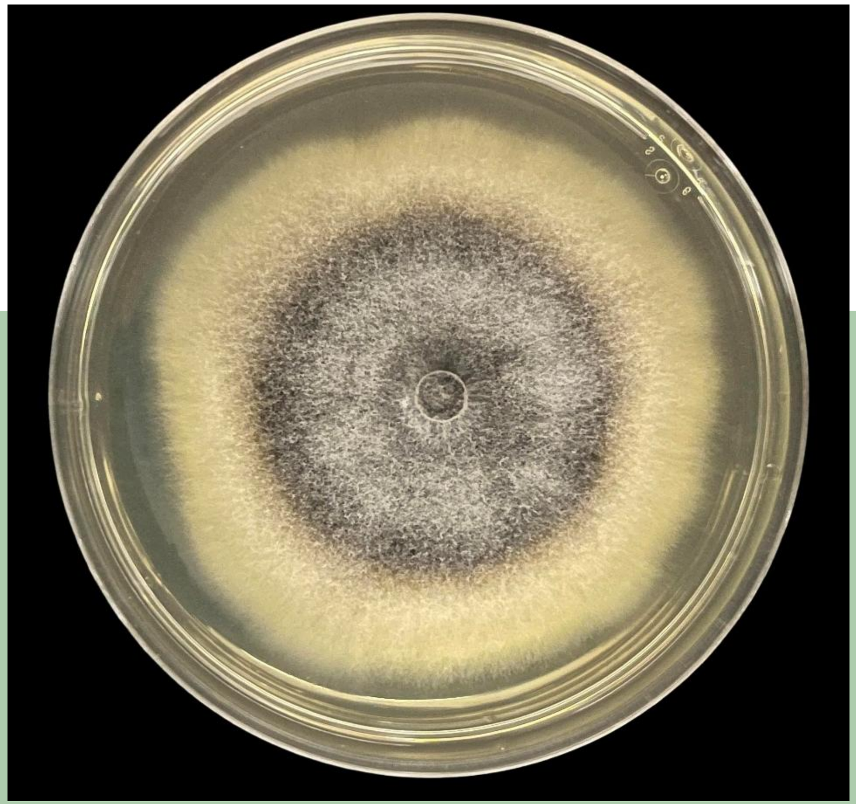
Double Trouble in Canola:

Understanding the interaction between *Verticillium longisporum* and *Leptosphaeria maculans*




Vidushika Madduma Bandara, **MSc student**

Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada, N2L 3C5

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Background

- Canola (*Brassica napus*) is one of the most commercially important oilseed crops in the world.

- Canada accounts for the world's largest canola production.

- The total economic impact of the Canadian canola sector for the average of the three years, 2020/21-2022/23 averaged C$ 43.7 billion per year (GlobalData, 2024).

- **Increasing stresses from fungal diseases are threatening the canola yield and quality.**

Blackleg disease

- **Causal agent:** Primarily by *Leptosphaeria maculans*

Healthy Diseased

Healthy Diseased

- **Yield losses:** 30-50% due to severe epidemics

(Wang et al., *Plants*, 2023)

- **Disease management:** Deployment of blackleg-resistant canola cultivars, diversified cropping systems, fungicidal treatment, and monitoring the race dynamics of *L. maculans*

Verticillium stripe disease

- **Causal agent:** *Verticillium longisporum*

Healthy Diseased

Healthy Diseased

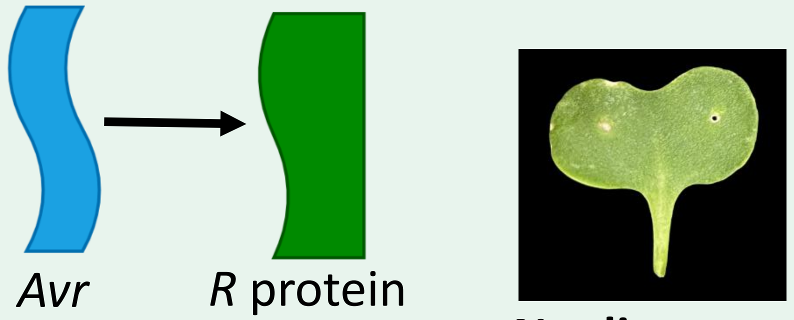
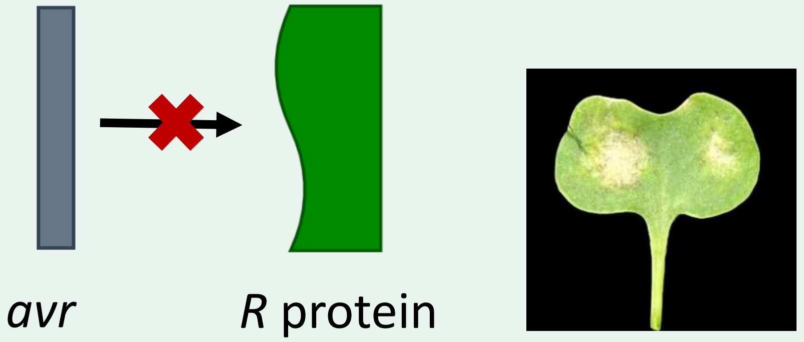
- **Yield losses:** between 10-50%

(Rimmer et al., *Compendium of Brassica Diseases*, 2007)

- **Disease management:** Incorporation of biosecurity measures and diversified cropping systems

No commercial canola varieties in Canada have been registered as resistant to Verticillium stripe!

