Characterization of New Strains of the Clubroot Pathogen in Alberta

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Clubroot, caused by *Plasmodiophora brassicae*, was first identified in western Canada in 2003, spreading to more than 2700 fields. Researchers conducted a five-year project to identify, characterize, and better understand strains of the clubroot pathogen that are able to overcome clubroot resistance, such as the new pathotype 5X. Overall, the results of this study have improved the understanding of the new strains of *P. brassicae* that have emerged in western Canada in recent years, and have resulted in the development of improved practices and tools for their identification and management. The results also make it clear that an integrated approach, combining other tools in addition to genetic resistance, will be needed for sustainable clubroot control.

Clubroot, caused by *Plasmodiophora brassicae*, was first identified in western Canada in 2003, with more than 2700 clubroot-infested fields now confirmed. While the outbreak is concentrated mainly in central Alberta, clubroot has continued to spread throughout the province, with isolated cases of the disease also found in Saskatchewan, Manitoba and North Dakota. Although the disease is managed primarily by the planting of clubroot resistant (CR) canola varieties, unfortunately, new strains of *P. brassicae*, such as pathotype 5X, have emerged in Alberta and appear to be highly virulent to all canola cultivars currently on the market.

Researchers in Alberta initiated a five-year project in 2013 to identify, characterize, and better understand strains of the clubroot pathogen that are able to overcome clubroot resistance. The objectives of the project were to: (1) monitor spread of this novel clubroot strain through surveys, (2) assess the potential of resistance-defeating pathotypes to reappear in further outbreaks, (3) characterize the pathotypes of *P. brassicae* present where resistance had broken down, (4) multiply inoculum of resistance-defeating pathotype(s) for screening in containers and or securely contained field facilities, and (5) search for molecular markers for novel clubroot pathotypes.

From 2014-2017, populations of *P. brassicae* representing 151 fields in Alberta were collected from galled roots of clubroot resistant (CR) canola plants and characterized for virulence on seven CR canola cultivars. One-hundred and one of these populations could overcome resistance in at least one CR cultivar and were evaluated further by inoculation on 13 Brassica hosts termed the Canadian Clubroot Differential (CCD) Set. The CCD Set included the differentials of Williams and Somé et al., selected hosts of the European
Clubroot Differential Set, and the *B. napus* cultivars ‘Brutor’, ‘Mendel’, ‘Westar’ and ‘45H29’. Each unique virulence pattern on the CCD Set represented a distinct pathotype and was identified with a letter. Five reference isolates, obtained prior to the introduction of CR canola, also were assessed.

A total of 17 pathotypes were detected using the CCD Set, compared with five pathotypes using the system of Williams and two with the system of Somé et al., suggesting that the CCD Set has a greater differentiating capacity. Pathotype A, a variant of pathotype 3 (as per Williams), which is able to overcome the resistance in CR *B. napus*, was predominant. The original pathotype 3, which is avirulent on CR canola, was classified as CCD pathotype H.

In addition to testing of the virulence phenotypes, restriction site-associated DNA sequencing (RADseq) was used to examine the genetic diversity of over 8750 variants within *P. brassicae* single-spore and field isolates collected from across Canada. The isolates included individuals that were either capable or incapable of causing disease on clubroot resistant canola cultivars. Population analysis indicated that most isolates belonged to one of two distinct populations of *P. brassicae* in Canada, suggesting multiple introductions of the pathogen into the country. The identification of this genetic variation will be important for future research and monitoring of the pathogen, including development and validation of additional markers specific for the new pathotypes. Populations of *P. brassicae* representing the key pathotypes have been made available to private and public breeders (subject to appropriate biosafety considerations) for screening purposes, in order to assist with the identification of effective resistance sources and the development of new CR canola products.

Finally, a targeted approach was taken to develop a molecular marker to identify pathotype 5X, which was the first of the resistance-breaking pathotypes of *P. brassicae* to be identified in Canada. In addition, PCR and qPCR assays developed as part of this project represent useful tools for the rapid and reliable diagnosis and quantification of new pathotype 5-like strains of *P. brassicae*. The molecular marker for pathotype 5X will facilitate screening of larger numbers of samples for the presence of this pathotype, and together with the genomic information could serve as the basis for development of additional markers for new pathotypes identified in this study and in the future.

Overall, the results of this study have improved the understanding of the new strains of *P. brassicae* that have emerged in western Canada in recent years, and have resulted in the development of improved practices and tools for their identification and management. The CCD Set, which is an important new tool for agronomists, breeders and researchers, will serve as an effective method to identify novel pathotypes and quickly determine their ability to overcome certain key sources of resistance. The results also make it clear that an integrated approach, combining other tools in addition to genetic resistance, will be needed for sustainable clubroot control.
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