SELECTION FOR PARTIAL RESISTANCE IN OIL PALM PROGENIES TO *Ganoderma* BASAL STEM ROT

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ABSTRACT

The development of Elaeis guineensis *progenies resistant to* Ganoderma *may provide the ideal long-term solution to basal stem rot, a major disease of oil palm in Malaysia and Indonesia. A study was conducted to select different oil palm progenies for resistance to* G. boninense *infection. In this study, 12-month-old seedlings from 23 progenies, namely, three DxD, six DxP, three OxO, one OxP, five TxP and five TxT were inoculated with* G. boninense *using the root inoculation technique. External foliar symptoms developing on seedlings were recorded for a period of 12 months. Twelve months after inoculation, all the seedlings were examined for internal symptoms based on the length of inoculated root lesioned, number of primary roots infected and extent of stem bulb tissues lesioned. Based on reisolation of* G. boninense *from inoculated seedlings, it was shown that all 23 progenies from the different oil palm crosses were infected by* G. boninense. *The uninoculated seedlings for each of progenies did not show any signs of disease symptoms or lesions and* G. boninense *was not present. Some 25.6% of the inoculated seedlings were dead due to* G. boninense *infection, and there were significant differences between the progenies tested for the severity of foliar symptoms measured. For internal symptoms, there was no significant difference in the length of inoculated roots lesioned. However, the number of primary roots infected and extent of stem bulb tissues lesioned were significantly different. Of the 23 progenies, the most susceptible progeny was PK 2724 [DxD, Deli (Elmina) x Deli (Elmina)], whilst a partially resistant progeny was PK 2567 (DxP, Congo x Cameroon). Partial resistance is expressed by low severity of foliar symptoms and slow progress of* Ganoderma *infection in the roots and stem tissues.*

Keywords: *Elaeis guineensis*, tolerant, susceptibility, stem rot, *Ganoderma*.

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INTRODUCTION

Host resistance is generally considered as an important and successful component in preventing and controlling plant diseases as this approach is relatively inexpensive, biologically safe and convenient for the farmer. Other means of protection measures are relatively disadvantageous to the farmers, as they require investment in machinery or labour and special training. The development of oil palm (*Elaeis guineensis*) genotypes resistant to *Ganoderma* may provide the ideal long-term solution to basal stem rot (BSR), the most important disease of oil palm in Malaysia and Indonesia. The disease has also been recorded in Africa, Papua New Guinea (Turner, 1981), Honduras (Chinchilla and Richardson, 1987), Colombia (Nieto, 1995) and Thailand (Tummakate and Likhitekaraj, 1994). In separate trials conducted in Sumatera, Indonesia, it was observed that African progenies of oil palm developed BSR at a much slower rate than local Deli progenies (Akbar *et al*., 1971; Hastjarjo and Soebiarpadia, 1975). This was attributed to a multiple gene effect (Akbar *et al*., 1971). The existence of resistant genotypes has also been indicated in trials

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of 20 *dura* x *pisifera* (DxP) crosses in Indonesia (Purba *et al*., 1994) and in *E. oleifera* x *E. guineensis* hybrids in Malaysia (Sharma and Tan, 1990; Chung *et al*., 1994). Lately, Franqueville *et al.* (2001) detected differences in susceptibility of oil palm germplasm planted in areas of high BSR incidence in Indonesia.

Although *Ganoderma* species had been identified as the causal organism of BSR of oil palm (Turner, 1981), formal proof of its pathogenicity has been difficult to obtain. Two groups of workers, one located in the Malaysian Palm Oil Board (MPOB), Bangi and the other at Universiti Putra Malaysia (UPM), Serdang addressed this problem and both developed reliable inoculation techniques, using two contrasting methods. Khairudin (1990) prepared inoculum by growing isolates on blocks (6 x 6 x 12 cm) of sterilized rubber wood. These were placed beneath germinating seedlings growing in soil in polybags. The seedlings showed progressive infection of roots and stem bases leading to foliar symptoms typical of BSR, and in a few instances, death after 10 months. Uninoculated controls remained healthy. The pathogen identified as *G. boninense* was reisolated from the diseased seedlings, confirming Koch's postulates. Similar results were obtained by Sariah *et al.* (1994). Ariffin and Idris (1990) had developed a method for inoculating isolated roots separately from the main root mass. This technique, called the root inoculation technique, involves withdrawal of a primary root through a slit made in a polybag containing an oil palm seedling. The root is then inserted into a tube (13 mm diameter x 100 mm length) containing a substrate colonized by the test pathogen. Using this technique, they showed that isolates of *Ganoderma* advanced along the length of a primary root at a speed of approximately 1.8 cm mth^{-1} (Ariffin and Idris, 2002), finally reaching the stem base and causing typical symptoms of BSR.

