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Mapping a Gene of the Blackleg Fungus *(Leptosphaeria maculans)* Conferring Host Specificity on Indian Mustard

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Leptosphaeria maculans causes blackleg disease of oilseed *Brassica* crops worldwide. Indian mustard (*Brassica juncea*) varieties are generally resistant to blackleg but recently *L. maculans* isolates that cause stem cankers on *B. juncea* have been reported in Australia (Ballinger and Salisbury, 1996). These isolates are of concern as the Australian Grains Research and Development Corporation breeding program is developing Indian mustard for low rainfall areas, as this crop is better suited to such conditions than canola.

Crosses were set up between M1, a virulent blackleg isolate that attacks Indian mustard cultivars Stoke and Zaria, and C13 an avirulent isolate that cannot attack these cultivars, and resultant random ascospores and tetrads were isolated from individual pseudothecia. F_1 progeny showed 41:32 inheritance for the ability to produce cotyledonary and stem lesions on cv. Stoke which indicates the presence of a single gene ($Chi^2 = 1.65$; $0.10 < p < 0.20$). This was supported by tetrad analysis; four tetrads each had four virulent and four avirulent progeny (Chen *et al.* 1996). All isolates formed lesions on *B. napus* cv. Midas, which is not surprising as this cultivar is susceptible to all Australian isolates that have been tested.

In spite of the economic importance of blackleg disease and the amenability of this haploid fungus to genetic manipulation, little genetic analysis has been carried out on *L. maculans*. We are mapping the host specificity gene and developing a genetic map of this fungus using Amplified Fragment Length Polymorphic (AFLP) analysis, a recently developed Polymerase Chain Reaction - based strategy (Vos *et al.* 1995) which generates more markers and is more reproducible than techniques such as Random Amplified Polymorphic DNA (RAPD) analysis. We have scored more than 100 AFLP markers in 60 F_1 progeny which are polymorphic (different) between isolates M1 and C13. Six of these markers are polymorphic between bulked samples of avirulent and virulent isolates. Analysis using MAPMAKER version 3.0 (Lander *et al.* 1987) with a LOD score of 3.50 and maximum distance of 60.0 cM reveals 14 linkage groups, and 20 unlinked loci, including that of the host specificity locus.

The genetic map is being compared to the physical map of the fungus which we are determining by pulsed field gel electrophoresis. This technique is particularly useful for gene mapping, as markers can be assigned to particular chromosomes following hybridisation of the markers to Southern blots of the pulsed field gel profiles. *L. maculans* has 15 chromosomes sized between 0.7 to 3.2 million base pairs (Howlett, 1997). AFLP markers on the same linkage group as the host specificity locus will be used as probes on blots of pulsed field gels of *L. maculans* chromosomal DNA so that the chromosome containing this gene can be identified. This gene will be isolated by positional (map-based) cloning and tested for its identity as a host specificity gene by genetic complementation of isolates lacking it.

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