Expanding the repertoire of genes in chickpea that provides protection against Ascochyta blight

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SPG Contributions	Project Status	Duration/Timeline of Project (Year to Year)	Total Project Cost
$186,000.00	Completed	August 2010 – August 2013	$186,000.00

Project Description

For successful chickpea growing, control of ascochyta blight is critical, since it can reduce chickpea yield very significantly. Progress has been slow in determining the genetic basis of resistance, identification of pathogen races, and breeding of Ascochyta resistant cultivars in general.

Chickpea has very limited genetic variation on which improvements can be made. The study is needed to identify new germplasm with Ascochyta resistance and molecular markers linked to resistance genes for use in chickpea breeding programs. Improved Ascochyta resistance should be one of the highest priorities, if this crop is to be grown at a large scale in Saskatchewan and elsewhere. These varieties would reduce the need for fungicide application, thereby benefitting growers economically.

1) There is chickpea germplasm with Ascochyta resistance not yet utilized in cultivar improvement.
2) Molecular markers linked to Ascochyta resistance will be helpful for chickpea breeders in development of better varieties.

1. The initial step was to acquire new chickpea germplasm from around the world for inclusion in the Canadian gene bank, Plant Gene Resources Centre (PGRC).

2. We plan to utilize the pulse crop genomic data base being produced in a 'next generation' DNA sequencing project at PBI to find and map Ascochyta resistance genes specifically. We also need to determine the molecular interaction between host and pathogen leading to resistance. A breakthrough in pulse crop genomics, disease screening, molecular host-pathogen interaction, and new germplasm could place the Canadian research contribution at the forefront of chickpea improvement. Nine mapping populations, developed at AAFC or received from collaborators, and two populations of recombinant inbred lines (RIL), were studied. Each plant in these populations was screened for Ascochyta resistance. Ten mapping populations were genotyped with 220 simple sequence repeat markers (SSR) published previously, while one was genotyped with 500 Single Nucleotide Polymorphism (SNP) markers identified at AAFC. Mapping software was used to generate individual linkage maps which were then integrated.

3. Another population, an inter-specific population of 131 RIL from a cross between cultivated chickpea C. arietinum ICC4958 and wild chickpea C. reticulatum PI489777, was obtained from Fred Muehlbauer (United States Department of Agriculture). This population had clearly differentiated responses when assessed with three different Ascochyta isolates, using the detached leaf assay, meaning that it could be used to isolate race-specific resistance genes. This population was genotyped with 100 SSR markers, and this information was integrated with data generated in work published from other labs.

Outcome

1. An extensive survey of chickpea accessions held in various gene banks worldwide was conducted and 433 new accessions were brought to PGRC. A single seed was selected from each line, and selfed once or twice to decrease heterozygosity. For 230 of the new genotypes, detached leaflets were inoculated with one strain of Ascochyta (3279a, a Saskatchewan isolate) and the disease progress assessed. More than 70 genotypes showed some degree of disease resistance. A subset of 192 chickpea accessions was selected from the above study for mapping of loci contributing to ascochyta blight resistance. Quantitative trait loci (QTL) for various traits can be mapped in collections of diverse plant germplasm using statistical software. DNA from the selected lines was genotyped using a 1536 SNP chip (Illumina).

2. The mapping of eleven selected populations resulted in identification of 30 QTL conferring resistance to ascochyta blight. The most significant QTL mapped to linkage group (LG) 2, 4, and 6 explaining 20-38% of resistance. LG4 harbored 30% of the QRL; LG 2 and LG6 17% each. We also compiled information on 32 Ascochyta QTL identified in 16 chickpea populations from other labs and integrated all these sources of information, providing one map of resistance sources. Some QTL were shared by two or more chickpea lines due to common descent, an indication of the narrow genetic base of chickpea varieties. One line, Amit, appeared to contribute a number of regions of non-specific resistance, suggesting that this line could contribute resistance which is not specific to particular pathotypes.

3. The interspecific cross contributed further data on race-specific resistance. Integration of all the available data showed eleven QTL which interacted with one or two of the isolates. This provides evidence that a specific host pathogen interaction exists at some QTL – an important issue which has been unclear in the past. Of the 1328 mapped loci, 1064 also had gene sequence information, and 555 of these matched with 1558 genomic regions in Medicago. This allowed us to search the gene banks for annotated genes and their possible function. A major discovery was a direct match of six QTL with genes encoding serine/threonine kinase proteins involved in pathogen recognition, innate immune response, programmed cell death and/or signal transduction. Genes at the remaining QTL were associated with plant defense pathways such as thaumatin, chalcone stilbene, peroxidase and ethylene, which are known to be involved in broad range defense against pathogens. It might be possible to use the specific interaction of three Ascochyta isolates with selected lines in this RIL population to characterize other isolates. However, the genetics of resistance may be based on too many quantitative as well as qualitative genes, a phenomenon which is also encountered in other host-pathogen relationships.

Three major milestones were achieved contributing to genetic control of ascochyta blight. First, new sources of resistance to A. rabiei were identified in chickpea accessions from 15 countries. Most of these have not been utilized previously and will therefore expand the repertoire of resistance available for plant breeding. Second, a meta-analysis was used to generate an integrated linkage map with data from 10 populations developed at AAFC and 16 other populations in the literature. A total of 62 QTL for ascochyta blight resistance were consolidated into 13 meta-QTL. The map consists of 203 SSR markers shared by researchers internationally, and 268 new SNP markers developed at AAFC. Third, resistance was shown to rely both on genes encoding serine/threonine kinases receptors responsible for isolate-specific recognition, as well as genes in the thaumatin, chalcone-stilbene, peroxidase, and ethylene mediated pathways known to be involved in broad range defense against

pathogens. This was achieved by screening of a mapping population with different A. rabiei pathotypes, which allowed us to integrate data and identify gene function using the Medicago and NCBI genomic data bases. The research showed that resistance to A. rabiei is controlled both by genes for recognition of specific pathogen isolates and genes involved in common defense pathways. Consequently, we propose that resistance breeding is best achieved by combining both types of resistance in new chickpea varieties. It will be wise to expand the repertoire with the new sources of resistance identified in this project.

For chickpeas, improved resistance to ascochyta blight is one of the highest priorities. Improved resistance will reduce cost of fungicides treatment, increase yield, increase the commodity prices, improve quality to local value-added industry, and provide more reliable quantities of seed for export, as well as better seed quality. Exposure to fungicides for both the environment and humans will also be reduced.

Research Objective

OBJECTIVE 1
To obtain new chickpea germplasm with Ascochyta resistance from diverse geographical regions.

OBJECTIVE 2
To develop integrated chickpea linkage map and consolidate meta-QRL (Quantitative Resistance Loci) for Ascochyta resistance.

OBJECTIVE 3
To identify genes related to Cicer – Ascochyta rabiei recognition and general defense pathways.