Turner (1981) considered that resistance to BSR would quickly be overwhelmed by the large volume of inoculum present in many plantations but genetic improvement has provided resistance for the diseases prevalent in oil palm nursery stocks. Thus, blast disease caused by *Pythium* and *Rhizoctonia* (Blaak, 1969) and vascular wilt caused by *Fusarium oxysporum* f. sp. *elaeidi*s (Meunier *et al*., 1979) can be reduced using resistant materials. For some of these diseases, a relatively rapid screening method is available (Mepsted *et al*., 1995), which facilitates breeding programmes. In contrast, observations on resistance to BSR have hitherto depended on the incidence of natural infections in the field, a process that takes many years. The root inoculation technique could provide an alternative method of screening. Using this technique, Ariffin *et al.* (1999) were able to detect significant differences in susceptibility among 20 commercial DxP materials. The work described here was undertaken to screen 23 oil palm progenies of different crosses, *viz*. *dura* x *dura* (DxD), *dura* x *pisifera* (DxP), *E. oleifera* x *E. oleifera* (OxO), *E. oleifera* x *pisifera* (OxP), *tenera* x *pisifera* (TxP), and *tenera* x *tenera* (TxT), for resistance to *G. boninense*.

MATERIALS AND METHODS

Source of Oil Palm Progenies Used

Germinated seeds of 23 oil palm progenies were provided by the Breeding and Genetics Group of MPOB, three DxD, six DxP, three OxO, one OxP, five TxP and five TxT (*Table 1*). The germinated seeds were planted in small black polypropylene bags (6 x 9 cm) for three months in the glasshouse and then transferred to big black polypropylene bags (38 x 50 cm) containing a mixture of sand, soil and organic matter (3:2:1). The bags were placed in the main nursery at 90 cm triangular spacing. The seedlings were maintained and watered (twice daily) using a sprinkler system and treated with fertilizer and pesticides applications as appropriate.

The 23 progenies were laid out in a completely randomized block design (RCBD) at eight seedlings per progeny with five replicates (blocks). A total of 40 seedlings per progeny were inoculated with *G. boninense* at 12 months old. For the control, the roots of the seedlings were inserted into tubes containing POPW substrate [mixtures of paddy and oil palm wood sawdust supplemented with sucrose, $(NH_4)_2SO_4$, Ca (SO_4 H_2O and bacto-peptone)] (Idris, 1999), but without any inoculum of *G. boninense.*

Preparation of *Ganoderma* Inoculum, Inoculation Technique, Assessment of Disease and Data Analysis

An isolate code SEL 28/1 was obtained from a *Ganoderma*-infected palm, planted on peat soil at Jalan Kebun, Klang, Selangor. A 5 x 5 mm piece taken from the growing margin of the disease stem tissues was plated on the *Ganoderma*-selective medium (GSM) (Ariffin and Idris, 1991). After incubation at 28°C for five days, the hyphal tips of the fungus growing from the cube were subcultured on potato dextrose agar (PDA). The isolate was maintained on PDA slants. This isolate was identified as *G. boninense* (Idris, 1999; Yamoaka, M., National Institute of Bioscience and Human Technology (NIBH), Japan; pers. comm., 1999). This isolate had previously been shown to be pathogenic to oil palm seedlings (Ariffin and Idris, 1990) and reconfirmed as pathogenic (Idris, 1999).

The *G. boninense* inoculum was raised on a substrate of POPW in test tubes (Idris, 1999). The mouths of the tubes were covered with autoclavable polypropylene plastic and subjected to autoclaving

