The inheritance of restriction fragment length polymorphisms in the flax rust *Melampsora lini*

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Summary. Random cDNA sequences synthesized from poly A+ RNA extracted from germinated urediospores of the flax rust fungus, *Melampsora lini*, were used as probes to detect restriction fragment length polymorphisms (RFLPs) in three races of *M. lini* originating from cultivated flax, *Linum usitatissimum*, and one race originating from Australian native flax, *L. marginale*. Fourteen out of 22 probes tested detected RFLPs in the three races from cultivated flax while 19 of the probes detected polymorphisms between these three races and the race from *L. marginale*. The segregation of seven RFLPs was determined in a family of 19 F2 progeny derived from a cross between two of the rust races. With six of these the inheritance was consistent, in each case, with the segregation of alleles at a single locus. Inheritance of the seventh was unusual and an explanation involving two loci with null alleles at each was proposed. No linkage was detected between any of the RFLP loci and nine unlinked loci specifying avirulence.

Key words: Flax -- Rust -- RFLP -- Genetic segregation

Introduction

Genetic studies of phytopathogenic fungi, including rusts, are frequently directed towards understanding the extent and mechanisms involved in the generation of new virulence specificities. Genetic markers for such studies have been largely limited to an analysis of virulence specificities and more recently to isozyme variation. In rust fungi these markers have been used to study the sexual transmission of genes (Johnson 1954; Flor 1955; Wilcoxson and Paharia 1958; Luig and Watson 1961; Samborski and Dyck 1968, 1976; Haggag et al. 1973; Burdon et al. 1986), as well as somatic events, such as heterokaryosis and nuclear exchange (Flor 1964; Watson and Luig 1959; Burdon et al. 1981) and parasexual recombination (Watson and Luig 1959; Ellingboe 1961). In general, isozyme markers have shown relatively little useful genetic variation.

Restriction fragment length polymorphisms (RFLPs) present an alternative source of markers for the measurement of genetic variation in phytopathogenic fungi (Michelmore and Hulbert 1987). The inheritance of RFLP markers and the construction of linkage maps have been reported for the downy mildew of lettuce, *Bremia lactucae* (Hulbert and Michelmore 1988), and the powdery mildew of wheat, *Erysiphe graminis* (Christiansen and Giese 1990). RFLPs have been observed in both maize rust (*Puccinia sorghi*) and wheat-stem rust (*P. graminis tritici*) using cDNA sequences derived from polyA+ RNA purified from germinated urediospores, as probes to detect RFLPs (Anderson and Pryor 1991 b, c). This work demonstrated that cDNA probes showed little cross species homology and no hybridization between genera.

The present paper reports the use of cDNA probes in detecting RFLPs in three races of flax rust (*Melampsora lini* (Ehrenb) Lév) originating from cultivated flax (*Linum usitatissimum* L.) and one race originating from Australian native flax, *L. marginale* Cunn. The segregation for seven of the RFLPs amongst F2 progeny derived from a cross of two of the races from cultivated flax is also described. Since these F2 progeny were segregating for avirulence at nine unlinked loci (Lawrence et al. 1981) a test for linkage between the RFLP loci and the avirulence loci was undertaken.
Materials and methods

Rust races

Four races of *M. lini*, designated C, H, I and LMS, were used in this study. The first three originated from cultivated flax, *L. usitatissimum*, whereas LMS was isolated from the Australian native flax, *L. marginale*. The origin of races C, H and I is described by Lawrence et al. (1981). The races C and H were crossed to produce the hybrids CH4, CH5 and CH6. The hybrid, CH5, was self-fertilized to produce a family of F₂ individuals (Lawrence et al. 1981), 19 of which were used in this study. The method of crossing *M. lini*, and the maintenance of the races, is described by Lawrence (1988). All the races of *M. lini*, including the hybrids and the F₂ progeny, were individually increased on the universally susceptible flax variety, 'Hoshangabad'.

Extraction of DNA, poly A⁺ RNA and cDNA synthesis

Rust germination and collection, DNA and RNA extraction, poly A⁺ RNA purification, and cDNA synthesis, were all carried out according to the methods described for the maize rust, *P. sorghi* (Anderson and Pryor 1991a, b). The cDNA library was synthesized from 5 μg of polyA⁺ RNA extracted from race CH5 and cloned with EcoRI linkers into the bacterial plasmid vector pGEM 3Zf(+) (Promega). The total library was stored as aliquots of bacterial cells in 7% DMSO at -80°C.

Identification of RFLP loci

Gel electrophoresis of digested DNAs, blotting, hybridization and labelling of DNA probes were conducted as previously described (Anderson and Pryor 1991 b). Probes were made from the cDNA insert isolated from each clone. Genomic DNA from the various rust races was digested with the restriction endonucleases EcoRI, HindIII and BamHI (Pharmacia). Those restriction enzymes which detected RFLPs in the parent races C and H were used to digest DNA from the hybrids and F₂ progeny for segregation analysis.

Analysis of data

To compare the complexity of the hybridization patterns, the number of digests which contained one, two, three, and more

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Fig. 1a–c. Autoradiographs of the same Southern blot containing genomic DNA from races of *M. lini* hybridized sequentially to three cDNA clones: pMLc37 (a), pMLc15 (b), pMLc35A (c). Lanes 1–5 contain genomic DNA from races LMS, CH5, I, H and C respectively, cut with EcoRI, HindIII and BamHI as shown.