Exploiting fungal genome sequences to develop novel approaches to disease control

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ABSTRACT

Molecular genetic techniques are being applied to understand strategies that *Leptosphaeria maculans, L. biglobosa 'canadensis'* and *Sclerotinia sclerotiorum* use to attack canola and juncea-canola. Our overall objectives are to develop improved strategies for disease control. The availability of complete genome sequences of these fungi is aiding in achievement of these aims.

Key Words: *Leptosphaeria maculans* - *Sclerotinia sclerotiorum* - *Brassica napus* - genome sequencing

INTRODUCTION

Control of fungal diseases usually relies on crop management, fungicide application and breeding disease-resistant varieties. Deployment of additional strategies requires comprehensive knowledge of both plant defence mechanisms and fungal disease processes. Little is known about these processes in the interaction between canola and its two major pathogens in Australia, the blackleg fungus, *Leptosphaeria maculans* and the stem rot fungus, *Sclerotinia sclerotiorum*. Recently we discovered another pathogen, *Leptosphaeria biglobosa 'canadensis'* in Australia (Van de Wouw et al. 2008). This fungus was cultured from stubble of canola-quality juncea. These two *Leptosphaeria* species form similar-sized lesions on cotyledons of canola and canola-quality juncea. However, *L. biglobosa* causes much less crown cankering than *L. maculans* and, thus less yield loss. We are analysing these three fungi in order to gain the fundamental knowledge about disease processes that underpins the development of novel anti-fungal strategies.

RESULTS AND DISCUSSION

Over the last five years we have identified several genes in the blackleg fungus that are crucial for the fungus to cause disease. These include genes involved in fungal nutrition and metabolism *in planta*, and cell wall structure (Van de Wouw et al. 2009). We have also shown that a toxin sirodesmin, is important for the fungus to cause stem cankers (Elliott et al. 2007). *Sclerotinia sclerotiorum* is a much more difficult fungus to manipulate experimentally than the blackleg fungus. Our approach has been to identify genes induced highly during infection, as these probably play an important role in disease. Such genes include multicopper oxidase, involved in iron uptake during infection, alcohol oxidase, alanine dehydrogenase and chorismate mutase, which have roles in amino acid biosynthesis. Other genes include alcohol dehydrogenase, transcriptional regulator *CipA*, and a zinc-dependent hydrolase. We are further investigating their role in disease.

The ability to exploit the molecular approaches described above is greatly aided by availability of the complete genome sequences of fungus and plant. A multinational *Brassica* genome project with a goal of producing a sequence for the A genome of *B. napus* is underway with an international consortium of researchers (http://www.brassica.info/resource/sequencing.php). This should lead to the cloning and characterisation of disease resistance genes. Complementary to this, initiatives are underway to sequence and characterise genomes of *L. maculans* (http://urgi.versailles.inra.fr/projects/lmaculans/index.php) and of *S. sclerotiorum* (http://www.broadinstitute.org/annotation/genome/sclerotinia_sclerotiorum/MultiHome.html).

Initial findings from the *L. maculans* genome initiative are that the genome is 44 Million bases (30 times smaller than that of canola) and has >10,000 genes. Surprisingly about a third of the DNA is repetitive and does not encode proteins. Genes important in disease, such as

avirulence genes, are dispersed within this repetitive DNA and three such genes have been identified by Rouxel's group (Fudal et al. 2007; Gout et al 2006; Parlange et al. 2009).

Knowledge of the genome sequence is allowing:

a) development of > 300 microsatellite markers which we are using to map fungal genes complementary to canola genes conferring resistance derived from *B.rapa* sylvestris, and a gene enabling the fungus to attack *B. juncea*. Characterisation of such loci allows understanding of how virulence of fungal populations change sunder selection pressure from extensive sowing of *Brassica* varieties with a similar source of resistance.
b) identification of genes responsible for the biosynthesis of a toxin, phomalide that causes necrosis on *B. juncea*.
c) comparative analyses with genome sequences of closely related fungi including *L. biglobosa 'canadensis''* (to identify genes involved in canker formation), and wheat pathogens *Stagonospora nodorum* and *Pyrenophora tritici repentis* (to identify genes involved specific to canola rather than wheat infection).

Knowledge of the *Sclerotinia* genome sequence has enabled us to clone a *S. sclerotiorum* glucosyltransferase that can detoxify antimicrobial phytoalexins such as brassinin in canola by adding a glucose residue to the phytoalexin. This is the first report of a fungal gene detoxifying plant defence molecules via glucosylation and indicates an important role for this enzyme during canola infection (Sexton et al. 2009).

The application of molecular technologies to these fungi is generating new knowledge regarding host recognition and disease mechanisms. The next few years should see major advances in our understanding of these important diseases. Approaches to exploit this knowledge for disease control are long term, high benefit and high risk. However, given that disease constrains canola production worldwide, and that bans on GMO canola in NSW and Victoria are lifted, we feel these strategies are justified. The genes and fungicide targets thus discovered, as well as protecting canola, also may be exploited to protect other crops against fungal diseases.

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