MOLECULAR ANALYSIS OF THE BLACKLEG FUNGUS *LEPTOSPHAERIA MACULANS*, THE CAUSAL AGENT OF BLACKLEG OF RAPESEED.

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ABSTRACT

We have pursued a molecular approach to examine *Leptosphaeria maculans* which causes blackleg of canola. Mutants of nitrate reductase, a key enzyme in the nitrate assimilation pathway have been isolated. The gene encoding this enzyme has been sequenced; this gene is the first protein-encoding gene characterised from *L. maculans*. Australian field isolates have highly variable karyotypes (chromosome complements) and novel-sized chromosomes are produced during crossing. The effect of this 'genome plasticity' on pathogenic variablity of the fungus is unknown.

INTRODUCTION

Leptosphaeria maculans causes blackleg, a serious disease of canola worldwide (Salisbury *et al.*, 1995). In spite of the economic importance of this disease, very little is known about *L. maculans* at the molecular level. In the past few years research groups in Canada, Europe and Australia have begun to apply molecular approaches to examine this fungus. In Australia we are developing genetic markers in the nitrate assimilation pathway. This pathway offers an excellent system to initiate molecular analysis of uncharacterised fungi. Mutants in genes encoding enzymes of the pathway can be easily selected and characterised. For instance, nitrate reductase mutants defective in either the structural gene encoding nitrate reductase, or in genes encoding

cofactors for this enzyme, can be selected by their chlorate-resistance. We are also studying genome organisation of this fungus using pulsed field gel electrophoresis.

EXPERIMENTAL

The gene encoding nitrate reductase has been isolated from *L. maculans* by screening a genomic DNA library with the *Aspergillus nidulans* nitrate reductase gene. This gene is the first protein-encoding gene characterised from *L. maculans*. It encodes a predicted protein of 893 amino acids (Williams et al. 1994). Mutants in nitrate reductase and molybdenum cofactor gene(s) of *L. maculans* have been isolated. Complementation of a *L. maculans* nitrate reductase mutant with the nitrate reductase gene, restored wild-type growth on nitrate as a sole nitrogen source. This gene will be useful as a chromosome-specific probe for mapping studies and the mutants will be valuable as markers for crosses.

Pulsed field electrophoresis experiments reveal that novel-sized chromosomes are produced during meiosis (Plummer and Howlett 1993). Homologous chromosomes of *L. maculans* were identified on the basis of their binding to chromosome-specific probes and changes in size of homologues in tetrads were followed during meoisis. In the progeny, novel-sized chromosomes were present that varied in size from the parental chromosomes from between 50 to 100 kb (Plummer and Howlett 1995). We propose that chromosomal length polymorphisms are produced during meiosis by parental homologous chromosomes of unequal sizes undergoing reciprocal recombination. This suggests that the genome of the fungus is 'plastic' (can readily change), and experiments are underway to see whether this genome plasticity is involved in generating pathogenic variability of the fungus.

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