ACPFG

Co-evolution of the host pathogen interaction between *Leptosphaeria maculans* and *Brassica* species

GRDC

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Australian Government
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Host pathogen co-evolution

ARC Discovery Project 2009-2013:

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Aims and objectives

- **To characterise the extent of diversity of *L. maculans* resistance genes in *Brassica napus* and wild *Brassica* relatives**
- **To characterise the genomic diversity of *L. maculans* populations in relation to geographic area and agronomic practice**
- **To determine the extent of linkage disequilibrium (LD) in the *L. maculans* genome, specifically around avirulence gene clusters**
- **To characterise the specificity of the gene for gene interaction between the plant host and pathogen at the genomic level**

Methodology

- **Identify candidate resistance genes from the *Brassica* genome sequencing projects.**
- **Assess the genetic diversity of these genes by sequencing alleles from diverse *Brassica* spp. including cultivars with known and unknown resistance.**
- **Assess the allele specific expression of these genes using RTPCR to determine whether gene expression regulation is associated with resistance gene evolution.**
- **An initial genetic diversity study to identify the extent of LD in *L. maculans* and define populations for association mapping studies. Association mapping will then be applied for the identification of novel avirulence genes that have been under recent selective pressure. Alongside this, the extent of variation of the pathogen will be determined across different sites in Australia as well as in international collections.**
- **Defining the gene for gene interaction between species at the level of sequence variation.**

Resistance gene diversity

- **Amplify, clone and sequence from *B. napus* lines genes associated with isolate specific responses to *L. maculans*. Corresponding gene promoter regions will also be sequenced using redundant primers sets derived from the *Brassica* genome sequence.**
- **Analysis of these sequences will identify conservation of gene and promoter regions that may be associated with resistance in canola cultivars.**

Resistance gene expression

- **The expression of each of the disease resistance genes will be assessed using RTPCR.**
- **An initial assessment will use RNA extracted from a time course experiment of *Brassica* leaves infected with *L. maculans*.**
- **Further expression studies will include RNA extracted from different *Brassica*-*L. maculans* interactions, to assess the influence of host-pathogen genotypes on resistance gene expression.**
- **This will determine whether variation in resistance gene expression is an important mechanism in the evolution of plant disease resistance genes.**

Association mapping

- **Association mapping is an efficient approach for linking heritable phenotypic observations with underlying genome sequence variation. Through a genome wide genetic analysis of diverse populations, markers are identified that are in LD with the gene responsible for the trait, enabling rapid cloning and sequencing of the underlying the gene.**
- **The application of association mapping requires two stages, an initial study of diversity followed by a detailed LD study.**
- **An initial assessment of genetic diversity defines the population structure for a detailed LD study and association mapping.**
- **Due to the high degree of selection pressure associated with avirulence and resistance genes, LD around these genes would be high. These features make association mapping the most appropriate means to identify avirulence and resistance genes undergoing selection pressure**

Association of resistance and avirulence genes

Assessment of the interaction between isolates possessing defined avirulence genes, with *Brassica* cultivars maintaining specific resistance genes will confirm the sequence basis for the plant-pathogen gene for gene interaction

Updated plans

- **'second generation' whole genome sequencing of Brassica species and blackleg isolates and populations of interest**
- **Work closely with collaborators to link the phenotypic and genetic analysis with the genomic analysis**