

Basal stem rot (BSR) caused by some species of *Ganoderma*, is the most destructive disease of oil palm in Southeast Asia (Turner and Gillbanks, 2003). The disease can infect oil palm at all stages of its growth. Infected, immature palms normally die within 6-24 months of the first foliar symptoms, while mature palms may survive two to three years or more. Various molecular and immunological methods have been described for detecting the disease in oil palm, including the DNA-polymerase chain reaction and use of polyclonal antibodies (Bridge *et al.*, 2000; Utomo and Niepold, 2000; Idris *et al.*, 2003). This article reports the use of polyclonal antibodies (PABs) against the crude mycelial proteins of *G. boninense* to serologically detect the fungus by indirect enzyme-linked immunosorbent assay (ELISA). ELISA is a technique for rapid quantification of a particular protein in a protein mixture using highly specific antibodies. The technique offers greater simplicity and requires less equipment than using DNA probes.

PRINCIPLE OF THE TEST

The sample of oil palm tissue (root, leaf or stem) is finely grounded, diluted with extraction buffer and centrifuged. The total protein concentration is determined using the Bradford assay (1976) with bovine serum albumin (BSA) as the standard. Indirect ELISA is performed using the method of Alexopoulou *et al.* (1998). A summary of the ELISA-PAB procedure is presented in *Figure 1*.

Each column of the ELISA microtitre plate is coated with the antigens diluted in coating buffer (50 μ l volume) and incubated overnight at 4°C. The plates are washed with phosphate buffered saline (PBS) plus 0.05% phosphate buffer saline-Tween (PBS-T), and serial dilutions of the primary antibody (from 1:10 to 1:1000) prepared in assay buffer

PBS-T are applied down each column in 50 μ l volumes and incubated for 1 hr at 37°C. The plates are again washed with PBS-T and the secondary antibody (goat anti-rabbit IgG-horseradish peroxidase diluted 1/5000 in assay buffer) applied in 50 μ l volumes to each well. After 1 hr incubation, the plates are put through a final wash cycle with PBS. Goat anti-rabbit immunoglobulin G labelled with horseradish peroxidase is used as the immunoconjugate and hydrogen peroxide with 2',2'-azino-di-(3-ethyl-benzthiazoline sulpho-nate) (ABTS), and then 50 μ l/well as the substrate and chromogen. Absorbance of the hydrolyzed substrate is measured at 405 nm with an ELISA reader. If the average absorbance values of the samples plotted on a standard curve graph do not fall on the standard curve, the samples are considered as positive for *Ganoderma* infection (*Figure 2*).

SPECIFICITY AND CROSS REACTIVITY TESTS

Four isolates of *Ganoderma* and saprophytic fungi were grown on potato dextrose agar (PDA). After 7-10 days' incubation, plugs were cut from the actively growing mycelium and transferred to sterile conical flasks, each containing 100 ml potato dextrose broth (PDB), and incubated at 28°C for 14 days. The mycelial cultures were harvested by vacuum filtration, rinsed with distilled water and blotted dry using sterile Whatman No. 1 filter paper. The fresh mycelia were then frozen at -20°C before being lyophilised in a freeze dryer. The samples were stored at -20°C until required. A sample of freeze-dried mycelium (0.5 g) was soaked in liquid nitrogen for 15 – 30 min and then ground in a pre-cooled sterile mortar and pestle in 1.5 ml extraction buffer. The ground tissues were placed in micro centrifuge tubes which were then vortexed thoroughly for a few seconds, and then centrifuged at 9000 rpm for 20 min at 4°C. The clear supernatant, free of cellular debris, was

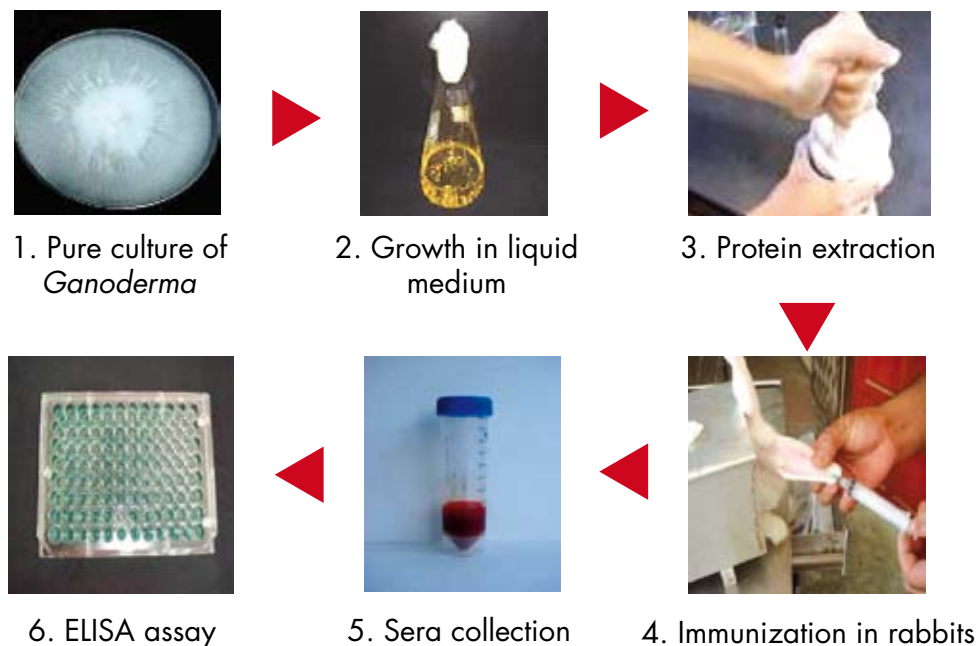


Figure 1. The ELISA-PAb.

