8.3 Screening for Resistance to *Phytophthora*

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Abstract

Identifying and evaluating disease resistance depends on rapid, reliable and robust bioassays that can rapidly screen large numbers of genotypes and breeding progenies. We developed seedling, leaf and stem bioassays to screen durian germplasm from Thailand, Vietnam and Australia for resistance to *Phytophthora palmivora*. Detached leaf assays segregated durian cultivars into classes consistent with field observations, and are recommended as an early screen in breeding programs. Durian cultivar Chanee emerged as the least susceptible cultivar in Thai and Vietnamese tests.

Screening Germplasm for Tolerance to *Phytophthora*

Disease-resistant varieties are central to the integrated management of *Phytophthora palmivora* in durian. Lim (1998a) suggested that wild *Durio* spp. evolving in damp, low-lying areas may be potential sources of genes for disease resistance against *Phytophthora*. The relatively few resistance studies reported suggest that resistance in durian is polygenic (Lim 1998b). One of the major aims of Australian Centre for International Agricultural Research (ACIAR) Project PHT/1995/134, 'Management of *Phytophthora* diseases in durian', was to develop a rapid and reliable resistance screening bioassay to identify sources of resistance in the germplasm collections of Thailand, Vietnam and Australia.

The resistance screening of a perennial crop such as durian might involve pot trials in which whole plants are artificially inoculated, or field trials in which trees at infested sites are assessed over time for disease development and survival. These tests are time-consuming and expensive, and considerable savings could be made if more rapid assays enabled more cultivars to be screened. Preliminary screening bioassays designed to identify cultivars with promising disease-resistance characteristics, or with high levels of susceptibility, have been successfully developed for other crops using detached plant organs.

One of the major diseases of cocoa is black pod, caused by *Phytophthora* spp., and screening bioassays have been developed using detached whole leaves, leaf-discs (Nyasse et al. 1995) and detached cocoa pods (Iwaro et al. 1997). Such bioassays have been used to expedite the identification of resistant genotypes that are suitable for cocoa breeding programs, or susceptible genotypes that should be excluded. Cocoa typically produces two pod flushes a year, with the main cropping season lasting up to six months. With such long production cycles, cocoa pods can be available for screening experiments most of the year. The distinct and relatively short fruiting period of durian makes fruit bioassays less practical as a

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routine tool. Additionally, the large size and the high value of durian fruit can make the design of statistically valid screening experiments difficult.

The variation in the pathogen population means that testing of cultivars at more than one place is necessary. At present, it is unclear if different pathogenic races or differences in aggressiveness occur among *P. palmivora* populations in Southeast Asia and Australia. In addition to differences in pathogen populations, we also have to consider differences in environmental conditions and soil types which occur at a local level and may have a significant influence on the expression of resistance in durian cultivars.

Bioassay Development

Entire leaf versus leaf-strip

Some durian cultivars have very large leaves, making the use of entire leaves in a bioassay unwieldy. Leaf-strips (approximately 6 cm long by 2.5 cm wide) cut from either side of the main vein can be used as an alternative. Although we found no difference in the rate or magnitude of lesion development between entire leaves and leaf-strips, there were disadvantages using leaf-strips. Fungal contamination at the cut edge of the leaf-strip was common, particularly if the leaves had been sourced from an orchard rather than from glasshouse-grown seedlings. We reduced contamination by surface-sterilising leaf-strips in a mixture of 10% ethanol and 3% a.i. sodium hypochlorite for 1 minute, followed by thorough rinsing in sterile deionised water before inoculation. However, the production and surface sterilisation of individual strips makes this a time-consuming process.

Wounded versus non-wounded leaf material

Ideally a bioassay includes wounded and non-wounded treatments so that tissue susceptibility to penetration and infection can be assessed independently. However, in bioassay experiments in Australia (Tan 1999) and Thailand, non-wounded durian leaves did not develop disease symptoms reliably when inoculated with *P. palmivora.* Consequently, a wounding device was designed to deliver a consistent wound to leaves (Figure 8.3.1) before inoculation with an agar plug from the edge of a colony of *P. palmivora*.

