

## Exploiting the genome sequence of *Leptosphaeria maculans* to minimise blackleg disease of canola

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### ABSTRACT

Control of fungal diseases relies on crop management, fungicide application and breeding disease-resistant varieties. Deployment of additional strategies requires comprehensive knowledge of both plant defence and fungal invasion mechanisms. Little is known about these processes in the interaction between canola and the blackleg fungus, *Leptosphaeria maculans*. The application of molecular technologies and genomic sequencing of *L. maculans* is generating new knowledge regarding disease mechanisms. With French colleagues, we have acquired the genome sequence of the blackleg fungus. The genome has a unique structure; it is compartmentalised into discrete alternating blocks that are either gene-rich or gene-poor. The gene-poor regions mainly comprise non-coding repetitive DNA, which accounts for almost 40 per cent of the total DNA in the genome.

These gene-poor regions have few active genes but those that are present play important roles in disease; for example, the effector avirulence genes, which are complementary to resistance genes in canola are present. Other regions of the genome are gene-rich and contain the 'housekeeping' genes, the genes necessary for an organism to survive. The location of the avirulence genes within the non-coding repetitive DNA allows the genes to be readily mutated, lost or gained. This enables the highly evolving blackleg fungus to cause disease outbreaks on canola varieties with particular resistant genes and explains the rapid breakdown of sylvestris-derived resistance on the Eyre Peninsula in 2003.

Markers for these avirulence genes have been developed and are now being used in high throughput methods for analysis of virulence of blackleg populations from stubble collected across Australia. Sexual spores (ascospores) released from stubble are trapped on tape in a Burkard liberation tunnel and then fungal DNA on the tape is PCR-analysed for virulence profiles of populations. For instance, allele profiles for two avirulence genes, *AvrLm1* and *AvrLm6*, corresponding to resistance genes *Rlm1* and *Rlm6* respectively, are consistent with the resistance gene complement of the cultivar from which stubble was derived. These findings are consistent with disease severity assessment of these cultivars. Our data support the concept of rotating different sources of resistance to minimise risk of breakdown of disease resistance and thus maximise durability of resistance genes.

**Key words:** *Leptosphaeria maculans* – blackleg – genome – avirulence genes

### INTRODUCTION

*Leptosphaeria maculans*, the casual agent of blackleg (phoma stem canker) disease, is responsible for major yield losses of canola (oilseed rape, *Brassica napus*) worldwide. The interaction between *L. maculans* and *B. napus* at the seedling stage follows Flor's 'gene for gene' hypothesis, where a resistance gene in the plant is complementary to an avirulence gene in the fungus (Ansan-Melayah *et al.*, 1995). Ten effector avirulence (*AvrLm*) genes that are involved in recognition of *L. maculans* by *Brassica* species have been identified (Balesdent *et al.* 2005; Van de Wouw *et al.* 2009) with three of them, *AvrLm1*, *AvrLm6* and *AvrLm4-7*, cloned (Gout *et al.* 2006; Fudal *et al.* 2007; Parlange *et al.* 2009). These three avirulence genes are all located in regions of the genome that are riddled with non-coding repetitive DNA. The location of these avirulence genes within the non-coding repetitive DNA allows the genes to be readily mutated, lost or gained. This enables the highly evolving blackleg fungus to cause disease outbreaks on canola varieties with particular resistant genes and explains the rapid breakdown of sylvestris-derived resistance on the Eyre Peninsula in 2003.

## RESULTS and DISCUSSION

With French colleagues, we have acquired the genome sequence of the blackleg fungus (Rouxel et al. 2011). The size of the *L. maculans* genome is 45.1 Mb, significantly larger than the closely related wheat pathogens, *Stagonospora nodorum* (36.6 Mb) and *Pyrenophora tritici-repentis* (37.8 Mb). This difference in genome size is due to the presence of non-coding repetitive DNA in the *L. maculans* genome, which accounts for 36% of the total DNA. This non-coding repetitive DNA has given the *L. maculans* genome a unique structure; it is compartmentalised into discrete alternating blocks that are either gene-rich or gene-poor. The gene-poor regions mainly comprise non-coding repetitive DNA and contain only 5% of the genes in the genome. However, those genes that are present play important roles in disease such as the avirulence genes. On the other hand, the gene-rich regions of the genome contain the remaining 95% of the genes including the 'housekeeping' genes, which are necessary for an organism to survive.

The location of disease related genes in gene-poor compartments of the genome allows targeting of these regions to look for avirulence genes. Once identified, markers for these avirulence genes can be developed and used in high throughput methods to analyse virulence of blackleg populations from stubble collected across Australia. We have developed molecular markers for two avirulence genes, *AvrLm1* and *AvrLm6*, corresponding to resistance genes *Rlm1* and *Rlm6*, respectively. Sexual spores (ascospores) released from stubble are trapped on tape in a Burkard liberation tunnel and then fungal DNA on the tape is PCR-analysed using markers for *AvrLm1* and *AvrLm6* to determine the virulence profiles of the populations.

The allele profiles for these two avirulence genes were determined for a range of fungal populations and were consistent with the resistance gene complement of the cultivar from which stubble was derived. For example, in populations collected from stubble of cultivars Garnet and 45Y77, which both contain resistance gene *Rlm1*, the frequency of *avrLm1*, the virulent allele of *AvrLm1* corresponding to *Rlm1*, was > 90%, whilst in populations from cv. Beacon and *B.junceae* Dune, which both lack *Rlm1*, the frequency of such isolates was only 43 and 35%, respectively. These data suggest that fungal populations exposed to selection from resistance gene *Rlm1* have a high frequency of virulent isolates, whilst populations not under selection pressure from this resistance gene do not. Thus selection pressure from specific resistance genes can greatly influence virulence of fungal populations and furthermore, this finding supports the concept of rotating different sources of resistance to minimise risk of breakdown of disease resistance and thus maximise durability of resistance genes.

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