the fungus. Although *Ganoderma* had been associated with BSR (Thompson, 1931), proof of its pathogenicity to satisfy Koch's postulate was only achieved in the early 1990s by inoculating oil palm seedling roots (Ariffin and Idris, 1991) or by using rubber wood blocks (Khairuddin, 1990; 1991; Sariah *et al*., 1994; Lim *et al*., 1992). With these two techniques, it has become fairly established that *G. boninense* is the main species pathogenic to oil palm. However, Idris (1999) has shown that two other species, *G. zonatum* and *G. miniatocinctum*, are also pathogenic, but *G. tornatum*, *G. lucidum*, *G. philippii*, *G. applanatum*, *G. pfeiifferi* and *G. oregonense* are not. This paper reports a reliable and quick technique for testing the pathogenicity of the *Ganoderma* fungus by inoculating oil palm germinated seeds.

EXPERIMENT 1: EFFECT OF DIFFERENT SIZES OF RUBBER WOOD BLOCKS AS SOURCES OF INOCULUM FOR INOCULATING GERMINATED SEEDS WITH *G. boninense*

This study was conducted in a nursery (shaded with two layers of polynet 30/70) at MPOB-UKM Research Station, Bangi, Selangor. Oil palm germinated seeds (commercial DxP standard cross) from the Breeding and Genetics Group of MPOB were used. The seedlings were maintained with regular watering, manuring and pesticide application. Three sizes of rubber wood blocks (RWB) as sources of *Ganoderma* inoculum were tested: 1.5 x 1.5 x 1.5 cm (3.3 cm^3), 3 x 3 x 3 cm (27 cm^3) and 6 x 6 x 6 cm (216 cm^3). The RWBs were washed and put in polypropylene bags containing 2% malt extract (incubated for 12 hr) and autoclaved for 1 hr at 121°C. After sterilization and cooling, the RWBs in the polypropylene bags were inoculated with *G. boninense*. The RWBs were then incubated in the dark (at 27°C) for 30 to 60 days. Fully colonized RWBs were used for inoculation, carried out by planting the germinated seeds in the polythene bags (size 15 x 23 cm) containing a soil mixture (two parts soil:one part sand:one part organic matter). The seeds were placed about 2.5 cm away from the RWB inoculum. Some seeds were planted without inoculum as the control. The layout of the experiment was a randomized complete block design with 20 replicates. The infection of *G. boninense* and the disease development were assessed monthly for six months based on visual foliar symptoms including progressive yellowing or desiccation (browning) of the oldest to youngest fronds and death of the seedlings with or without *Ganoderma* fructifications. At six months, the seedlings were harvested and cut open to check for internal symptoms of the pathogen. Cultures were made on *Ganoderma* selective medium or GSM (Ariffin and Idris, 1991), from root or stem tissues (bole) to confirm the presence of the causal fungus.

The results of infection at six months after inoculation are presented in *Table 1*. No foliar symptoms were observed in the control seedlings and also in the seedlings inoculated with *G. boninense* raised on RWB of size 3.3 cm^3 but foliar symptoms were apparent on all the seedlings inoculated with *G. boninense* raised on the larger substrates of 27 cm^3 (25%) and 216 cm^3 (60%). The first foliar symptoms was observed two months after planting the seeds. The symptoms of *Ganoderma* infection observed were progressive yellowing and desiccation (browning) of leaves from the oldest to the youngest (*Figure 1*). A fruiting body may or may not have developed on the infected seedlings before or after appearance of the foliar symptoms (*Figure 1*). No internal

ISSN 1511-7871
9 771511 787001

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TABLE 1. EFFECT OF DIFFERENT SIZES OF RUBBER WOOD BLOCK (RWB) INOCULUM ON INFECTION OF SEEDLINGS WITH *G. boninense* AT SIX MONTHS AFTER INOCULATION[#]

RWB inoculum	% of seedlings infected	
	Based on visual foliar symptoms	Based on reisolation of *Ganoderma* on GSM
1.5 x 1.5 x 1.5 cm (3.3 cm^3)	0	10
3 x 3 x 3 cm (27 cm^3)	25	60
6 x 6 x 6 cm (216 cm^3)	60	100
Uninoculated (control)	0	0
Chi-square analysis	$p<0.05$	$p<0.05$

Note: [#]No. of germinated seeds/treatment: 20.

disease symptoms were observed in the control seedlings and also in those seedlings inoculated with *G. boninense* raised on substrate of RWB size of 3.3 cm^3 but internal disease symptoms (*Figure 2*) were apparent on those seedlings inoculated with the larger substrates. However, *Ganoderma* was isolated from the stem or roots of all the seedlings inoculated with *G. boninense* - RWB of 3.3 cm^3 (10%), 27 cm^3 (60%) and 216 cm^3 (100%). The study suggests that *Ganoderma* requires a threshold size inoculum for infection.