Incubation conditions

Where ambient temperatures were too cold or variable for infection to occur, incubation was carried out in constant-temperature cabinets at 26˚C. Tissue desiccation was successfully avoided by incubating whole detached leaves on wire mesh platforms over free water, in sealed Tupperware® containers. However, incubating leaf-strips over free water, as described above, did not prevent desiccation. While desiccation was reduced by laying the leaf-strips on paper-towel moistened with sterile water, cross-contamination was common due to accidental contact between the leaf-strips, or colonisation of the towel by the pathogen. Tissue desiccation and cross-contamination were prevented when leaf-strips were inoculated at one end and the non-inoculated ends were placed vertically into slots made in a layer (75 mm deep) of solidified water agar and incubated in a sealed Tupperware® container (Figure 8.3.2). Although more time-consuming, an additional advantage of placing the strips vertically rather than horizontally was that many more strips could be accommodated in a single tray, increasing the number of samples that could be tested in a single bioassay.

Figure 8.3.1 Wounding device, constructed from a clothes peg and thumb-tack, designed to standardise the wounding of leaves.

Figure 8.3.2 Inoculated durian leaf-strips standing vertically in water agar to keep them turgid during incubation.

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Symptom assessment in leaves

Depending on incubation conditions, it may take up to three days from inoculation to the appearance of the first disease symptoms. Measurement commences as soon as symptoms appear. When entire leaves are inoculated, lesion diameter is measured. As lesions are often not concentric, it is recommended that the diameter be measured in more than one direction, then averaged. In leaf-strips, the length of the lesion from the wound to the leading edge of the lesion should be measured.

Stem bioassay

Detached-stem bioassays are better for comparing clonal lines of *Eucalyptus marginata* for susceptibility to *Phytophthora cinnamomi* (Hüberli 2002) than for comparing pathogenicity between isolates of the pathogen (Hüberli 2001). Durian stems are readily available, can be obtained from large trees without undue injury, and as such should be suitable for use in a bioassay. However, attempts to develop a bioassay for durian using detached stems were unsuccessful.

Green stems (stems in which periderm formation had not yet occurred), with diameters 0.50–1.25 cm, were obtained from durian orchards in northern Australia. Each stem was cut to a length of 15 cm before surface sterilisation for 2 minutes in the solution described above. The holes in non-draining test-tube racks were half filled with washed/sieved sand and 2 mL water that contained 50 μg/mL benzimidazole. The rack was autoclaved and a stem placed upright into each of the holes. A plug of inoculum mycelium/sporangia was placed onto the end of each stem and the rack was then put into a Tupperware® container and sealed for incubation.

Despite a more rigorous surface sterilisation, the stems were rapidly colonised by secondary invaders. Unlike *E. marginata*, lesions were not visible from the outside of the inoculated durian stem. Even when the epidermis was scraped away, it was difficult to see the lesions, and, if visible, to determine the lesion boundary. When the stems were split longitudinally, the pith often appeared orange but this may have been due to oxidation of the exposed tissues. Due to the difficulty of definitively identifying and measuring lesions, the stem was dissected into 1 cm segments, which were plated sequentially onto selective agar to calculate how much of the tissue was colonised by the pathogen. A bioassay using excised stems as described above is time-consuming, expensive and consequently considered unsuitable as a rapid and inexpensive screen for resistance in durian.

In summary, leaves are the most practicable durian organ to use in a detached-organ screening bioassay. Where incubation space is not limiting, the use of entire leaves is recommended due to the labour-intensiveness of producing strips or discs. Where incubation space is limiting, leaf-strips or discs can be used but surface sterilisation must be rigorous to minimise contamination and interference by secondary pathogens.

Germplasm Screening in Thailand

Field observations in Thailand indicate that durian cultivar Chanee is moderately resistant to infection by *P. palmivora*, while Kadoom, Kanyao and Monthong are susceptible. The four cultivars were screened in controlled experiments using the following methods:

- attached leaves, wound inoculated with mycelium/sporangia
- attached stem, wound inoculated with mycelium/sporangia
- detached fruit, wound inoculated with mycelium/sporangia
- attached unwounded root, inoculated with a sporangial suspension for five days
- measurement of zoospore production from sporangial suspension into which seedling roots were immersed (Figure 8.3.3).

