Researchers conducted a three-year study focusing on four key components using multiple commercial canola varieties to reveal the mechanism of race-specific and non-specific blackleg resistance. The overall findings showed that many R-rated canola cultivars carry a level of quantitative resistance (QR) or race nonspecific resistance to blackleg in Canada. This study also identified for the first time the molecular mechanism associated with QR against blackleg of canola. These resistance resources can be valuable to blackleg management in Canada due to different modes of action as opposed to major-gene resistance. The information from the study will be shared with all stakeholders, including growers, breeding companies and agronomists, to show the value of race nonspecific resistance against blackleg in western Canada.

Most canola cultivars grown in western Canada carry the major resistance (R) gene Rlm1 and/or Rlm3, while the current pathogen population of Leptosphaeria maculans generally lacks the corresponding avirulence genes AvrLm1 and disrupted effect of AvrLm3, indicating that these R genes are no longer effective. Despite the situation, severe blackleg occurs only on a relatively small percentage of canola fields (<10% annually). Researchers at Agriculture and Agri-Food Canada in Saskatoon, SK conducted a three-year study using multiple commercial canola varieties to reveal the mechanism of for race-specific and non-specific blackleg resistance. There were four key components/objectives to the study.

1. Characterizing blackleg resistance associated with common canola cultivars used in western Canada.

Eight R-rated (blackleg resistant) common cultivars carrying Rlm1 and/or Rlm3 were evaluated for nonspecific blackleg resistance by inoculation with virulent isolates of L. maculans (without the corresponding AvrLm1 and AvrLm3). “Westar” was used as a susceptible control. Three of the varieties were assessed in more detail based on the infection and spread of fungal hyphae in inoculated cotyledons using a 0-9 scale and fluorescence microscopy. The amount of L. maculans DNA in the petioles and stems linked to hyphal spread and growth was quantified using droplet digital PCR (ddPCR) at 14 days post inoculation (dpi).

The study showed that most of the R-rated canola cultivars also have a level of race nonspecific resistance not directly involving R gene-Avr gene interaction. The results indicate that quantitative resistance (QR) plays a role for these R-rated canola cultivars by reducing the spread of fungal hyphae from infected cotyledons into stems (lower disease
incidence) and/or limiting the damage to the stem after the pathogen gets in there (lower disease severity). QR was confirmed with cotyledon inoculation of several canola cultivars using multiple virulent *L. maculans* isolates.

Fig 1 Stem infection by *L. maculans* (blackleg) via leaf petiole inoculation on R-rated Canadian canola cultivars (CCC) vs. susceptible control (Westar). There was no involvement of R gene-Avr interaction in these resistant reactions. Arrows show the scars of fallen inoculated leaves.

2. Understanding the molecular mechanisms of the resistance gene *Rlm1* based on RNA sequencing.

Although blackleg resistance has been widely studied through genetics, molecular mechanisms underlying the host–pathogen interaction remain largely unknown. In this study, transcriptome analysis was carried out using a double haploid (DH) *B. napus* line carrying the resistance (R) gene *Rlm1* inoculated with a virulent or avirulent isolate of *L. maculans* on cotyledons.

The study results showed that the resistance mediated by *Rlm1* in response to *L. maculans* carrying *AvrLm1* is a localized defense response and cannot be translocated to other parts of the plant. The comparative study of transcriptome provided a repertoire of candidate genes involved in the regulatory networks for blackleg resistance mediated by the R gene *Rlm1*, and contributed to a better understanding the molecular basis for blackleg resistance by a specific R gene. The study also indicated that major-gene resistance, as shown by
Rlm1 and LepR1, may have quite different molecular mechanisms, including up-regulation of genes involved in jasmonic acid and salicylic acid pathways.

Although major R genes like Rlm1 can completely halt blackleg infection at the infection site, the resistance can be overcome rapidly by shifts in the pathogen population. Although the mechanisms underlying quantitative resistance (QR) are unknown, QR is of interest against blackleg, especially in western Canada where the crop season is much shorter than many canola-growing regions in the world. To identify genes and gene functions, researchers used RNA-Seq on infected cotyledons of “74-44 BL” a Canadian cultivar with QR against a range of L. maculans isolates.

This study identified for the first time the molecular mechanism associated with QR against blackleg of canola. It is substantially different from those found with major R genes, including Rlm1 and LepR1. The study found that many genes showed differential expression in inoculated 74-44 BL relative to inoculated Westar, and it appears that the QR is through increased programmed cell death (PCD) as a way of limiting the biotrophic growth of L. maculans in the cotyledons of 74-44 BL. The QR mechanisms for PV 530 G and 45H29 are also being studied, and these cultivars are used to represent Canadian canola cultivars in this study.

4. The quantitative resistance (QR) against blackleg remains effective under high-temperature conditions.
QR, also known as adult-plant or race nonspecific resistance, has the potential to provide a more durable, if less complete, protection of canola against blackleg. However, the effectiveness of QR may also vary widely in the field, and it has long been suspected that elevated temperatures, such as heat waves during the prairie summers, may negatively affect the expression of QR. To test the impact of high temperatures, blackleg infection was assessed on three common canola cultivars with QR (74-44 BL, PV 530 G and 45H29), with and without the treatment of 7-h daily exposure to 32°C for one week during early plant flowering under controlled-environment conditions. This treatment was compared with that under a moderate temperature at 22°C day-time high. Westar was used as a control.

The study showed that QR against blackleg remains effective under high-temperature conditions, with little impact of elevated temperature observed. Therefore, QR traits of common canola cultivars would likely be stable under a wide range of field temperatures during a crop season in western Canada.

The overall findings from the four components of the study showed that many R-rated canola cultivars carry a level of race nonspecific resistance to blackleg in Canada. There was also no effect of elevated temperature on the performance of nonspecific resistance to blackleg during plant flowering stages. These resistance resources can be valuable to blackleg management in Canada due to different modes of action as opposed to major-gene resistance. The information from the study will be shared with all stakeholders, including
growers, breeding companies and agronomists, to show the value of race nonspecific resistance against blackleg in western Canada. There is a need and opportunity to screen and identify nonspecific resistance sources efficiently in canola germplasm/breeding lines for introducing good background resistance into canola hybrids.

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