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Ability of a *Leptosphaeria maculans* isolate to form stem cankers on Indian mustard (*Brassica juncea*) segregates as a single locus

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Abstract

Australian isolates of the blackleg fungus *Leptosphaeria maculans*, that form cankers on two Indian mustard (*Brassica juncea*) varieties (Stoke and Zaria) are described. This ability to form cankers on var. Stoke segregates as a single locus in both F_1 and backcross progeny from a cross between two *L. maculans* isolates.

Blackleg, caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. et de Not. [anamorph = *Phoma lingam* (Tode: Fr.) Desm.], is the most serious disease of oilseed *Brassicas*, particularly canola or oilseed rape (*Brassica napus* L. and *B. campestris* L.) worldwide. This fungus consists of several strains or pathotypes that are morphologically similar but may be different species, as they do not interbreed and are distinct at a molecular level [Williams, 1992]. Two of these pathotypes are termed 'highly virulent' and 'weakly virulent' based on their ability to form stem cankers, the major cause of blackleg-associated yield loss, on canola. These pathotypes can also be discriminated by molecular characters including the number and size of chromosomes (karyotype) [Morales et al., 1993]. Within the 'highly virulent' pathotype, individual isolates can be virulent or avirulent on particular host varieties.

Indian mustard (*Brassica juncea*) varieties are generally resistant to blackleg and accordingly, this plant has been used as a source of blackleg resistance for canola. However some isolates of the 'weakly virulent' pathotype infect the pith of *B. juncea*, but do not produce stem cankers [Johnson and Lewis, 1994]. Recently *L. maculans* isolates that cause stem cankers on *B. juncea* have been reported in Canada [Kutcher et al., 1993] and in Australia [Salisbury and Ballinger, 1993]. In a collection of Australian *L. maculans* isolates from the major canola-growing regions, all were of the 'highly virulent' pathotype and 15% of them formed stem cankers on the two Indian mustard varieties (Stoke and Zaria) tested [Salisbury and Ballinger, 1993]. These isolates concern plant breeders as programs in Australia are underway to develop edible oils from Indian mustard. Canola breeders are now using additional sources of blackleg resistance other than *B. juncea* for their breeding programs.

We examined 16 isolates collected by P. Salisbury and D. Ballinger that could be classified as either virulent or avirulent, based on whether or not they caused stem cankers on *B. juncea* varieties Stoke or Zaria, when pycnidiospores were sprayed onto seedlings [Salisbury and Ballinger, 1993]. We confirmed these two groupings using a stem-prick inoculation assay (Figure 1). One group (virulent) caused lesions with a mean length ranging from 2 to 5 cm (isolates V4, M1, 18, 10, V1, A13, P2, A11, P10) and another (avirulent) caused lesions of less than 0.4 cm, often with a dark coloured spot surrounding the site of inoculation (isolates MC2, C13, GA2, MD2, G2, X2, P5) on

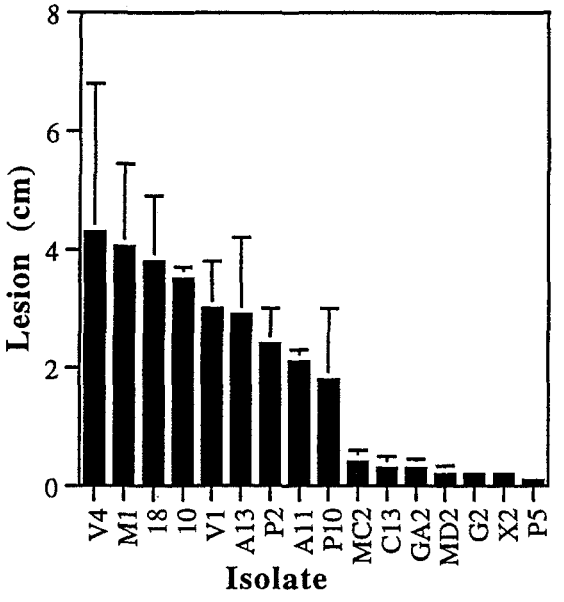

Figure 1. Length of lesions on *B. juncea* var. Stoke caused by *L. maculans* isolates. A droplet (10 μl) containing 10^4 pycnidiospores was placed onto the axil of the first leaf of seedlings at the four leaf stage. The stem was then pierced with a needle (25 gauge) through the droplet. Five weeks later symptoms were assessed. Virulent isolates caused dark coloured sunken lesions that partially girdled the stem, whilst avirulent isolates caused small necrotic spots around the site of infection [Plummer et al., 1994]. Data represent the mean external lesion length on eight plants ± standard deviation. This experiment was carried out twice.