TABLE 1. THE 23 OIL PALM PROGENIES USED IN THIS STUDY

Progeny	Cross	Pedigree background	Genetic
PK 2626	DxD	0.212/704 x 0.212/524	Deli (Elmina) x Deli (Elmina)
PK 2556	DxD	0.212/6 x 0.212/332	Deli (Elmina) x Deli (Elmina)
PK 2724	DxD	0.212/6 x 0.212/652	Deli (Elmina) x Deli (Elmina)
PK 2567	DxP	0.221/1340 x 0.219/1371	Congo x Cameroon
PK 2640	DxP	0.212/3 x 0.200/812	Deli (Elmina) x (SP 19/29.36)
PK 2574	DxP	0.212/627 x 0.182/297	Deli (Elmina) x AVROS
PK 2572	DxP	0.212/437 x 0.182/297	Deli (Banting) x AVROS
PK 2571	DxP	0.212/71 x 0.182/297	Deli (Banting) x AVROS
PK 2550	DxP	0.218/1153 x 0.174/955	Cameroon x AVROS
PK 2635	OxO	0.211/970 x 0.211/970	Honduras x Honduras
PK 2558	OxO	0.211/1098 x 0.211/970	Colombia x Honduras
PK 2585	OxO	0.211/1801 x 0.211/970	Panama x Honduras
PK 2629	OxP	0.211/2480 x 0.174/955	Panama x AVROS
PK 1922	TxP	0.218/1336 x 0.174/211	Cameroon x AVROS
PK 1768	TxP	0.151/638 x 0.182/357	Nigeria x AVROS
PK 1867	TxP	0.149/11745 x 0.174/304	Nigeria x AVROS
PK 1595	TxP	0.151/330 x 0.182/357	Nigeria x AVROS
PK 2005	TxP	0.149/11526 x 0.174/211	Nigeria x AVROS
PK 1894	TxT	0.219/299 x 0.219/299	Cameroon x Cameroon
PK 1708	TxT	0.218/1006 x 0.219/299	Cameroon x Cameroon
PK 2048	TxT	0.149/12279 x 0.150/4352	Nigeria x Nigeria
PK 1511	TxT	0.149/2704 x 0.151/1418	Nigeria x Nigeria
PK 1672	TxT	0.218/2108 x 0.219/299	Cameroon x Cameroon

at 1.4 kg cm^{-2} at 121°C for 30 min. After cooling, each tube was inoculated with two plugs (7 mm diameter) of a 5-7 days old *Ganoderma* culture grown on PDA and incubated at 28°C for 20-30 days. This medium was used as inoculum when the mycelium had completely covered the substrate in the tube.

The inoculation technique followed that described by Ariffin and Idris (1990). A small incision was made at the side of the polypropylene bag containing the seedlings, revealing some roots. One of the primary roots exposed was then pulled through the opening, washed with water to remove the adhering soil, the distal end excised and placed into a test tube containing inoculum. Approximately 2-3 cm of the root was inserted into the medium and the tube sealed with parafilm. The whole tube was covered with brown paper to maintain darkness.

After inoculation with *G. boninense*, the oil palm seedlings were assessed for external and internal symptoms over 12 months. External symptoms including the number of dead seedlings and foliar discolouration were recorded at three-months' interval. The severity of foliar symptoms was assessed according to Sariah and Zakaria (2000): severity of foliar symptoms (%) = [(ax1) + (bx0.5)]/c x 100, where *a* the number of desiccated (browned/wilted) leaves, *b* the number of yellowing leaves, *c* the total number of leaves, and *1* the index for desiccated leaves and *0.5* the index for yellowing leaves. Destructive sampling of all seedlings was done at 12 months after inoculation to assess for internal symptoms. The length of the inoculated root infected was measured by cutting 1 cm long pieces of the root and plating them onto GSM to check for the presence or absence of *G. boninense.* The whole plant was split longitudinally at the inoculated root to examine the extent of stem bulb tissue lesioned using a 1 cm^2 grid. The number of primary roots infected was also counted. The data were analysed using SAS Release 6 (SAS Institute Inc., 1990). Analysis of variance (ANOVA) was performed to test the differences between the progenies, followed by comparison of means using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The data relating to external and internal symptoms for eight seedlings of each progeny within each of the five blocks were recorded after 12 months' inoculation. Using the root inoculation technique, all the progenies from the 23 different crosses were found to be infected by *G. boninense*. There were also no differences in the length of inoculated roots infected, which were generally completely decayed by the time they were destructively sampled. Plating the inoculated roots, infected roots and stem bulb

tissues lesioned on GSM consistently yielded *G. boninense*, confirming the disease and causal pathogen. The uninoculated progenies did not show any sign of disease symptoms or lesion and *G. boninense* was not present.

Some 25.6% of the inoculated seedlings were dead due to *G. boninense* infection (*Table 2*). The severity of the foliar symptoms, which developed on inoculated seedlings varied significantly ($p<0.05$) between the progenies tested (*Table 2*). In the seedlings of some progenies, the lesions in the inoculated roots extended into the stem bulb and then other primary roots. There were significant differences ($p<0.05$) in the incidence and extent of stem bulb tissues lesioned between progenies (*Table 3*). Differences between progenies were also observed in the number of additional primary roots infected from the stem base ($p<0.05$).

