Exploring Genetic Resistance to Sclerotinia Stem Rot in Canola Through *VQ* Gene Expression Profiling

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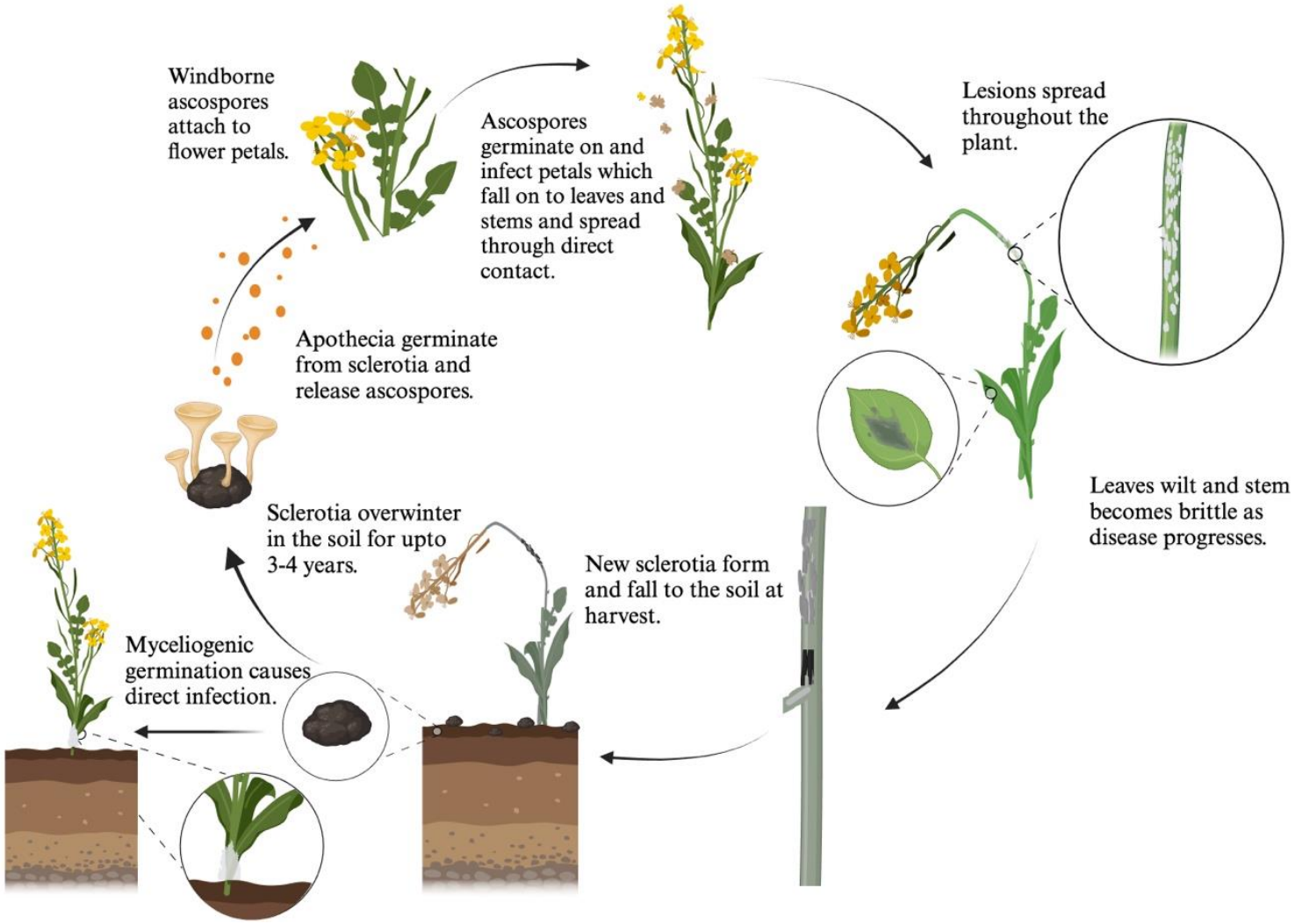
Canola (*Brassica napus*)

- Canola is a major Canadian cash crop.
- Canola is the third most utilized crop for oil production, worldwide.
- The canola sector contributed an average C$43.7 billion per year to the Canadian economy during the period of 2020/21 to 2022/23.
- Canola: cultivars of oilseed rape that yield seed oils containing less than 2% erucic acid and meal products with less than 30 μmol glucosinolates per gram.