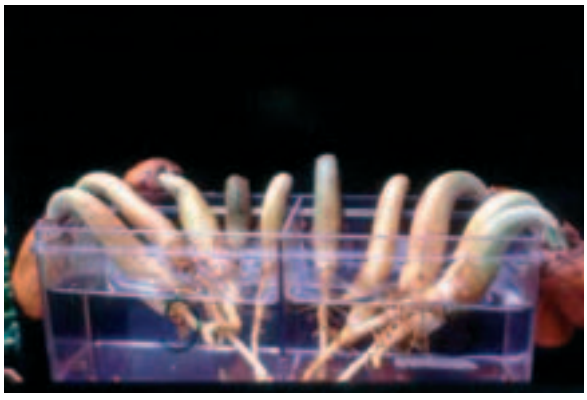

Figure 8.3.3 Germinated durian seeds immersed in a sporangial suspension.

Controls were inoculated with sterile agar or water. Percentage disease incidence was measured in leaf, stem and fruit by estimating the amount of the tissue covered by lesions. In roots, disease incidence was calculated by plating sequential segments of the roots onto selective media and calculating the number of pieces from which the pathogen grew. Additionally, colonisation of the root was assessed through examination under a dissecting microscope,

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looking for mycelium and sporangia and expressed as a percentage of the root examined.

Symptoms were similar for all cultivars in that lesions produced on leaves were dark brown, and on fruit were light brown and soft. Lesions did not develop at the point of inoculation on stems, rather the terminal part of the inoculated branch wilted and leaves abscised.

The disease incidence in the screening bioassays agrees with the field performance of cultivar Chanee. Leaf, stem, fruit and root tissues were less susceptible than Kadoom, Kanyao or Monthong (Table 8.3.1). Similarly, *P. palmivora* colonised significantly fewer Chanee roots, and produced fewer zoospores.

Germplasm Screening in Vietnam

A leaf-strip bioassay was performed on durian cultivars Chanee, D2, D6, D101, Goc Ghep, Hat Lep Dong Nai, Hat Lep Tien Giang, Kho Qua Xanh, La Queo, Monthong, Ri6, Sua Hat Lep Ben Tre and Tu Quay. The cultivars were screened against three isolates of *P. palmivora* obtained from (i) soil, (ii) stem canker and (iii) leaf in diseased orchards of Tien Giang Province. Controls were inoculated with sterile agar. A second bioassay in which leaf-strips and detached stems were screened against the soil isolate was conducted on the same cultivars, with the replacement of cultivar Goc Ghep with Kho Qua V. Controls were inoculated with sterile agar. In both bioassays, lesions were measured five days after inoculation.

The soil isolate was more virulent than either the canker or the leaf isolates, and in general the canker isolate was more virulent than the leaf isolate (Figure 8.3.4). Based on the symptoms produced by the virulent soil isolate, cultivars Tu Quy, Chanee and La Queo were less susceptible to the pathogen. The commercially popular Ri6 and Sue Hat Lep Ben Tre emerged as two of the most susceptible cultivars.

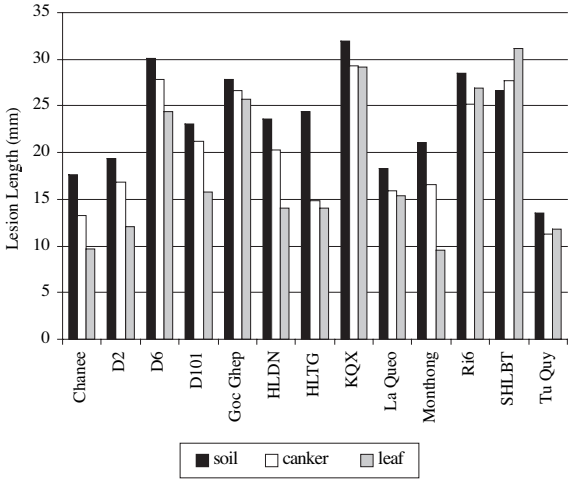

Figure 8.3.4 The length of lesions (mm) on leaf strips of durian cultivars Chanee, D2, D6, D101, Goc Ghep, Hat Lep Dong Nai (HLDN), Hat Lep Tien Giang (HLTG), Kho Qua Xanh (KQX), La Queo, Monthong, Ri6, Sua Hat Lep Ben Tre (SHLBT) and Tu Quay, five days after inoculation with isolates of *Phytophthora palmivora* from either soil, canker or leaf. Controls were inoculated with axenic agar.