Resistance *(R)* genes in *B. napus*

Pathogen genotype	Host plant genotype	
	R	*r*
Avr **(Avirulent gene)**	 *Avr* *R* protein **No disease** Incompatible interaction	 *Avr* *r* protein **Disease** Compatible interaction
avr **(Virulent gene)**	 *avr* *R* protein **Disease** Compatible interaction	 *avr* *r* protein **Disease** Compatible interaction

- Qualitative resistance is controlled by single dominant *R* genes in *B. napus.*
- Involves a gene-for-gene interaction.

Research questions

- Verticillium stripe can co-exist with blackleg.
- Co-inoculation of *V. longisporum* and *L. maculans* increased blackleg severity and yield losses in canola under both field and greenhouse conditions (Wang et al., *Plants*, 2023).

1. Does the presence of Verticillium stripe in canola break down the blackleg disease resistance determined by major *R* genes?
2. Are there any key genes in *B. napus* that are involved in the response to both fungal diseases and their interactions?

Objectives

1. Investigate how *R* genes in canola and *Avr* genes in *L. maculans* interact with *V. longisporum*

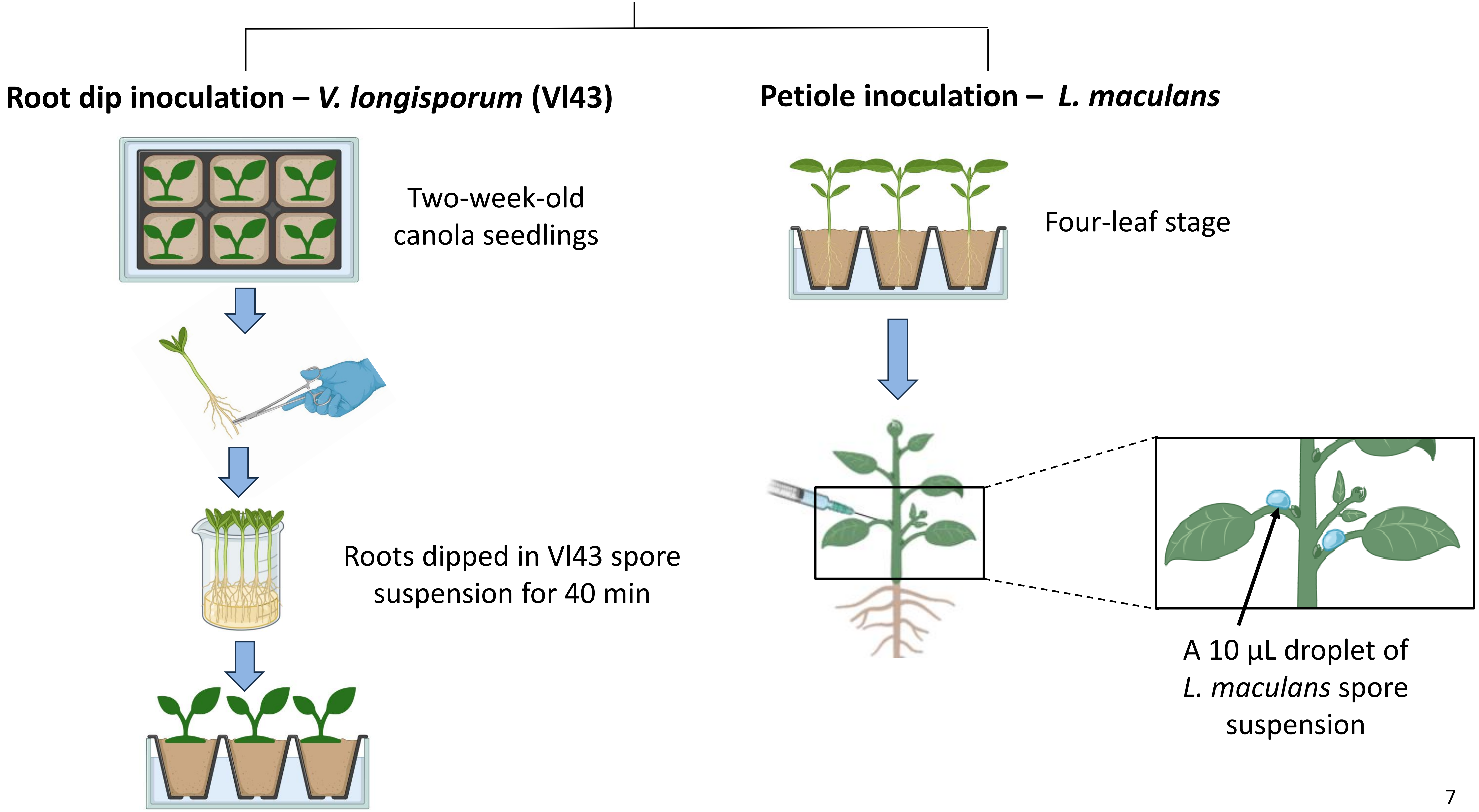
2. Understand the transcriptome changes in *B. napus* due to the interaction of *R* and *Avr* genes with the *V. longisporum* pathogen.

