

Identifying Virulence Factors in *Leptosphaeria maculans*, the Cause of Blackleg Disease of Canola

Hossein Borhan, Agriculture and Agri-Food Canada, Saskatoon SK

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*Researchers in Saskatchewan conducted a three-year project to identify virulence genes in the *Leptosphaeria maculans* genome, the pathogen that causes blackleg in canola. The main priority of this research was to develop tools (markers) for the known virulence (*avr*) genes as they are the targets of major blackleg R genes currently available in commercial canola cultivars, and to monitor pathogen populations in the field. As a result of the project, researchers produced a catalogue of LM virulence genes expressed during infection and successfully developed markers for LM race determination and species differentiation.*

Blackleg is a major disease of canola in Canada and world-wide. Although breeders have been successful in developing canola varieties with resistance to blackleg disease by incorporating resistance (R) genes into commercial varieties; these resistance sources have been overcome by the emergence of new races of the pathogen. Emergence of new virulent races is due to mutation(s) in the pathogen effectors. Effectors are small proteins and molecules injected by the pathogen into plant to overcome plant immunity.

Researchers in Saskatchewan conducted a three-year project to identify effector (virulence) genes in the *Leptosphaeria maculans* (*Lm*) genome. The main objective of this research was to develop tools (markers) for the known virulence (*avr*) genes as they are the targets of major blackleg R genes currently available in commercial canola cultivars.

Researchers developed a pipeline to identify effector genes from the genome of *Lm* isolate 00-100, previously sequenced by AAFC, and produced a catalogue of *Lm* effectors that were present during infection of canola. Using genomic tools, researchers initially identified 668 *Lm* effectors. Additional criteria such as high level of expression during infection, predicted virulence function and similarity to virulence genes reported from other pathogens were applied and a final list that consisted of 80 *Lm Avr* genes were determined.

The final objective of the project was to develop molecular markers to monitor the pathogen population in the field and to identify new emerging isolates. Researchers developed and tested markers for known *Avr* genes, and generated over 61,000 SNP markers across *Lm* genome. The majority of the named *L. maculans Avr* genes have been cloned, either through work of other research groups or by the researchers themselves.

The researchers have also developed a set of KASP markers for all of the cloned *Avr* genes. Additional markers will be developed as information about the remaining known *Avr* genes become publicly available.

Marker accuracy was tested on a set of more than 100 well-characterised *Leptosphaeria* isolates from around the world, with the markers proving to be extremely accurate at predicting the pathotype of each isolate. In addition to developing markers for race determination of *L. maculans*, the researchers also developed KASP markers that could differentiate *L. maculans* from *L. biglobosa*, which is less virulent on canola in Canada and reported to be the only species present on rapeseed in China. Therefore the use of species-specific KASP markers could have a significant application in monitoring the export of canola seeds for potential contamination with blackleg. The blackleg race testing system has now been rolled out to commercial testing labs across western Canada, so that producers can have samples collected from their own fields tested and use the information to select a canola variety with appropriate resistance for their farm.

Scientific publications.

- Ghanbarnia K, Ma L, Larkan NJ, Haddadi I, Fernando WGD, Borhan MH. 2018. *Leptosphaeria maculans* AvrLm9: a new player in the game of hide and seek with AvrLm4-7. *Mol Plant Pathol*. doi: 10.1111/mpp.12658.
- Parham Haddadi, Lisong Ma, Haiyan Wang, and M. Hossein Borhan. 2016. Genome-wide transcriptomic analyses provide insights into the lifestyle transition and effector repertoire of *Leptosphaeria maculans* during the colonization of *Brassica napus* seedlings. *Molecular Plant Pathology* 17 (8), 1196-1210.
- Kaveh Ghanbarnia, Isabelle Fudal, Nicholas J. Larkan, Matthew G. Links, Marie-Hélène Balesdent, Bronislava Profotova, W.G. Dilantha Fernando, Thierry Rouxel, and M. Hossein Borhan. 2015. Rapid identification of the *Leptosphaeria maculans* avirulence gene *AvrLm2* using an intraspecific comparative genomics approach. *Molecular Plant Pathology* 16 (7), 699-709.