Table 8.3.1 Disease incidence (%) in attached leaves, attached stems and detached fruits of durian cultivars Chanee, Kanyao, Kadoom and Monthong inoculated with *Phytophthora palmivora*, as well as disease incidence and colonisation of the roots, and zoospore production (time in minutes to zoospore release and numbers of zoospores) in 0.5 mL sporangial suspension in the presence of roots.

Durian cultivars	Field observations	Attached leaves	Attached stems	Detached fruit	Attached roots		Zoospore production	
					Incidence	Colonisation	Number of zoospores	Time to zoospores
Chanee	MR	47a	20a	20a	10a	60a	76a	45
Kanyao	S	100b	100b	100b	45b	100b	119b	30
Kadoom	S	100b	100b	100b	50b	100b	124b	15
Monthong	S	100b	100b	100b	50b	100b	190c	15

Note: means followed by the same letter are not significant difference at the 5% level by Duncan's multiple range test (DMRT); MR = moderately resistant, S = susceptible.

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In the second bioassay, pathogen growth in the detached stems was limited (Figure 8.3.5). However, taken together with the results of the first screening, results from the detached leaves indicate that Tu Quy and Chanee may be suitable for use as rootstocks, while Ri6 is inappropriate because of its susceptibility (Figure 8.3.5).

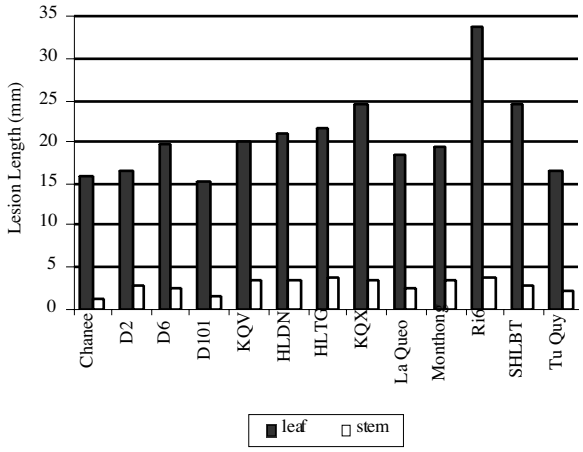

Figure 8.3.5 The length of lesions (mm) on leaf strips and detached stems of durian cultivars Chanee, D2, D6, D101, Kho Qua V (KQV), Hat Lep Dong Nai (HLDN), Hat Lep Tien Giang (HLTG), Kho Qua Xanh (KQX), La Queo, Monthong, Ri6, Sua Hat Lep Ben Tre (SHLBT) and Tu Quay, five days after inoculation with an isolate of *Phytophthora palmivora* soil. Controls, which were inoculated with axenic agar, did not develop lesions and are not shown in this graph.