Sclerotinia Stem Rot (SSR)

- Caused by the fungi *Sclerotinia sclerotiorum*.
- Can infect over 500 plant species.
- Present across the world and occurs within most canola-producing regions.
- One of the most devastating diseases to affect canola in Canada.
- Sclerotia can remain dormant in soil for over 5 years.
- Can cause major yield loss.
- Can cause the downgrading of seeds and lower their export value.

Disease Cycle



Disease Control

- Predictive models have been established to evaluate SSR risk using environmental data.
- Cultural practices:
  - Crop rotation
  - Planting density
  - Stubble management
- Fungicides
- Biocontrol agents
- Use of partially resistant varieties.

BnVQ Genes

- Valine-Glutamine (VQ) containing proteins, encoded by *VQ* genes, play an important role in the defense responses to both abiotic and biotic stressors in plants.
- Involved in plant defense as well as seed development and growth.
- Currently, 118 *VQ* genes have been identified in *B. napus*.
- *VQ* genes code for a specific class of transcriptional co-factors that have a conserved VQ-motif structure and are involved in transcriptional regulation.
- Both up- and down-regulation of these genes have been observed in response to biotic and abiotic stress.

Objectives

- **Objective 1**:

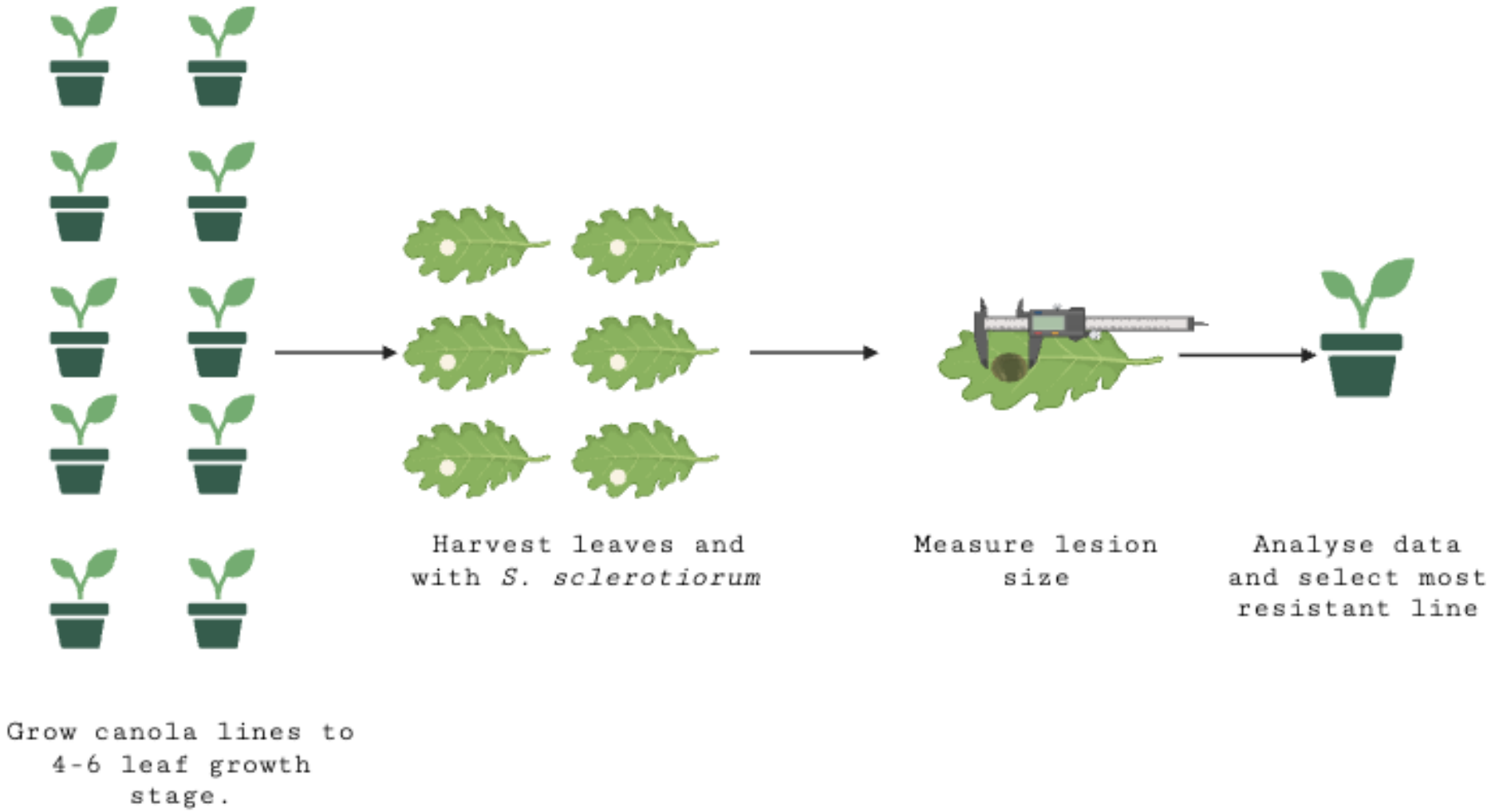
  Characterization of resistance to *S. sclerotiorum* in *B. napus* lines to identify a candidate with strong partial resistance.