Materials and Methods

Table 1: *Brassica napus* genotypes used in the study

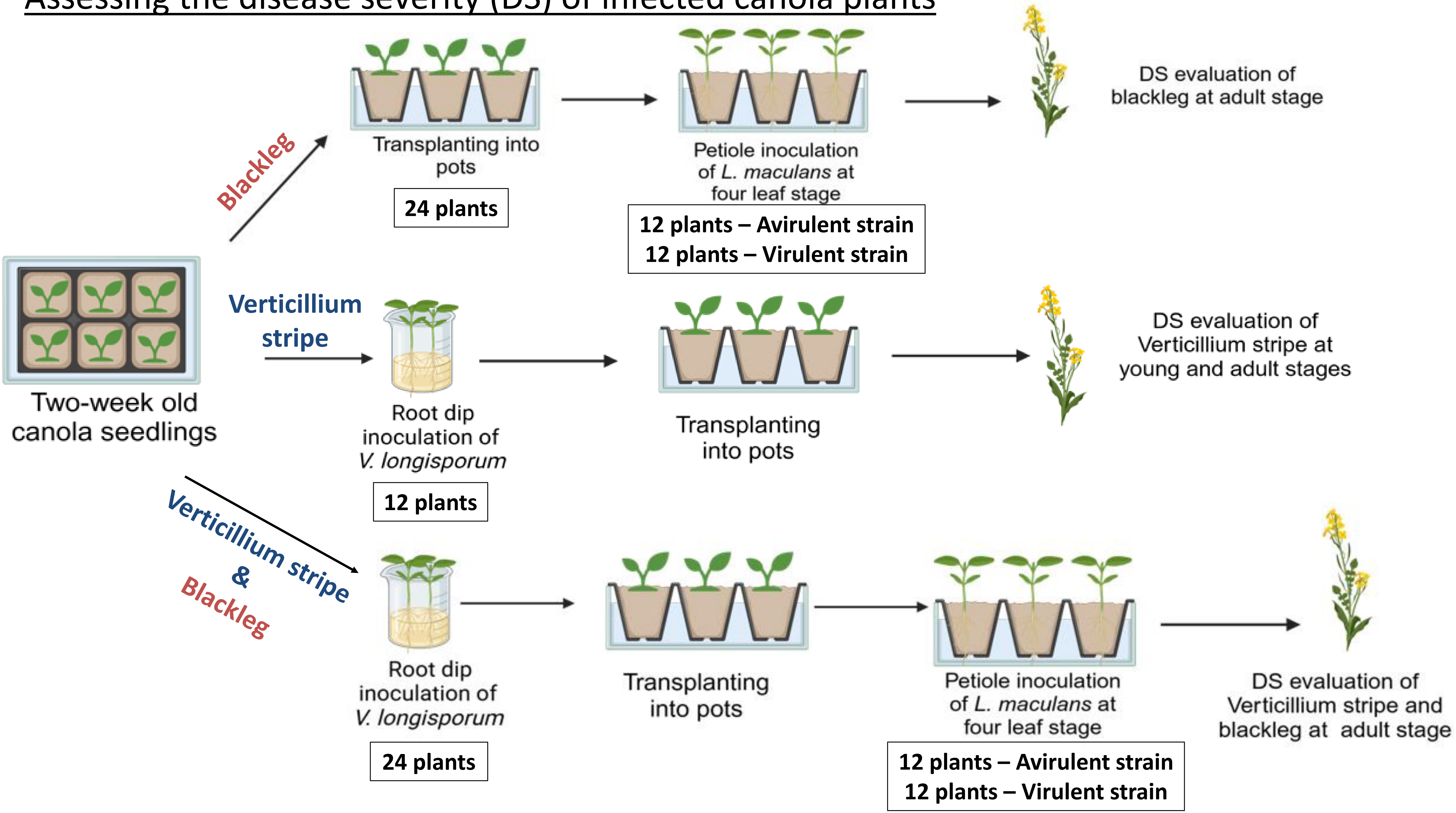
Canola variety	Resistance genotype	Reference
Westar	No *R* gene	Balesdent et al., 2002
Quinta	*Rlm1, Rlm3*	Kutcher et al., 2010
Jet Neuf	*Rlm4*	Gout et al., 2006
Surpass 400	*Rlm1, RlmS*	Van de Wouw et al., 2009
01-23-2-1	*Rlm7*	Dilmaghani et al., 2009
Goéland	*Rlm9*	Balesdent et al., 2006
Glacier	*Rlm2, Rlm3*	Balesdent et al., 2001
02-22-2-1	*Rlm3*	Gout et al., 2006
MT29	*Rlm1, Rlm9*	Delourme et al., 2008
1065	*LepR1*	Kutcher et al., unpublished
1135	*LepR2*	Kutcher et al., unpublished

Inculation of plant material



Objective 1 :

Assessing the disease severity (DS) of infected canola plants



1. Assessment of Verticillium stripe severity

- At the young stage – at 7,14,21, and 28 days post inoculation (dpi)
  (Eynck et al., *Journal of Plant Diseases and Protection,*2009)
- At the adult stage – at 105 dpi
  (Cui et al., *Canadian Journal of Plant Pathology,* 2023)