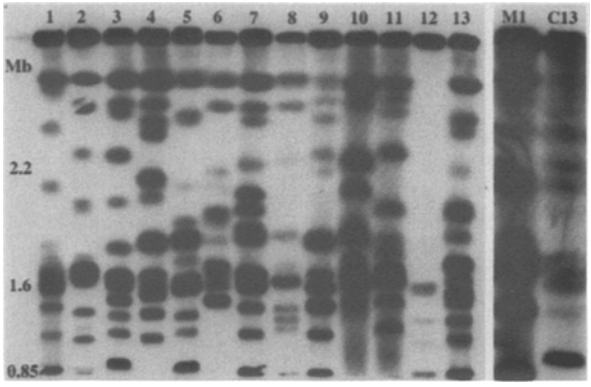

Figure 2. Southern analysis of chromosomal DNA of *L. maculans* isolates probed with the repetitive element, LMR1. Electrophoresis conditions: 0.8% agarose gel (Sigma type II); 0.5 X TBE buffer; switching interval, 500 s for 72 h followed by 420 s for 48 h; 100 V. Blots were hybridised and washed as described by Plummer and Howlett [1995]. Lanes 1 to 8 contain chromosomal DNA from isolates that are virulent on varieties Stoke and Zaria (A13, A11, 18, V1, V4, P9, P10, P2); lanes 9–13 contain chromosomal DNA from isolates avirulent on varieties Stoke and Zaria (GA2, MD2, X2, MC2, G2). Parents of progeny shown in Figure 3, isolates M1 (virulent) and C13 (avirulent), were electrophoresed in a separate experiment. All isolates contain LMR1. The sizes of several *Saccharomyces cerevisiae* chromosomal DNA bands are marked.

var. Stoke (Figure 1) and var. Zaria (data not shown). All isolates caused lesions of between 1 to 4 cm on a universal suscept of *B. napus* (var. Midas) (data not shown).

The Australian isolates we tested had about 15 resolved chromosomal DNA molecules ranging in size from about 0.6 to 3.5 Mb as shown by pulsed field gel electrophoresis and contained a repetitive DNA element LMR1, a pattern indicative of the 'highly virulent' pathotype (Figure 2) [Morales et al., 1993; Taylor and Borgmann, 1994]. Isolates that caused lesions on varieties Stoke and Zaria, and those that did not, could not be discriminated by their electrophoretic karyotype (Figure 2).

Crosses were set up, as described by Plummer and Howlett [1995], between an *L. maculans* isolate (C13, avirulent) that produced lesions on var. Stoke of less than 0.4 cm, and an isolate (M1, virulent) that produced lesions of more than 2 cm. Random ascospores and tetrads were isolated from individual pseudothecia. F_1 progeny showed 1:1 inheritance for the ability to produce stem lesions on both varieties Stoke (Figure 3A) and Zaria (data not shown). A similar segregation ratio was obtained with members of a F_1 tetrad (Figure 3B). Three tetrads, consisting of backcross progeny, also showed 1:1 segregation (Figure 4). *L. maculans* tetrads comprise four pairs of twins with each pair having a distinct electrophoretic karyotype [Plummer and Howlett, 1995]. For the four tetrads in the current study (one in Figure 3B and three in Figure 4), identity of twins was determined by pulsed field gel electrophoresis (data not shown); twins always had the same virulence phenotype on var. Stoke.

Our data suggest the presence of a single *L. maculans* locus that conditions pathogenicity on *B. juncea* var. Stoke and Zaria. As yet we cannot say if this trait is an avirulence gene in isolate C13, or a virulence gene in isolate M1. Currently we are carrying out experiments to discriminate between these two possibilities.

