

Identifying genetic diversity of avirulence genes in *Leptosphaeria maculans* using whole genome sequencing

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Abstract Next generation sequencing technology allows rapid re-sequencing of individuals, as well as the discovery of single nucleotide polymorphisms (SNPs), for genomic diversity and evolutionary analyses. By sequencing two isolates of the fungal plant pathogen *Leptosphaeria maculans*, the causal agent of blackleg disease in *Brassica* crops, we have generated a resource of over 76 million sequence reads aligned to the reference genome. We identified over 21,000 SNPs with an overall SNP frequency of one SNP every 2,065 bp. Sequence validation of a selection of these SNPs in additional isolates collected throughout Australia indicates a high degree of polymorphism in the Australian population. In preliminary phylogenetic analysis, isolates from Western Australia clustered together and those collected from *Brassica juncea* stubble were identical. These SNPs provide a novel marker resource to study the

genetic diversity of this pathogen. We demonstrate that re-sequencing provides a method of validating previously characterised SNPs and analysing differences in important genes, such as the disease related avirulence genes of *L. maculans*. Understanding the genetic characteristics of this devastating pathogen is vital in developing long-term solutions to managing blackleg disease in *Brassica* crops.

Keywords *Leptosphaeria maculans* · SNPs · Re-sequencing · Molecular markers · Blackleg disease · *Brassica*

Introduction

The most economically damaging pathogen of *Brassica* species, particularly canola (*Brassica napus*; rapeseed; oil-seed rape), is *Leptosphaeria maculans* (Howlett et al. 2001). *L. maculans*, a filamentous ascomycete, is the causal agent of phoma stem canker, commonly referred to as blackleg. In late stages of infection, it spreads through the stem vasculature causing lesions and leading to poor growth, lodging and eventually plant death. This fungus is found in canola-growing regions worldwide such as Australia, Canada and Europe. Increased production of canola in these regions has led to a rise in the severity of the disease. In Australia alone, *L. maculans* infection is responsible for an estimated Australian \$100 million in crop losses each year, with average losses ranging from 15–48 % and significant efforts are underway to improve resistance to this disease (Tollenaere et al. 2012; Kaur et al. 2009).

Understanding the characteristics of *L. maculans* is vital for developing an effective and sustainable approach to the management of blackleg disease on *Brassica* species. Given the threat imposed by this pathogen on the global rapeseed

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