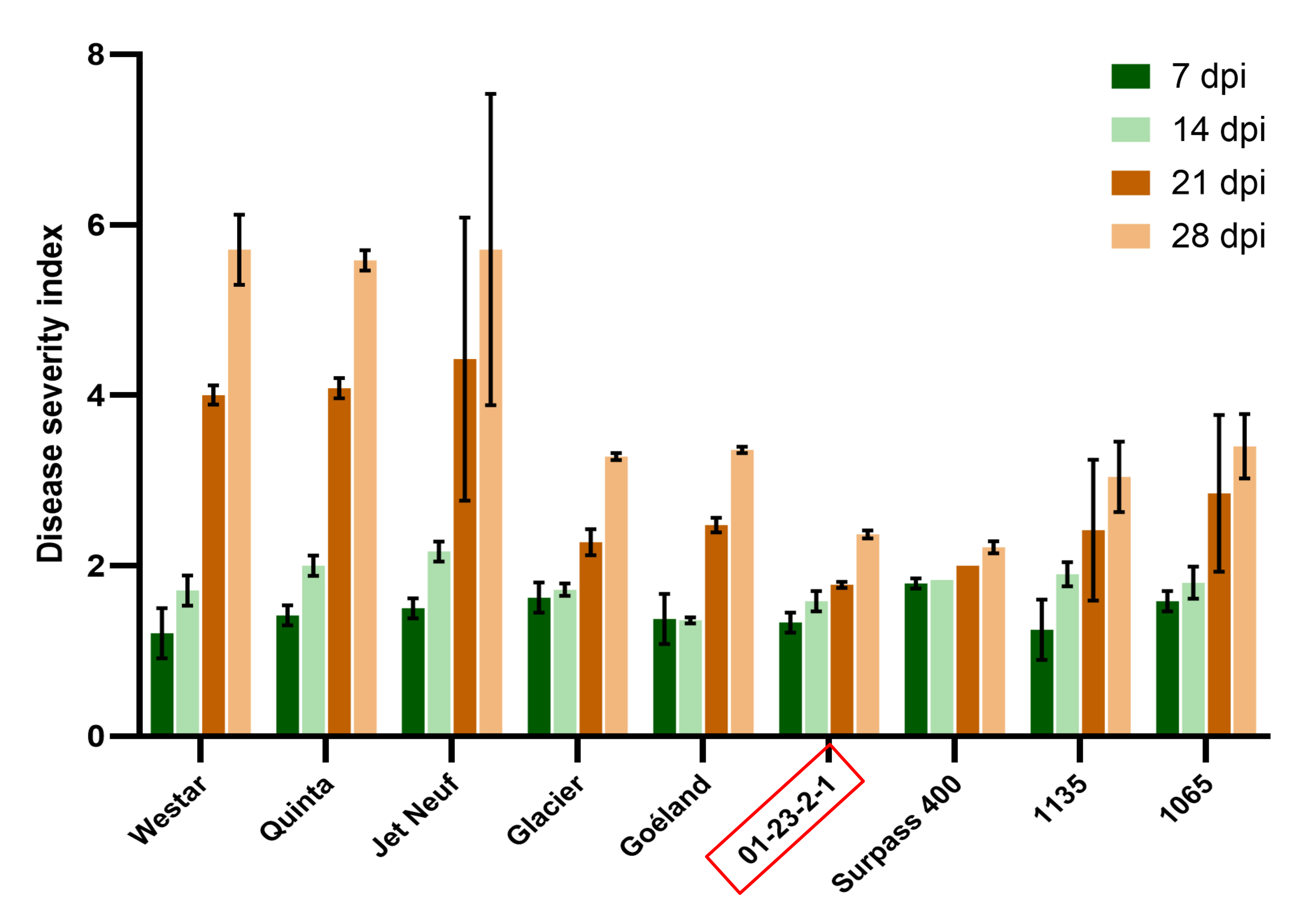
2. Assessment of blackleg severity

- At the adult stage – at 105 dpi
  (Guo and Fernando, *Plant Disease*,2005)

Figure 1: Disease rating scale for adult plants infected by *Verticillium longisporum*

Results

Assessment of DS of Verticillium stripe at the young stage



Disease severity index (DSI) =

$$\frac{(1 \times N_1) + (2 \times N_2) + (3 \times N_3) \ldots\ldots\ldots + (9 \times N_9)}{N_1 + N_2 + N_3 \ldots\ldots\ldots\ldots + N_9}$$

N_n = number of plants in the respective class

(Eynck et al., 2009)

7 dpi

Inoculated Water control

Westar

Inoculated Water control

01-23-2-1

Inoculated Water control

Quinta

Inoculated Water control

Jet Neuf

21 dpi

Inoculated Water control

Westar

Inoculated Water control

01-23-2-1

Inoculated Water control

Quinta

Inoculated Water control

Jet Neuf

Objective 2:

Understanding the transcriptome changes in *B. napus*

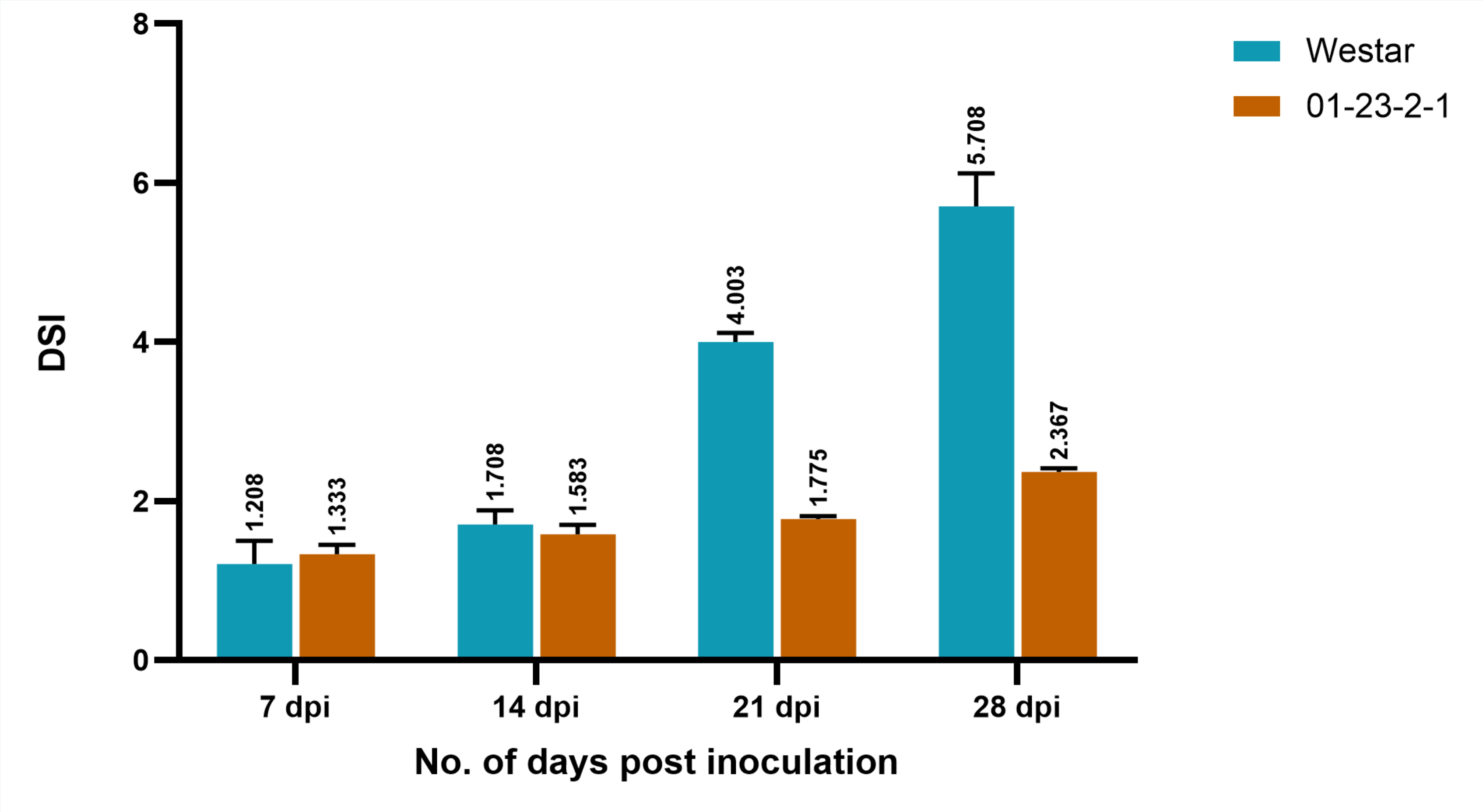
RNA extraction and RNA sequencing of samples from **01-23-2-1 (*Rlm* 7)** with different disease treatments were carried out.

Why 01-23-2-1 canola genotype?

1. The resistance in 01-23-2-1 to the avirulent isolate (UMAvr7) was broken down, possibly due to the presence of *V. longisporum*.

2. Shows better resistance to Verticillium stripe at the young stage compared to the susceptible genotype Westar.

DSI for Verticillium stripe at the young stage



21 dpi

Westar **01-23-2-1**

3. The *L. maculans* strains used to inoculate 01-23-2-1 (*Rlm 7*) have the same genetic background except the avirulence gene *AvrLm7*, which was knocked out **(Zou et al., *Frontiers in Microbiology,* 2020).**

UMAvr7
(with *AvrLm7*)

umavr7
(without *AvrLm7*)

