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Studies on the genetic and molecular basis for clubroot resistance in canola

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Background:

In Canada, the emergence of clubroot as a canola disease has led to large-scale screening of *Brassica* germplasm for clubroot (*Plasmodiophora brassicae*) resistance by both public institutions and private industry; these efforts have led to the release of clubroot resistant (CR) canola hybrids by several companies. The resistance in these hybrids, however, is most likely based on single CR-genes, and single gene-based resistance will eventually become eroded or breakdown altogether. Indeed, the fairly complex race or pathotype structure of *P. brassicae* in Alberta suggests that regional pathogen populations could adapt rapidly in response to the selection pressure imposed by the cropping of CR canola, resulting in the loss of the effectiveness of the resistance trait. Therefore, as part of a proactive strategy for durable clubroot resistance, it will be important to: (1) pyramid or stack resistance genes in canola cultivars and (2) rotate resistance genes in clubroot infested fields. It also will be important to understand the molecular basis for resistance and, conversely, for virulence in the pathogen, in order to enable a rational approach to resistance breeding. This project was aimed at enabling the implementation of such clubroot management strategies.

Objectives:

a) Identification of major resistance genes and development of molecular markers

b) Clarification of behavior of these genes in different genetic backgrounds

c) Evaluation of the feasibility of resistance gene pyramiding and rotation

d) Identification and investigation of the biological function of host and pathogen genes differentially expressed during the infection process

What we did:

The project consisted of several related research activities. Doubled haploid (DH) populations were developed from crosses between clubroot resistant and clubroot susceptible canola genotypes. The DH populations were then evaluated for resistance to selected strains of *P. brassicae*. Molecular markers were identified to facilitate marker assisted selection. The durability of resistance in different host genotypes was assessed in greenhouse experiments using a population and single-spore isolate of the pathogen. A transcriptomics analysis was conducted to evaluate differential gene expression in some of the resistant and susceptible DH lines generated earlier. A subset of the differentially expressed genes was further examined by real-time PCR in order evaluate changes in gene expression associated with pathogenesis or host resistance. Histological and morphological comparisons of host and non-host resistance were carried out, and the role of primary and secondary zoospores in pathogenesis was investigated.

Three key results:

Phenotypic evaluation of about 200 DH lines from two crosses between a clubroot susceptible and two clubroot resistant lines indicated that a major gene is involved in the control of clubroot

resistance in these two populations. A region of chromosome A3 carrying resistance to the predominant pathotype 3 of *P. brassicae* housed 12 markers linked to the resistance introgressed from the European winter oilseed rape 'Mendel. Additional studies with a population stemming from another cross between a susceptible and a resistant parent identified a resistance locus that is linked to the clubroot resistance gene *CRa*.

Under greenhouse conditions, genetic resistance in some host genotypes was significantly eroded in as few as two cycles of exposure to the same single-spore isolate or population of the pathogen.

Primary zoospores were observed to cause secondary infection when the host already was under primary infection, suggesting that *P. brassicae* uses primary infection to overcome the basal resistance of the plant to cortical infection.

Take home message for the industry:

The molecular markers that were identified will be useful in breeding programs, including in CR gene pyramiding and map-based cloning of the genes. The information on the durability of resistance and cross-infectivity of *P. brassicae* strains highlights the need for careful resistance stewardship, in particular longer rotations out of canola in fields where clubroot is an issue. An examination of the impact of resistant cultivars on soil inoculum levels also demonstrated how even relatively small percentages of susceptible plants within a resistant crop can help to maintain inoculum levels, reducing the effectiveness of rotations. Insights obtained on the role of primary and secondary infection on pathogenesis, host and non-host resistance, and the molecular and histological changes associated with *P. brassicae* infection will facilitate efforts to better understand the mechanisms of host resistance and pathogen virulence.

Value for the industry:

The canola industry contributes an average of $15.4 billion per year to the Canadian economy, including $8.9 billion in farm cash receipts alone. Clubroot represents a very significant threat to this industry, as a consequence of potential yield and quality losses and the costs associated with disease management, including forced rotations out of canola and efforts to exclude the pathogen from non-infested fields and regions. Hard numbers are lacking with respect to the total dollar value for losses associated with clubroot in Alberta, but working on the (conservative) assumption that approximately one-quarter of the traditional canola growing area in this province is at risk for the disease, and using a moderate estimate of 25% yield losses, then 25% of one-quarter of the provincial canola cash receipts could be lost. As such, research to understand and manage the disease is an important investment in mitigating the impact of clubroot. The financial support received for this project would represent approximately a 150-fold return on investment.

Value to the team:

In addition to the deliverables achieved and information obtained, this project played a very important role in the training of 16 highly qualified personnel at the University of Alberta, Alberta Agriculture and Forestry, and the Plant Biotechnology Institute, National Research Council of Canada. These included 2 research associates, 2 post-doctoral fellows, 4 technicians, 4 graduate students, and 4 summer students. Personnel received training in the areas of genetics, host-pathogen interactions, plant breeding, plant pathology, agricultural biotechnology, experimental design and statistical analysis.