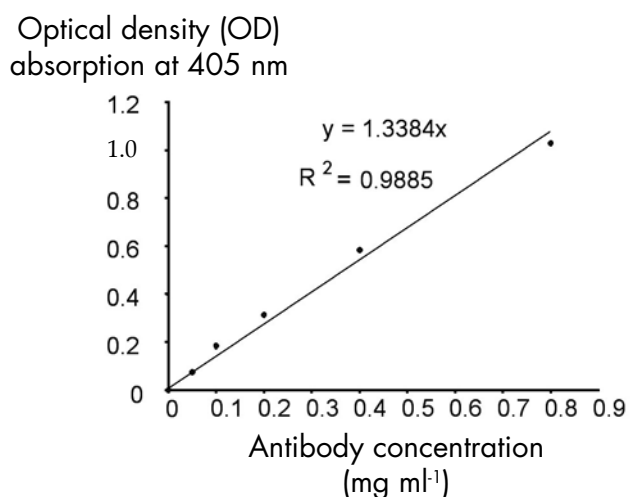


Figure 2. ELISA standard curve for detection of *Ganoderma*.

used for total protein determination by Bradford Assay. Indirect ELISA was performed as described previously. The ELISA-PAb developed was able to detect the pathogenic species of *Ganoderma* such as *G. boninense*, *G. zonatum* and *G. miniatocinctum* but not the non-pathogenic *G. tornatum* (Table 1 and Figure 3).

Cross reactivity tests with saprophytic fungi revealed that the ELISA-PAb cross reacted with

Penicillium but not with *Trichoderma* and *Aspergillus* (Table 2 and Figure 4).

DETECTION OF BSR USING THE ELISA-PAB DEVELOPED

Roots and leaf samples were collected from DxP oil palm seedlings artificially infected with *G. boninense* using the rubber wood block (RWB) sitting technique as described by Idris *et al.* (2006). All the samples were washed with distilled water, weighed and ground. Protein and indirect ELISA was performed as described previously. The samples were also placed in *Ganoderma* selective medium (GSM) to check for the presence or absence of *G. boninense*. The ELISA-PAb developed was able to detect BSR in both roots and leaves of 30 seedlings artificially infected with *G. boninense* (Table 3), whereas GSM was only able to confirm the disease from the root samples.

Trunk and leaf samples were also collected from healthy and diseased standing oil palm at MPOB Research Station Teluk Intan, Perak. The palms were 10 years old. The ELISA-PAb developed was able to detect the disease in both tissues of healthy-looking palms and diseased-standing palms (Table 4). On the other hand, GSM could only confirm the disease from the trunk samples.

TABLE 1. SPECIFICITY TESTS OF ELISA-PA_b FOR DETECTION OF FOUR SPECIES OF *Ganoderma*

Species of <i>Ganoderma</i>	Pathogenicity test	Results (+/-)
<i>G. boninense</i>	Pathogenic	+
<i>G. miniatocinctum</i>	Pathogenic	+
<i>G. zonatum</i>	Pathogenic	+
<i>G. tornatum</i>	Nonpathogenic	-

Note: positive detection (+) and negative detection (-).



1. *G. boninense*



2. *G. zonatum*



3. *G. miniatocinctum*



4. *G. tornatum*

Figure 3. Species of *Ganoderma*.

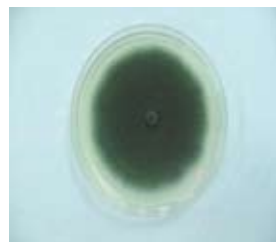
TABLE 2. CROSS REACTIVITY TESTS OF ELISA-PA_b WITH SAPROPHYTIC FUNGI

Saprophytic fungus	Results (+/-)
<i>Aspergillus niger</i>	-
<i>Penicillium</i> sp.	+
<i>Trichoderma virens</i>	-
<i>Trichoderma harzianum</i>	-

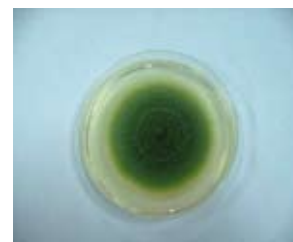
Note: positive detection (+) and negative detection (-).



1. *Trichoderma* sp.



2. *Aspergillus* sp.



3. *Penicillium* sp.

Figure 4. Saprophytic fungi.

TABLE 3. DETECTION OF BSR IN OIL PALM SEEDLINGS ARTIFICIALLY INFECTED WITH *G. boninense* USING ELISA-PAb

Sample	Inoculated seedlings (%) (N=30)		Uninoculated seedlings (%) (N=30)	
	GSM	ELISA-PAb	GSM	ELISA-PAb
Leaf	0	100	0	0
Roots	100	100	0	0

TABLE 4. DETECTION OF BSR IN MATURE OIL PALM USING ELISA-PAb

Sample	Healthy-looking palms (%) (N=12)		Diseased-standing palms (%) (N=12)	
	GSM	ELISA-PAb	GSM	ELISA-PAb
Leaf	0	50	0	16.7
Trunk	50	83.3	100	100

CONCLUSION

The ELISA-PAb method described here could detect BSR in all the tissues of oil palm tested—roots, trunk and leaves. Only a small sample of tissue (minimum 0.5 g) is required, but it would have to be preserved in liquid nitrogen immediately after sampling to keep it in good condition for analysis.

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For more information kindly contact:

Director-General
MPOB
P. O. Box 10620
50720 Kuala Lumpur, Malaysia.
Tel: 03-87694400
Website: www.mpob.gov.my
Telefax: 03-89259446