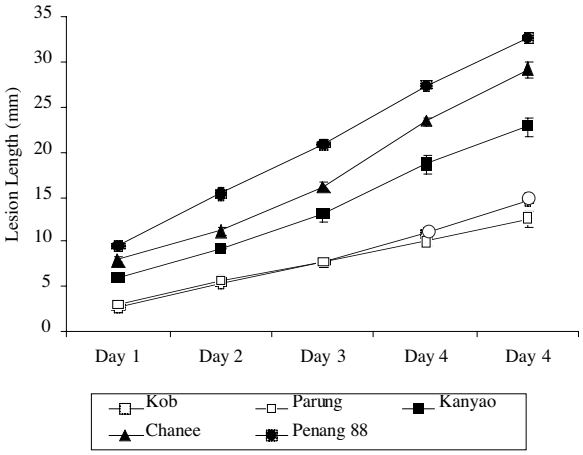

Figure 8.3.6 Mean lesion diameter (mm) in durian (*Durio zibethinus*) cultivars Chanee, Kanyao, Kob, Parung and Penang 88 in a detached-leaf bioassay. The remaining 14 cultivars screened —Chompoosee, D10, D24, D98, D102, D123, Kobyao, Kumpun, Hew 3, Kradoom, Luang, Limberlost, Red Prawn and Sunai — and *Durio macrantha* fell between Kanyao and Penang 88. Controls, which were inoculated with axenic agar, did not develop lesions and are not shown in this graph. Vertical bars are standard errors of the means.

Germplasm Screening in Australia

In summer 2000/2001 at the Centre for Wet Tropics Agriculture, *Durio macrantha* and 19 cultivars of *D. zibethinus* were screened in a detached-leaf bioassay against a locally obtained trunk-canker isolate of *P. palmivora*. The durian cultivars screened were Chanee, Chompoosee, D10, D24, D98, D102, D123, Kanyao, Kob, Kob Yao, Kumpun, Hew 3, Kradoom, Luang, Limberlost, Parung, Penang 88, Red Prawn and Sunai. Controls were inoculated with sterile agar. In autumn 2002, Chanee, D10, Kob, Hew 3 and Monthong were screened against the canker isolate and a root isolate, as well as a fruit isolate which showed low virulence in preliminary trials (Tan 1999). Controls were inoculated with sterile agar. In both bioassays, lesion extension was measured daily from two to six days after inoculation. The summer screening indicated that Kob and Parung were less susceptible to infection by *P. palmivora* than the other cultivars (Figure 8.3.6). The ranking of isolates that were screened twice was the same for the summer and autumn bioassays, from Kob, the least susceptible cultivar, to D10, the most susceptible cultivar, with Hew 3 and Chanee displaying intermediate susceptibility. In the autumn screening, the largest lesions were produced in Monthong, which is in agreement with published and anecdotal evidence stating that it is highly susceptible. The fruit isolate caused significantly smaller lesions than either the canker or root isolates (Figure 8.3.7), confirming the results of Tan (1999).

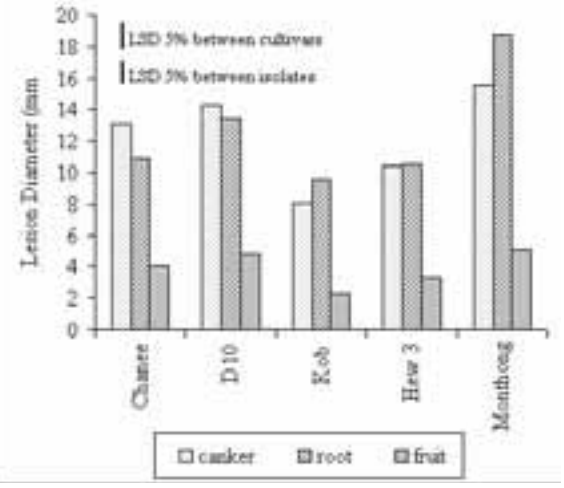

Figure 8.3.7 Mean lesion diameter (mm) in durian cultivars Chanee, D10, Kob, Hew 3 and Monthong in a detached-leaf bioassay with isolates of *Phytophthora palmivora* from either canker, root or fruit. Controls, which were inoculated with axenic agar, did not develop lesions and are not shown in this graph. Vertical lines represent the least significant difference (LSD).

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Chanee emerged as one of the most susceptible cultivars tested (Figure 8.3.6 and 8.3.7) in Australia, which contradicts the experimental evidence from Thailand and Vietnam. Cultivar D10 also developed extensive lesions indicating high susceptibility, which is in contrast with previous reports (Lim 1998b). As discussed earlier in this paper, these discrepancies could arise from pathogen differences between Australia and Thailand, or due to erroneous identification and labelling of durian germplasm imported into Australia in the 1970s and 1980s (Lim 1998a). DNA testing confirmed that the originally introduced Chanee had been misidentified on introduction to Australia (Zappala et al. 2002).

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