Incompatible interaction

Compatible interaction

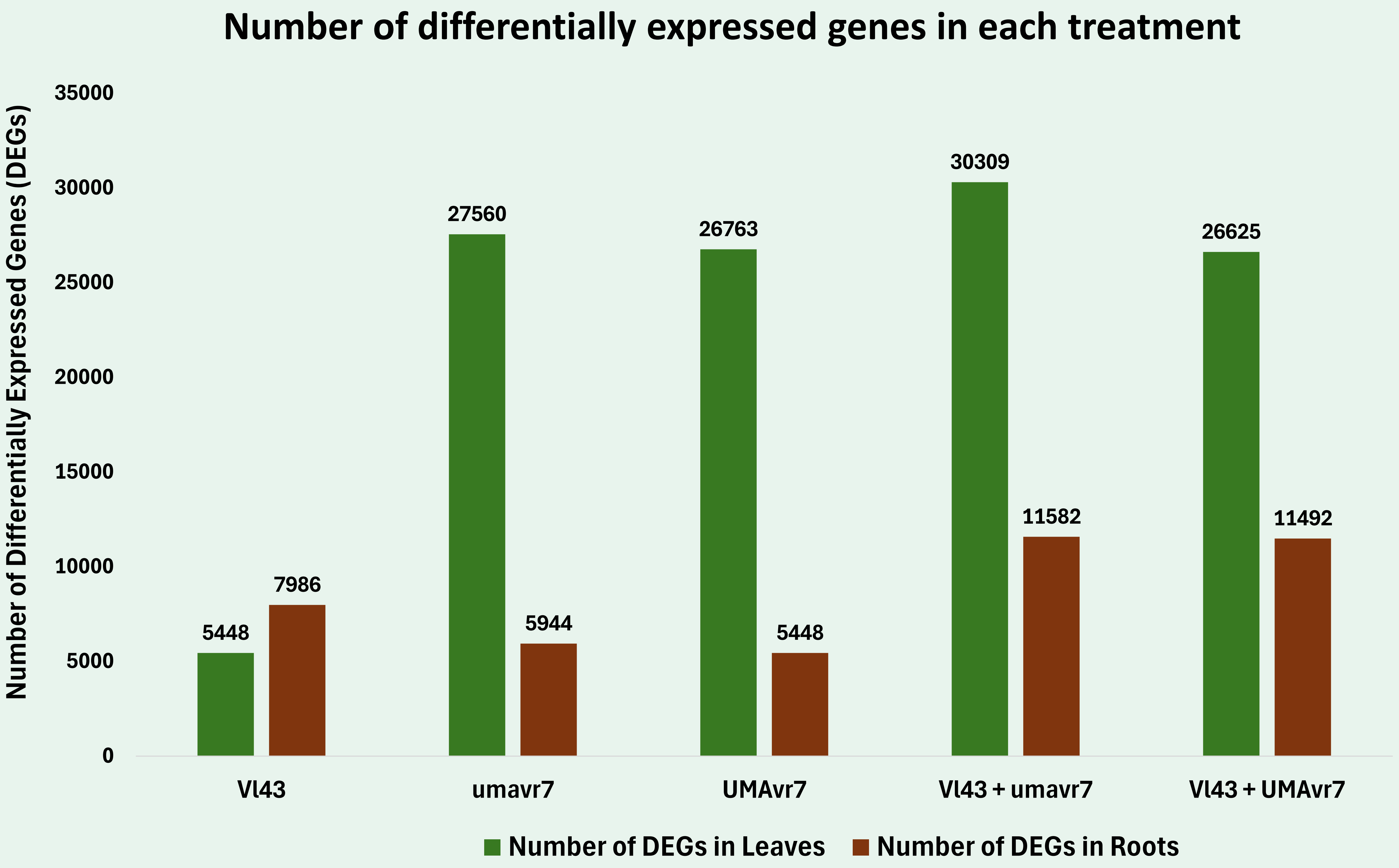
01-23-2-1 (with *Rlm 7*)
14 dpi

- Leaf and root samples were collected from **01-23-2-1 (*Rlm* 7)** canola genotype at **14 dpi with blackleg.**

Table 2: Number of tissue samples collected from each treatment

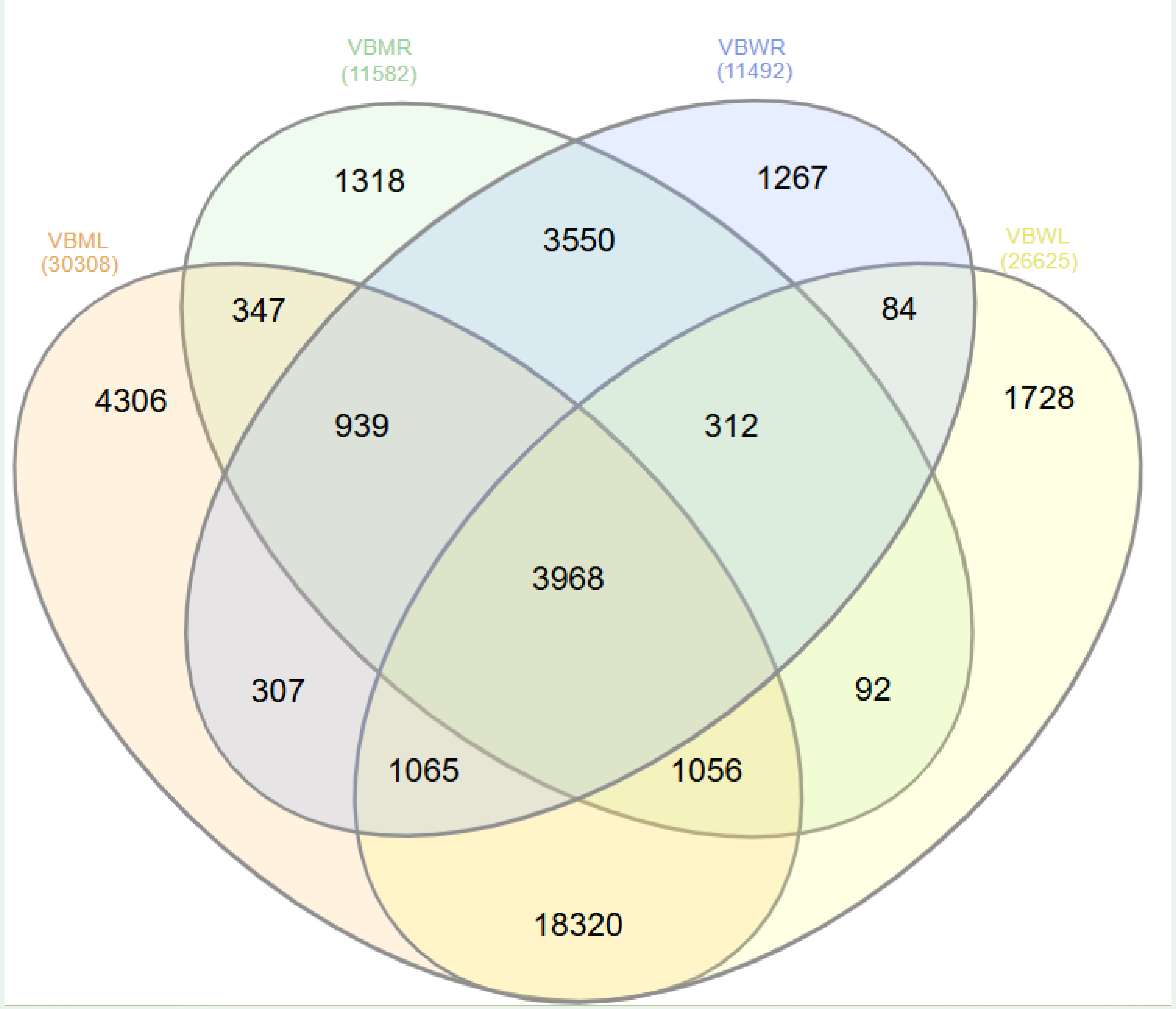
Treatment	Number of root samples collected (per replicate)	Number of leaf samples collected (per replicate)
1. Plants inoculated with Vl43	3	3
2. Plants inoculated with umavr7	3	3
3. Plants inoculated with UMavr7	3	3
4. Plants inoculated with Vl43 and umavr7	3	3
5. Plants inoculated with Vl43 and UMavr7	3	3
6. Non-inoculated water controls	3	3

