Molecular studies of ascochyta blight disease in chickpea

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Ascochyta blight disease is the major fungal disease limiting chickpea production in WA. It was first detected in commercial crops in WA during 1999. The ascochyta blight fungus (*Ascochyta rabiei*) is extremely aggressive, is particularly severe during cool, wet weather, and can be devastating to most chickpea crops. Scientists from ICARDA, ICRISAT and national programs in Turkey, Syria, India, Pakistan and the USA have been breeding for disease resistance for 15-20 years. However, despite frequent introductions of resistant genotypes, ascochyta blight continues to occur worldwide in chronic epidemic cycles. The absence of durable resistance has been attributed to the appearance of new pathotypes and high levels of polymorphism for aggressiveness in pathogen populations.

Resistance to ascochyta blight is available in the wild germplasm, however, little is known about its genetic control. In cultivated chickpeas, several minor genes, buffered by a few major genes, control resistance/tolerance to ascochyta blight. Identification of these genes and the regulatory events associated with resistance/susceptibility will greatly enhance our future capacity to develop new strategies for disease control. If chickpea is to remain a viable and profitable crop in Australia, it is important that we obtain a better understanding of the mechanisms responsible for defence in resistant genotypes and how these are overcome in susceptible genotypes. This information can then be used to enhance strategies for breeding resistance.

A new project has been established to identify the genetic mechanisms responsible for susceptibility and/or resistance to ascochyta blight disease in chickpea. The overall goal is to obtain a working knowledge of the defence mechanisms that control disease resistance and how pathogens overcome these systems in susceptible plants. The aim is to identify and characterise plant genes, gene regulatory events and biochemical pathways involved in pathogenesis. The specific aims of this project are to:

- examine the pathology of the chickpea-ascochyta interaction;
- develop isogenic chickpea lines for whole genome analysis;
- develop a range of molecular resources to examine gene expression in resistant/susceptible plants;
- undertake gene expression studies using cDNA microarray technologies;
- analyse the results using legume-specific bioinformatics data mining programs.

The information generated through these studies will ultimately lead to a much better understanding of the chickpea-ascochyta interaction and secure the knowledge required to form sound hypotheses for developing robust, durable resistance strategies for control of ascochyta blight disease in chickpea.

Preparation of chickpea and ascochyta germplasm for genomic analysis

Twelve elite chickpea lines of kabuli (6) and desi (6) types with demonstrated resistance/tolerance to ascochyta blight have been obtained from the international breeding programs. Five susceptible Australian commercial varieties have also been made available. Seed from these lines has been bulked up at Murdoch University for use in screening and characterisation of phenotypic and molecular responses to infection with the ascochyta blight fungus. The results will be used to select parental lines for developing near isogenic recombinant inbred lines (RILs) for molecular and genetic dissection of the disease responses in chickpea. The goal is to generate F8:9 populations that are morphologically and genetically characterised for suceptibility and resistance to ascochyta blight disease.

A range of *A. rabiei* isolates collected in 2001 from various sites in WA have been subcultured and used to reinfect chickpea in the glasshouse. Each isolate has been re-isolated and fresh *in vitro* cultures generated from single spores. These cultures have subsequently sporulated and large numbers of spores collected for disease screening experiments. Ten isolates have been chosen for disease screening experiments, the aim being to identify two isolates that differ widely in virulence or pathogenicity. These will be used in a larger scale screening experiment of resistant and susceptible lines to characterise plant response to infection (isolate x genotype). A small project is also being carried out to see if there are any genetic differences between isolates (sequencing ITS regions, AFLP profiling).

Molecular and bioinformatics resources for analysis of global gene expression

Plant biotechnology is currently experiencing a period of rapid change. There has been a shift away from the identification and manipulation of individual genes to the global characterisation of gene expression and gene function. These new technologies are providing us with the ability to analyse the co-ordinated expression of thousands of genes in a single experiment and thus, resulting in much quicker identification and characterisation of pathogenesis-related genes and resistance pathways. We have begun to assemble the chickpea molecular resources needed to carrying out these largescale gene expression studies. Of particular importance is the construction of the cDNA libraries needed for microarray experiments. The libraries are based on resistant/susceptible chickpea lines that have been challenged with either the ascochyta blight fungus or various chemicals that are known to induce resistance response pathways. Funding permitting, the cloned cDNAs will be characterised by single pass sequencing to generate a database of expressed sequence tags (ESTs) which will be characterised for gene function and their role in resistance responses. Library redundancy will be minimised using differential screening methods and the automated robotic instrumentation available throught the Centre for High-throughput Agricultural Genetic Analysis (CHAGA), Murdoch University.

Sophisticated and powerful bioinformatics tools are being developed for largescale comparative analysis of legume genomes. These computational tools have enabled us to compare hundreds of thousands of gene sequences from many different plants (and animals) in a single experiment. On this basis, we have developed an automated bioinformatics program that uses comparative genomics to select biologically uncharacterised genes for microarray analysis. The molecular resources identified in this work are currently being assembled to demonstrate biological 'proof of concept' for this tool and we expect to achieve this goal early in 2002. Of particular importance for this work has been the availability of large numbers of sequenced clones (> 150,000) from the model legume, *Medicago truncatula*, a plant that is closely related to chickpea in the evolutionarally sense. We have recently demonstrated that genes from these two plants share high sequence similarity and fully expect to be able to exploit the growing base of *M. truncatula* resources to fast-track our studies of chickpea.

In summary, the biological and technical resources required for this project are either in place now or will become available as the project evolves. The project outputs will provide a solid foundation for future studies of the molecular basis of disease susceptibility and resistance in chickpea. These resources will also prove valuable for other chickpea gene expression studies, e.g. cold tolerance. The agricultural industry will benefit from the flow of knowledge and genetic resources into chickpea improvement breeding programs.