Canola Verticillium Stripe Disease: Pathogenicity Variations, Coexistence with *Leptosphaeria maculans*, and Transcriptome Profile at Adult Plant Stage

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INTRODUCTION

- Canola (*Brassica napus*, Canadian oil, low-acid or oilseed rape, 2n = 38) is an integral crop for the Canadian economy, contributing $43.7 billion annually.[1]
- Canola was bred from *Brassica* species, including *Brassica rapa L.* (AA, 2n = 20) and *Brassica oleracea L.* (CC, 2n = 18) to create a product lower in erucic acid and glucosinolates.[2, 3]
- Canola is used as food oil, biofuel, cosmetics, and animal feed.[2]
- Canola production is threatened worldwide by fungal pathogens such as *Verticillium longisporum* and *Leptosphaeria maculans*, which cause Verticillium stripe and blackleg disease, respectively.[4]
- *V. longisporum* causes stunting, chlorosis, stem discoloration, premature senescence, and reduced oil content and grain size, whereas *L. maculans* causes lesions, stem cankers, and plant lodging.[4]
- *V. longisporum* was discovered in Manitoba, Canada, in 2014 and is now prevalent across Manitoba, Alberta, Saskatchewan, Ontario, and Quebec.[1]
- V. *longisporum* is derived from hybridization into three distinct lineages, A1/D1, A1/D2 and A1/D3, all of which infect Brassica species with the most aggressive being A1/D1.[3,5]
- *V. longisporum* is devastating to agriculture because there are currently no resistant cultivars, and the depth of its Canadian population structure and diversity, pathogenicity, and infection process remain largely unknown.
- *L. maculans* was discovered in Canada in the 1990s and has since become widespread.[6]
- Despite resistant cultivars being available, *L. maculans* can coexist in the plant with *V. longisporum*, exacerbating both diseases.
- *L. maculans* contains avirulence (*Avr*) genes that enable infection unless they interact with resistance (*Rlm*) genes in canola.[6]
- *L. maculans* capability to coexist with *V. longisporum* remains undiscovered.
- Since *L. maculans* infections depend on the the *Avr* genes present in a strain, knowledge regarding genotype synergism with *V. longisporum* is essential for disease resistance breeding.

OBJECTIVES

1. To characterize *V. longisporum* populations from canola-growing regions in the Canadian prairies and evaluate their pathogenicity on 'Surpass 400' (resistant) and 'Westar' (susceptible) varieties.
2. To compare transcriptome profiles of differing *V. longisporum* strains in *B. napus* at the adult plant stage.
3. To characterize *L. maculans* from *V. longisporum*-infected canola in the Canadian Prairies and identify their *Avr* genes through PCR and cotyledon inoculation in *B. napus* differentials.