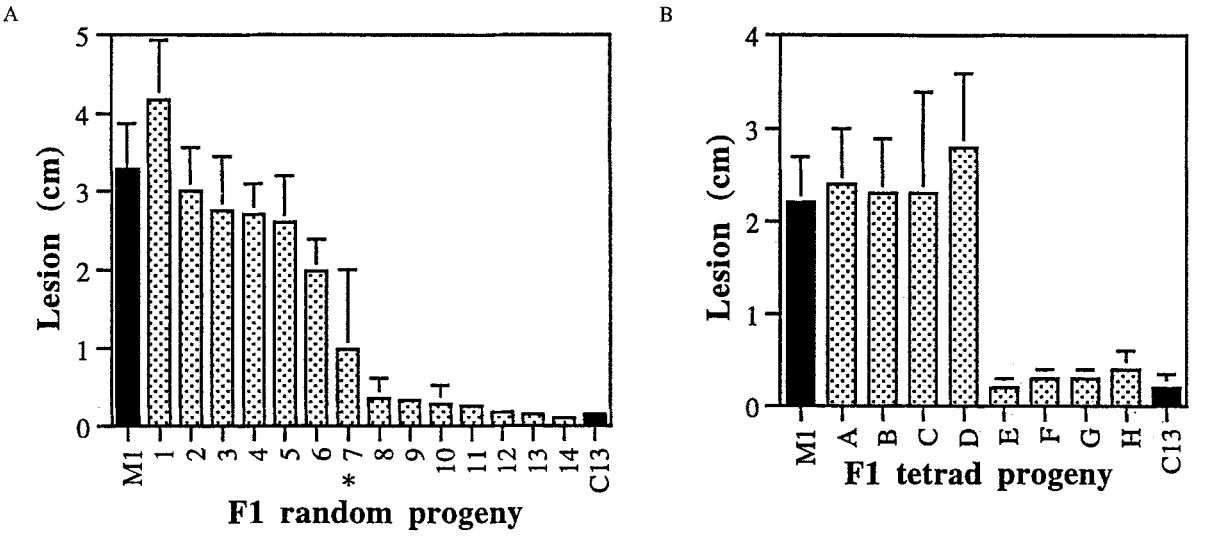

Figure 3. Length of lesions on *B. juncea* var. Stoke caused by cultures derived from random ascospores (A) and a tetrad (B) from a cross between *L. maculans* isolate C13 (avirulent) and M1 (virulent). (A) Data represent the mean lesion length on four plants ± standard deviation. * If isolate 7 is classified as virulent, there are seven virulent and seven avirulent progeny; if it is classified as avirulent, there are six virulent and eight avirulent progeny (Chi-square value (1:1) is 0.285; p = 0.75–0.5). (B) Isolates A & B; C & D; E & F; G & H are twins, as shown by pulsed field electrophoresis experiments. Data represent the mean lesion length on ten plants ± standard deviation.

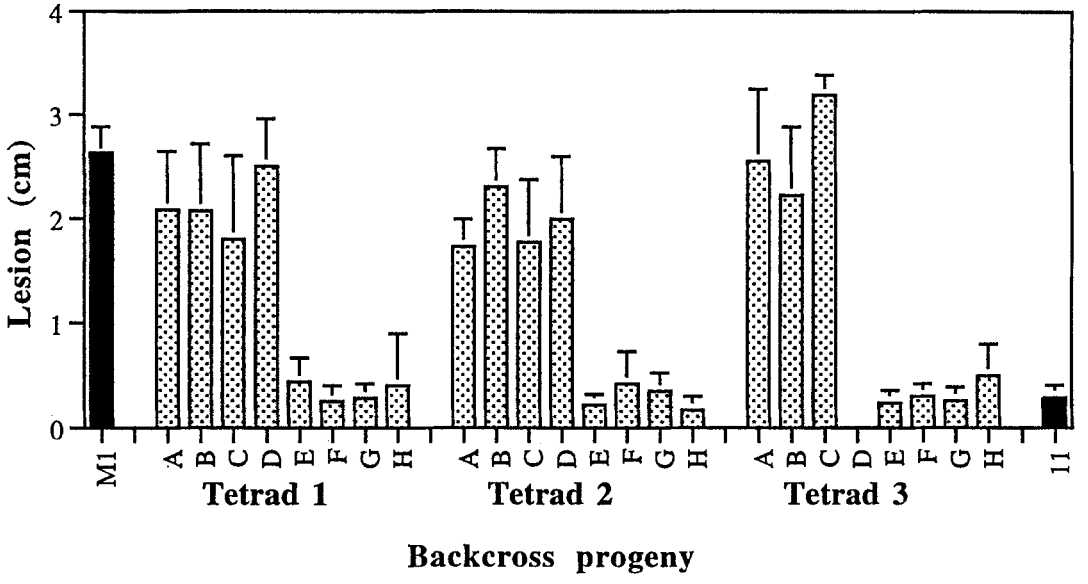

Figure 4. Length of lesions on *B. juncea* var. Stoke caused by cultures derived from three backcross tetrads from the cross between M1 (virulent) and 11 (avirulent) (see Figure 3). Isolates A & B; C & D; E & F; G & H are twins. The third tetrad is incomplete as only seven of the eight ascospores germinated. Data represent the mean of ten plants ± standard deviation.

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