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SHORT RESEARCH NOTES

A detached leaf bioassay to screen Durian cultivars for susceptibility to *Phytophthora palmivora*

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Abstract. A detached-leaf bioassay was developed and used to screen five durian (*Durio zibethinus*) cultivars against *Phytophthora palmivora* isolates from a trunk canker, root and fruit. The fruit isolate was less aggressive than the canker and root isolates. The bioassay using the canker isolate was later used to determine the variation in resistance of *D. macarantha* and nineteen cultivars of *D. zibethinus*. The cultivars displayed a range of responses with Parung and Gob being most tolerant, with Gaan Yaow, Chanee and Penang 88 being susceptible. The remaining germplasm fell between Gaan Yaow and Penang 88 in susceptibility. The leaf bioassay was found to be a rapid and reliable method for assessing the susceptibility of durian cultivars.

Additional keywords: root rot, trunk canker, fruit rot, cultivars, oomycete.

Durian (*Durio zibethinus*) is one of the most popular and widely eaten seasonal fruits in South East Asia (Lim 1990). *Phytophthora palmivora* is known to cause the most destructive and economically significant diseases of durian including leaf blight, fruit rot, trunk canker and root rot. Trunk canker is deemed to be the most devastating of these diseases (Lim 1990).

Durian is propagated by grafting onto seedlings of common commercial cultivars (Sangchote 2000), and the use of resistant cultivars will be central to the integrated management of *P. palmivora* in this crop. Research conducted by Sangchote (2000) showed that the disease incidence on attached leaves, stems and fruits corresponded with field performance, suggesting that the same genetic mechanisms for resistance are operating in these various plant parts. Resistance in durian is considered horizontal and under polygenic control (Sangchote 2000) and, to date, no systematic screening of germplasm has been reported. To achieve this, a detached leaf bioassay was developed and used to compare the virulence of isolates of *P. palmivora* obtained from durian trunk canker, roots and fruit. In a later experiment, the detached leaf bioassay was used to evaluate *Durio* germplasm for susceptibility to a trunk canker isolate of *P. palmivora.*

Five durian cultivars, Chanee, D10, Gob, Hew 3 and Monthong were screened against *P. palmivora* isolates from a trunk canker (BRIP 42474), root (BRIP 42475), and fruit (BRIP 42476). Healthy, mature leaves were obtained from durian trees growing in the South Johnstone region of far north Queensland. The leaves were surface sterilised in a mixture of 10% ethanol and 3% a.i. NaOCl for 1 min and then rinsed in sterile distilled water. Immediately prior to inoculation, each leaf was punctured four times (on either side of the midrib and at either end of the leaf) with a device that was designed to standardise the wounding process. The leaves were inoculated by placing a 4 mm plug of an axenic culture of *P. palmivora* mycelium/sporangia face down onto the wounds. Leaves used as non-inoculated controls were only wounded. Leaves were supported on a wire mesh platform over free water in an airtight Tupperware container and incubated at 26°C in the dark. The experimental unit (replicate) was the Tupperware container with one non-inoculated control and two inoculated leaves, with four replicate containers per cultivar. Diameters of lesions were measured daily using a micrometer from days 2 to 6 after inoculation. Lesion diameters for the inoculated leaves were averaged within each experimental unit. A split plot design and a repeated measures analysis of variance (using

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the Greenhouse Geisser adjustment) were used to analyse lesion development over time (Greenhouse and Geisser 1959). Sections of all leaf lesions were surface sterilised in 70% ethanol for 1 min, blotted dry with sterile paper then transferred to P_{10}ARP+H selective medium (Jeffers and Martin 1986). The plates were observed for growth of *P. palmivora* from the sections of leaf after incubation in the dark at 26°C for 72 h.

In a later experiment, 19 cultivars of *D. zibethinus*, and the species *D. macarantha* were screened against the virulent *P. palmivora* (BRIP 42474) trunk canker isolate using the method previously described. The durian cultivars screened were Gumpun, Luang, Gob Yaow, Gob, D98, Chompoosee, Limberlost, Sunan, Parung, Hew 3, Gaan Yaow, Chanee, Kradum-Tong, D10, D102, D123, Red Prawn, Penang 88, and D24. *Durio macarantha*, a semi-wild species, was included for its potential as tolerant rootstock (Lim 1997). An experimental unit consisted of the Tupperware container containing one non-inoculated control and three inoculated leaves, with four replicates per cultivar. Results in the *Durio* germplasm evaluation were expressed as the mean lesion size and standard error of the mean over 5 days for each cultivar. Isolation of *P. palmivora* from sections of leaves was carried out as described previously.

In the study of the virulence of isolates of *P. palmivora* from trunk canker, roots and fruit, all isolates caused lesions in all cultivars. However, the fruit isolate caused significantly smaller lesions in all five cultivars ($P < 0.05$) than either the canker or root isolates (Table 1), indicating it was the least virulent of the isolates tested. Leaf senescence started to occur 6 days after inoculation. *P. palmivora* was re-isolated from all the leaf lesions.