DISEASE BACKGROUND

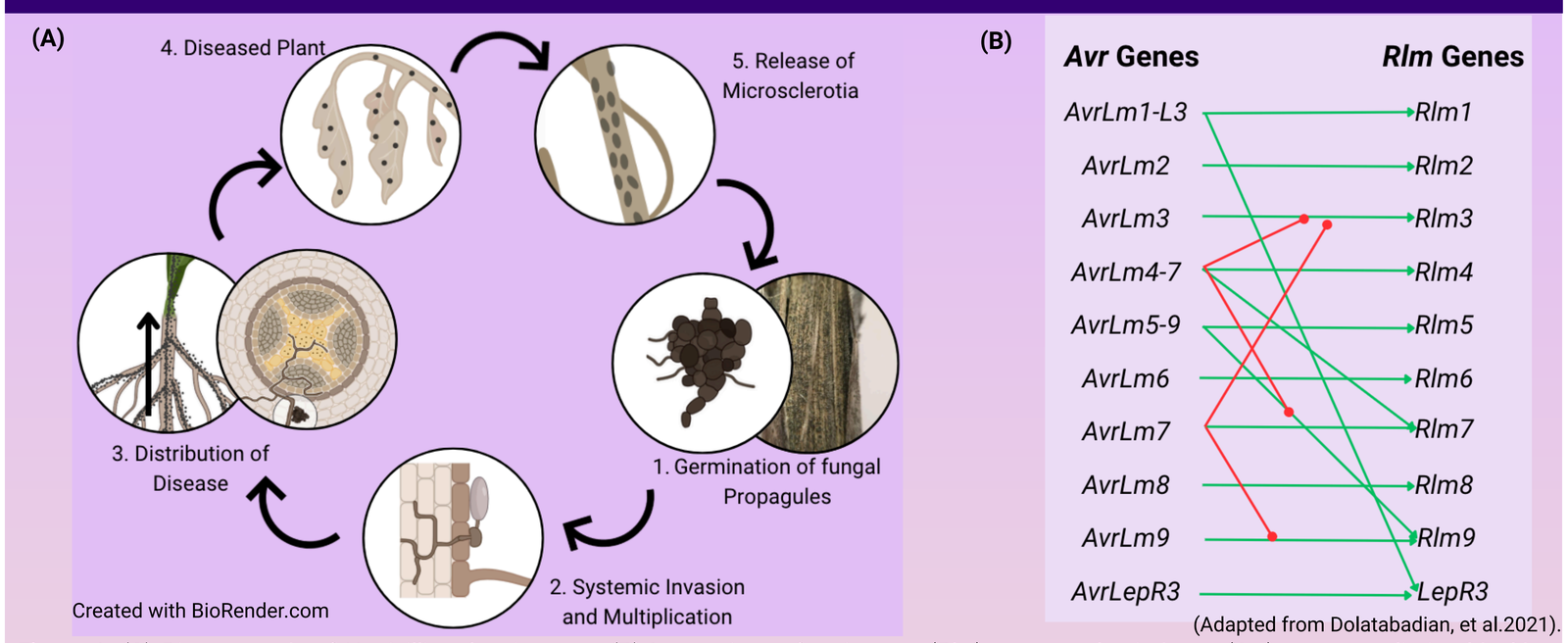
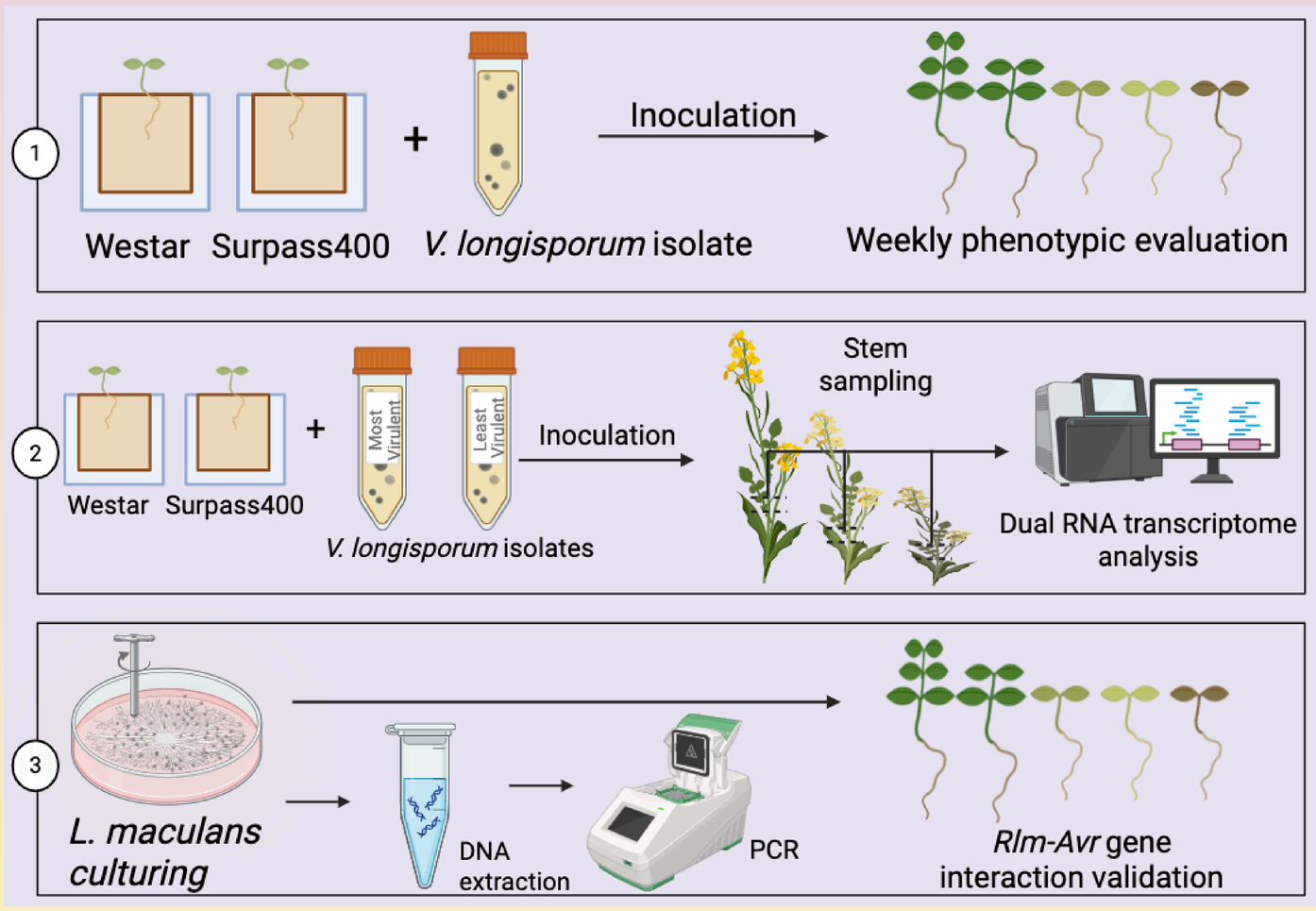