EXPERIMENT 2: PATHOGENICITY OF DIFFERENT SPECIES OF *Ganoderma* TESTED BY INOCULATION OF OIL PALM GERMINATED SEEDS

To test the pathogenicity of different *Ganoderma species*, RWBs (6 x 6 x 6 cm) were inoculated with the following isolates of *Ganoderma*: *G. boninense, G. zonatum, G. miniatocinctum, G. tornatum, G. lucidum* and *G. philippii* (Idris *et al*., 2000a, b). The inoculated RWBs were incubated (at 27°C) for approximately 60 days or until completely colonized by the fungus. Oil palm germinated seeds (commercial DxP standard cross) were inoculated using the technique in Experiment 1. The results are presented in *Table 2*. At six months, no disease symptoms were observed on the seedlings inoculated with *G. tornatum, G. lucidum* and *G. philippii* but apparent on those inoculated with *G. boninense* (45%), *G. zonatum* (45%) and *G. miniatocinctum* (25%). In addition, no internal disease symptoms were observed in the seedlings

Figure 1. Uninoculated seedling without disease symptoms (1A) and inoculated seedling with foliar symptoms and fruiting body of Ganoderma *(1B) at six months after inoculation.*

Figure 2. Inoculated with internal symptoms (2A) and uninoculated without internal symptoms (2B). Oil palm seedlings at six months after inoculation.

TABLE 2. PATHOGENICITY OF DIFFERENT SPECIES OF *Ganoderma* BY INOCULATION OF OIL PALM GERMINATED SEEDS USING RWB (6 x 6 x 6 cm) AT SIX MONTHS AFTER INOCULATION[#]

Ganoderma species	% of seedlings infected	
	Based on visual foliar symptoms	Based on reisolation of *Ganoderma* on GSM
G. boninense	45	90
G. zonatum	45	95
G. miniatocinctum	25	85
G. tornatum	0	0
G. lucidum	0	0
G. philippii	0	0

Note: [#]No. of germinated seeds/treatment: 20.

inoculated with *G. tornatum, G. lucidum* and *G. philippii* while attempts to reisolate the fungi on GSM failed. However, the seedlings inoculated with *G. boninense, G. miniatocinctum* and *G. zonatum* exhibited internal symptoms and *Ganoderma* was isolated (between 85% to 95%) from their stems and roots. Thus, *G. boninense, G. miniatocinctum* and *G. zonatum* were pathogenic to oil palm, but *G. tornatum, G. lucidum* and *G. philippii* are not.

EXPERIMENT 3: COMPARISON OF SUSCEPTIBLE (DxD), TOLERANT (DxP) AND COMMERCIAL (DxP) PROGENIES BY INOCULATION OF OIL PALM GERMINATED SEEDS WITH *G. boninense*

The inoculation technique developed was tested against germinated seeds of the following oil palm progenies known to be susceptible to *Ganoderma* DxD (Elmina D x Elmina D), tolerant DxP (Zaire x Cameroon) and commercial DxP (standard cross) as reported by Idris *et al.* (2004). *G. boninense* was raised on RWB of 27 cm^3. The inoculated RWBs were incubated (at 27°C) for approximately 45 days. The oil palm germinated seeds from all the progenies were then inoculated using the technique in Experiment 1. The results are presented in *Table 3*. At six months, foliar symptoms were observed in all progenies but with different incidences: DxD (40%), tolerant DxP (20%) and commercial DxP (25%). However, all progenies exhibited internal disease symptoms and *Ganoderma* was reisolated from stems or roots at 60% to 70%.

TABLE 3. INFECTION OF SUSCEPTIBLE, TOLERANT AND COMMERCIAL STANDARD CROSS OIL PALM BY *G. boninense* SIX MONTHS AFTER INOCULATING THE GERMINATED SEEDS WITH RWB (size 3 x 3 x 3 cm)[#]

Oil palm progeny	% of seedlings infected	
	Based on visual foliar symptoms	Based on reisolation of *Ganoderma* on GSM
Susceptible DxD (Elmina D x Elmina D)	40	70
Tolerant DxP (Zaire x Cameroon)	20	70
Commercial DxP (Standard cross)	25	60
Chi-square analysis	$p<0.05$	Not significant

Note: [#]No. of germinated seeds/treatment: 20.

TECHNIQUE FOR INOCULATION OF GERMINATED SEEDS WITH *Ganoderma*

Based on these studies, the following technique is recommended to inoculate germinated seeds of oil palm with *Ganoderma*:

Step 1 - Prepare RWB as a source of *Ganoderma* inoculum (*Figure 3A*).

Step 2 - Transfer RWB inoculum into the polythene bag containing unsterilized soil (*Figure 3B*).

Step 3 - Plant the germinated seed in the polythene bag containing RWB inoculum (2.5 cm between inoculum and geminated seed) (*Figure 3C*).

Step 4 - Assess disease development by the progress of *Ganoderma* infection (*Figure 3D*).

CONCLUSION

The technique developed, using RWB as substrate for *Ganoderma* inoculum, has infected oil palm seedlings artificially, thus, confirming the pathogenicity of *Ganoderma* as the causal agent of BSR. This technique should be useful to screen oil palm for resistance to *Ganoderma* and to study the host-pathogen interaction, infection biology and disease development.

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