TABLE 2. PERCENTAGE OF DEAD AND SEVERITY OF FOLIAR SYMPTOMS OF 23 OIL PALM PROGENIES, 12 MONTHS AFTER INOCULATION

Progeny	Cross	External symptoms[#, ##]	
		Dead (%)	Severity of foliar symptoms (%)
PK 2556	D x D	37.5 ab	38.5 a
PK 2626	D x D	32.5 abc	34.6 ab
PK 2724	D x D	40.0 a	40.9 a
PK 2640	D x P	22.5 abcd	23.0 abc
PK 2572	D x P	25.0 abcd	25.9 abc
PK 2571	D x P	27.5 abcd	27.8 abc
PK 2574	D x P	22.5 abcd	23.7 abc
PK 2550	D x P	27.5 abcd	28.6 abc
PK 2567	D x P	10.0 d	12.3 c
PK 2558	O x O	25.0 abcd	27.9 abc
PK 2585	O x O	35.0 abc	35.3 ab
PK 2635	O x O	27.5 abcd	27.8 abc
PK 2629	O x P	37.5 ab	38.3 a
PK 2005	T x P	30.0 abc	31.1 abc
PK 1922	T x P	20.0 bcd	22.4 abc
PK 1867	T x P	25.0 abcd	26.1 abc
PK 1768	T x P	22.5 abcd	24.9 abc
PK 1595	T x P	27.5 abcd	29.9 abc
PK 2048	T x T	20.0 bcd	22.6 abc
PK 1894	T x T	15.0 cd	17.1 bc
PK 1511	T x T	22.5 abcd	24.9 abc
PK 1672	T x T	22.5 abcd	24.4 abc
PK 1708	T x T	15.0 cd	17.1 bc
Mean		25.6	27.2

Notes: [#] Values represent the mean of 40 seedlings per progeny.

[##] Means with the same letters within a column are not significantly different ($p<0.05$) from each other by DMRT.

TABLE 3. MEAN NUMBER OF PRIMARY ROOTS INFECTED AND STEM BULB TISSUES (cm^2) LESIONED OF OIL PALM PROGENIES, 12 MONTHS AFTER INOCULATION

Progeny	Cross	Internal symptoms[#, ##]	
		No. of primary roots infected	Stem bulb tissues (cm^2) lesioned
PK 2556	D x D	23.0 a	10.35 ab
PK 2626	D x D	20.8 abc	9.98 abc
PK 2724	D x D	22.4 ab	10.58 a
PK 2640	D x P	12.5 cde	6.70 cd
PK 2572	D x P	11.7 de	6.68 cd
PK 2571	D x P	13.5 cde	7.13 bc
PK 2574	D x P	11.6 de	6.63 cd
PK 2550	D x P	15.0 abcde	7.15 bc
PK 2567	D x P	6.5 e	3.73 d
PK 2558	O x O	17.1 abcd	7.95 abc
PK 2585	O x O	16.4 abcd	8.35 abc
PK 2635	O x O	16.2 abcd	8.53 abc
PK 2629	O x P	16.7 abcd	8.70 abc
PK 2005	T x P	15.7 abcd	7.08 bc
PK 1922	T x P	16.1 abcd	8.03 abc
PK 1867	T x P	16.3 abcd	8.00 abc
PK 1768	T x P	16.0 abcd	8.10 abc
PK 1595	T x P	16.0 abcd	8.43 abc
PK 2048	T x T	11.4 de	7.30 abc
PK 1894	T x T	10.5 de	6.80 cd
PK 1511	T x T	13.8 bcde	7.90 abc
PK 1672	T x T	14.4 abcde	7.88 abc
PK 1708	T x T	11.1 de	6.53 cd
Mean		14.9	7.6

Notes: [#] Values represent the mean of 40 seedlings per progeny.
[##] Means with the same letters within a column are not significantly different ($p<0.05$) from each other DMRT.

The root inoculation technique showed differences in susceptibility between seedlings of different progenies but none showed absolute resistance. Generally, this related to the differences in the rate of *Ganoderma* spread in tissues of the roots and stem bulb as they became invaded by *G. boninense*, rather than to tolerance of invasion, as have been implied from field observations (Sharma and Tan, 1990; Chung *et al*., 1994; Purba *et al*., 1994; Franqueville *et al*., 2001). It is still not known whether complete resistance is present in any oil palm genotypes. Those screened so far did not represent the full range of genotypes available (Rajanaidu and Jalani, 1995; Kushairi *et al*., 1997). However, it seems unlikely that full resistance exists in any oil palm population and field observations to the contrary could be the result of disease escapes (Ariffin *et al*., 1993; 1996).

CONCLUSION

This study showed that the most susceptible progeny was PK 2724 [DxD, Deli (Elmina) x Deli (Elmina)], whilst the partially resistant progeny was PK 2567 (DxP, Congo x Cameroon). Low severity of the foliar symptoms and slow progress of *G. boninense* infection in the roots and stem tissues of oil palm expressed partial resistance. What is not known is whether the differences detected by this technique can be used to predict field performance, where the progress of the disease takes several years to develop visible symptoms. Obviously, this needs to be tested, possibly using the most susceptible and partially resistant progenies.

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