Figure 1. (A) Disease cycle of *Verticillium longisporum*. **(B)** Interactions of resistance (*Rlm*) genes and avirulence (*Avr*) genes mediating blackleg resistance in canola. Green lines exhibit gene-for-gene interaction. The red oval ended lines indicate *Avr* genes that mask other interactions

MATERIALS AND METHODS



- The first phase will involve growing 'Westar' and 'Surpass 400' canola varieties in germination pouches
- After inoculating both varieties with 72 isolates of *V. longisporum* collected from Manitoba, Saskatchewan, Alberta, and Ontario, disease severity will be recorded for the five weeks post-inoculation using a 1-9 scale.[7]
- After the first phase, the least and most virulent strains will be selected for the second phase.
- In the second phase, 'Westar' and 'Surpass400' canola will be grown and inoculated at two weeks with the selected *V. longisporum* isolates.
- Canola stems will then be sampled at different levels of disease severity to perform dual RNA-transcriptome analysis.
- In the final phase, canola stems, collected from *V. longisporum*-infected fields, are plated on V8 media, where *L. maculans* pycnidia may be present.
- The visible pycnidia will be isolated on V8 plates, and DNA from these isolates will be used for PCR amplification of selected *Avr* genes.
- Subsequently, canola cultivars with specified *Rlm* genes will be inoculated with those *L. maculans* isolates to confirm *Avr-Rlm* interactions.

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EXPECTED OUTCOMES

- The pathogenicity of predominant *V. longisporum* isolates across western Canada is more diverse.
- High-virulence A1/D1 *V. longisporum* isolates cause earlier, more extensive colonization and xylem infection than low-virulence isolates, especially in susceptible 'Westar' compared to resistant 'Surpass 400'.
- Specific *Avr* gene profiles of *L. maculans* can interact with *V. longisporum* in *B. napus*.
- Understanding the genetic mechanisms of *V. longisporum* disease progression can highlight genes or processes of interest for further research toward resistance breeding.

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ACKNOWLEDGEMENT

Canola Agronomic Research Program
ALBERTA CANOLA
NSERC CRSNG
INNOVATION Canada Foundation for Innovation / Fondation canadienne pour l'innovation
Manitoba Canola Growers
LAURIER WILFRID LAURIER UNIVERSITY
SASK OILSEEDS
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