The *Durio* cultivars and species screened against the trunk canker isolate showed various levels of disease severity (Fig. 1). The mean lesion diameters showed that cultivars Parung and Gob developed smaller lesions than Gaan Yaow, Chanee and Penang 88. The remaining germplasm developed lesions with diameters that fell between those produced on Gaan Yaow and Penang 88. Leaf senescence started to occur

Table 1. Overall mean lesion diameter (mm; days 2 to 6) on detached leaves of five durian cultivars inoculated with *Phytophthora palmivora* isolates from trunk canker, roots and fruit

Cultivar	Isolate		
	BRIP 42474 (canker)	BRIP 42475 (root)	BRIP 42476 (fruit)
Gob	8.11	9.54	2.26
Hew 3	10.42	10.50	3.31
Chanee	13.08	10.90	4.00
D10	14.26	13.44	4.83
Monthong	15.50	18.78	5.08

$(P < 0.05) = 1.66$ except when comparing means in rows, then $(P < 0.05) = 1.49$.

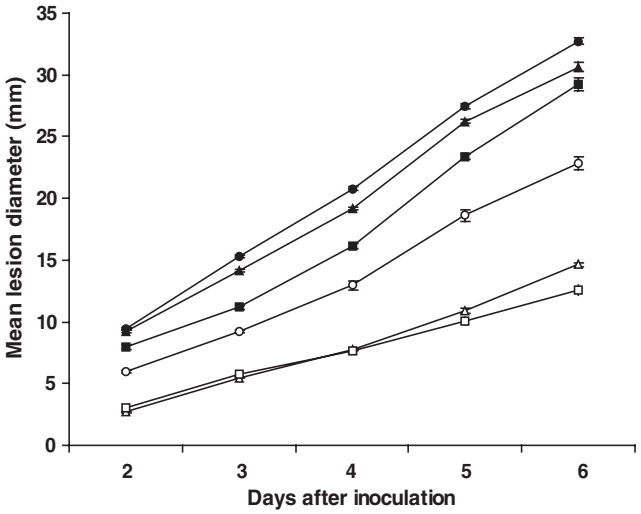

Fig. 1. Mean lesion diameter on durian cultivars Parung (□), Gob (△), Gaan Yaow (○), Chanee (■), *Durio macarantha* (▲) and Penang 88 (●) inoculated with *Phytophthora palmivora*. An additional 14 cultivars were screened and these fell between Gaan Yaow and Penang 88. Vertical lines are standard errors of the means.

6 days after inoculation. *P. palmivora* was re-isolated from all the leaf lesions.

The evaluation of durian germplasm for susceptibility to the trunk canker isolate of *P. palmivora* showed that there was good agreement between this study and the results from the initial virulence study. In the virulence study, the cultivar Gob showed promising levels of tolerance by developing significantly smaller lesions ($P < 0.05$), whereas lesion development was extensive in the commercially popular cultivars Monthong and Chanee. Lim (1997) in his review of *Phytophthora* resistance in field-grown durian cultivars reported Gob as tolerant, and Monthong and Chanee as susceptible to trunk canker. Pongpisutta and Sangchote (1993) also described Monthong and Chanee as being susceptible following wound inoculation of detached fruit. However, in our study the cultivar D10 proved susceptible to *P. palmivora*. This result contradicts research conducted at the Malaysian Agricultural Research and Development Institute, where D10 was described as being tolerant to *Phytophthora* in seedling inoculations and was used in a durian breeding programme (Tai 1971). This finding may be due in part to differences in virulence between isolates of *P. palmivora* in Malaysia and Australia. There is also some doubt as to the true identity of some of the durian cultivars imported into Australia.

The use of tolerant cultivars as resistant rootstocks is considered a most important component of the integrated management of *Phytophthora* diseases of durian. The development of the detached leaf bioassay has proven to be a rapid, reliable and non-destructive method of assessing *Phytophthora* susceptible germplasm in durian. However,

the real success of the leaf bioassay as a means of assessing cultivar susceptibility is determined by a strong correlation with field performance.

Lim (1997) referred to wild and semi-wild species of *Durio* and closely allied genera in the family Bombacaceae as possible sources of resistance. He alluded to the many *Durio* spp. which grow in low-lying marshy environments as having the greatest potential in the breeding of disease resistant rootstock. However, as durian cultivars are self incompatible with progeny segregating, any resistant parent material used in research will need to be cloned. Any future research into the management of *Phytophthora* diseases of durian in far northern Queensland would involve further use of the detached leaf bioassay to test *Durio* spp. from diverse growing environments, and the glasshouse and field evaluation of planting material with potential disease resistance.

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