Results



Vl43 – Verticillium only
umavr7– Blackleg only, virulent strain
UMAvr7– Blackleg only, avirulent strain
Vl43 + umavr7– Verticillium and blackleg, virulent strain
Vl43 + UMAvr7– Verticillium and blackleg, avirulent strain

Unique and shared DEGs of 01-23-2-1 canola genotype inoculated with Verticillium (Vl43) and blackleg virulent strain (umavr7) vs Vl43 and blackleg avirulent strain (UMAvr7)



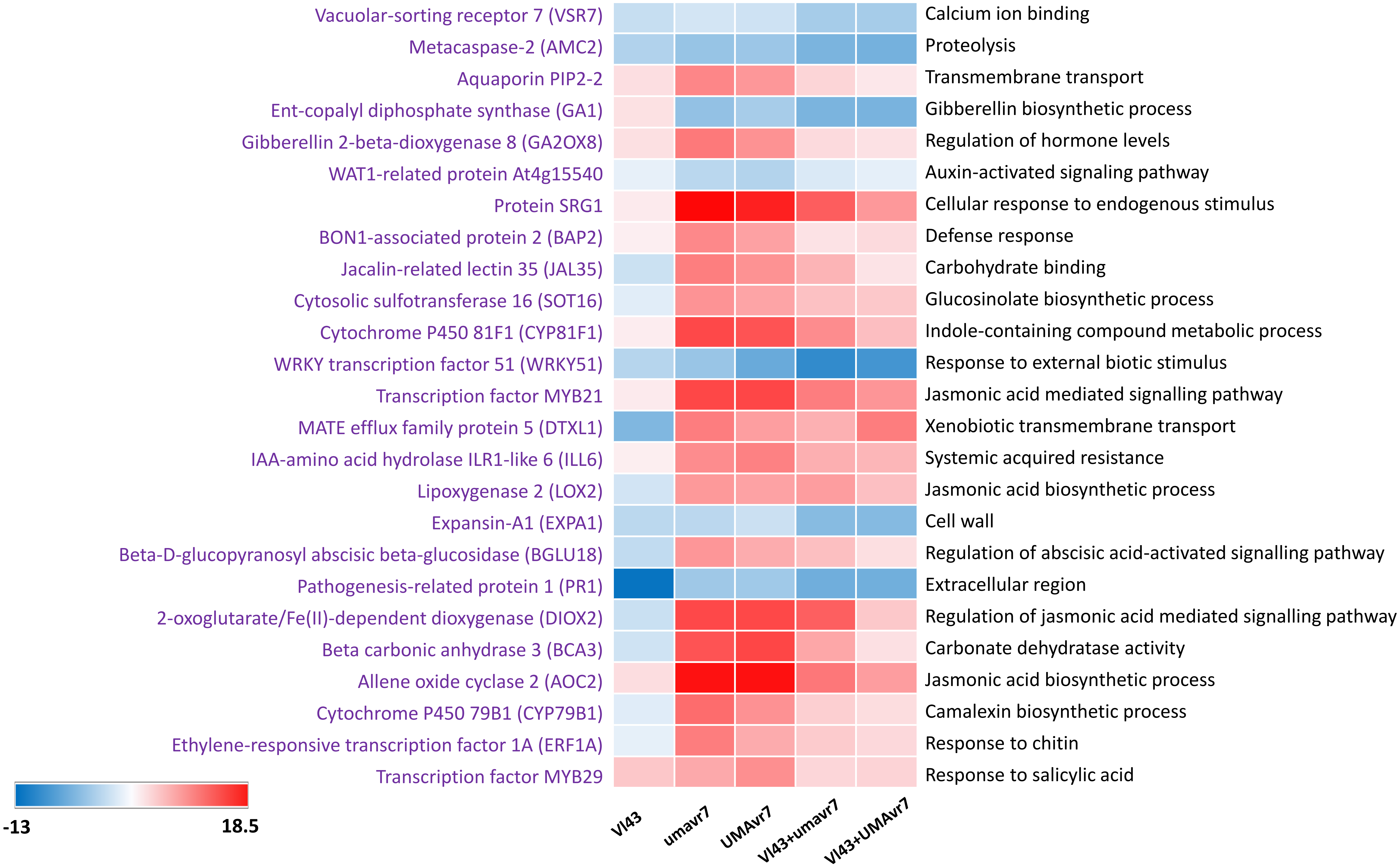
DEGs of leaves inoculated with Vl43 and UMAvr7 vs water control

DEGs of roots inoculated with Vl43 and UMAvr7 vs water control

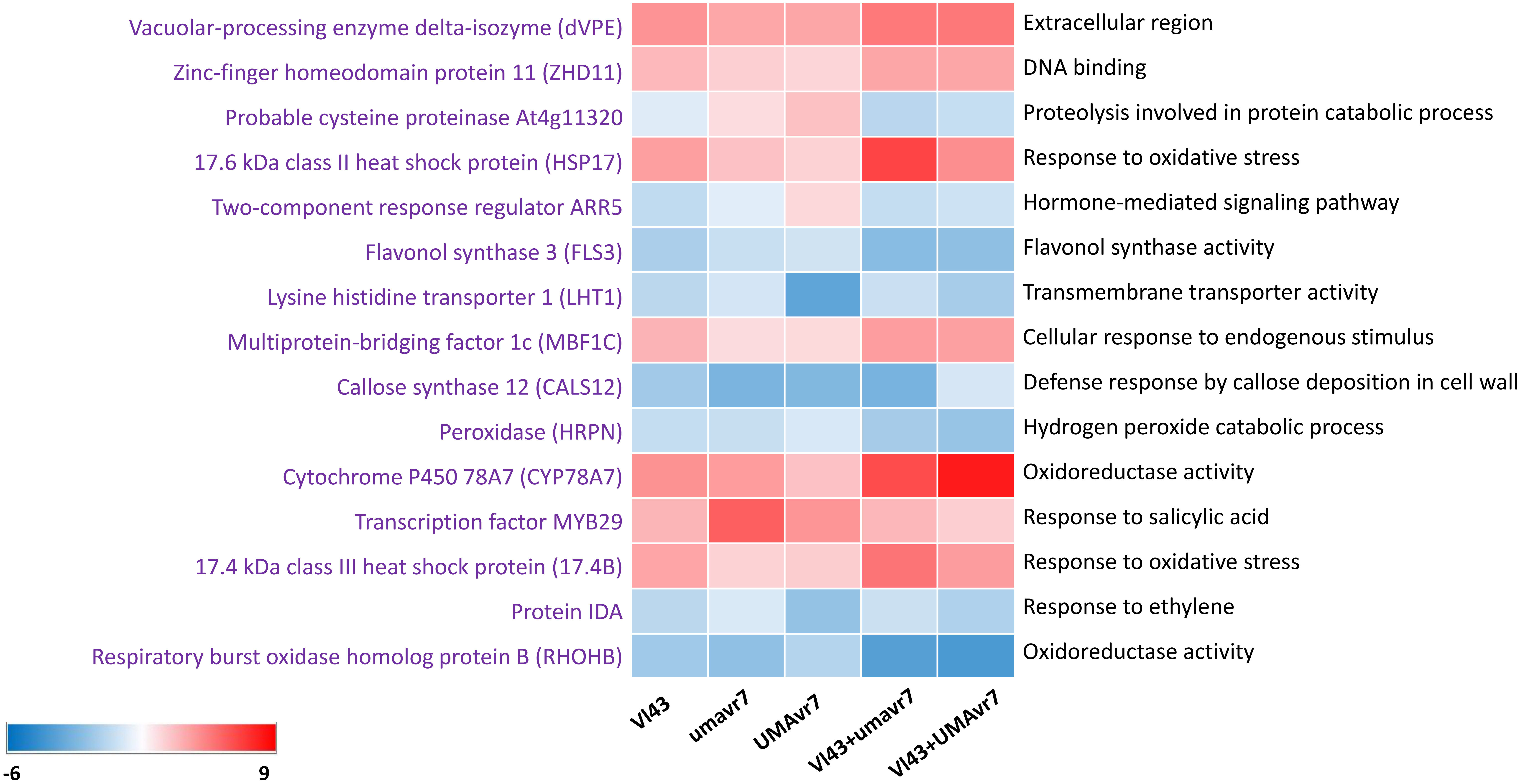
DEGs of roots inoculated with Vl43 and umavr7 vs water control

DEGs of leaves inoculated with Vl43 and umavr7 vs water control

Differential expression of defence related genes in leaves



Differential expression of defence related genes in roots



Conclusions

- The canola genotype 01-23-2-1 shows more resistant to Verticillium stripe at the young stage.

- Jet Neuf and Quinta are more susceptible to Verticillium stripe at the young stage.

- The number of DEGs of leaves were higher than that of roots in all the treatments except for plants inoculated only with Vl43.

Way Forward..

01 Assessing the adult stage DS of canola genotypes inoculated with both *V. longisporum* and *L. maculans.*

02 Validating the functions of candidate DEGs involved in key resistance pathways, such as plant hormone signal transduction pathways and production of antioxidant enzymes.

03 Identifying potential DEGs that can be utilized in breeding to improve resistance to both pathogens in canola.

Acknowledgments

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Dr. Gabriel Moreno-Hagelsieb (Wilfrid Laurier University)

Collaborators
Dr. Fei Liu (Henan University)
Professor Andreas von Tiedemann (Georg-August-University of Gottingen)

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