- **Objective 2**:

  Profiling differential *VQ* gene expression and other differentially expressed genes in response to *S. sclerotiorum* infection in resistant and susceptible

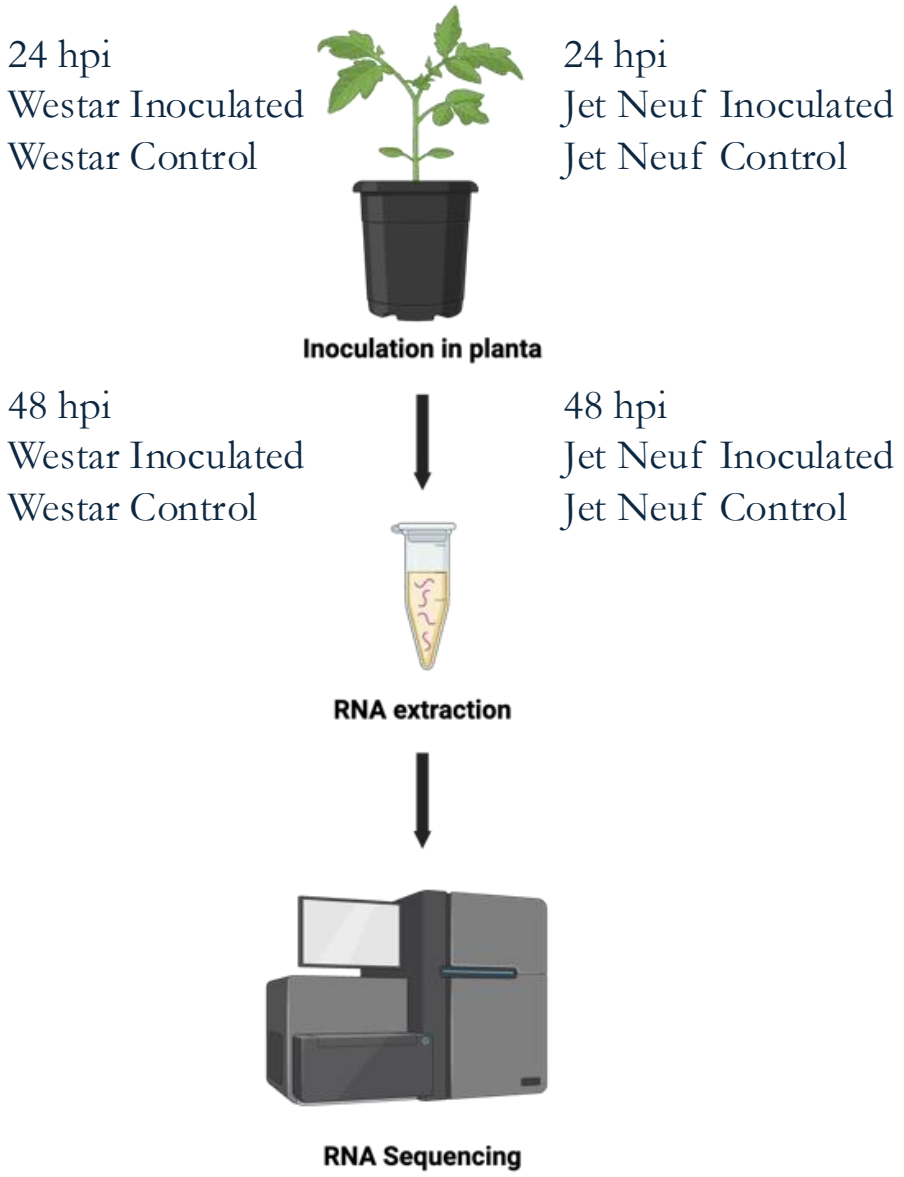
  B. napus lines.

Characterization of resistance to *S. sclerotiorum* in *B. napus* lines

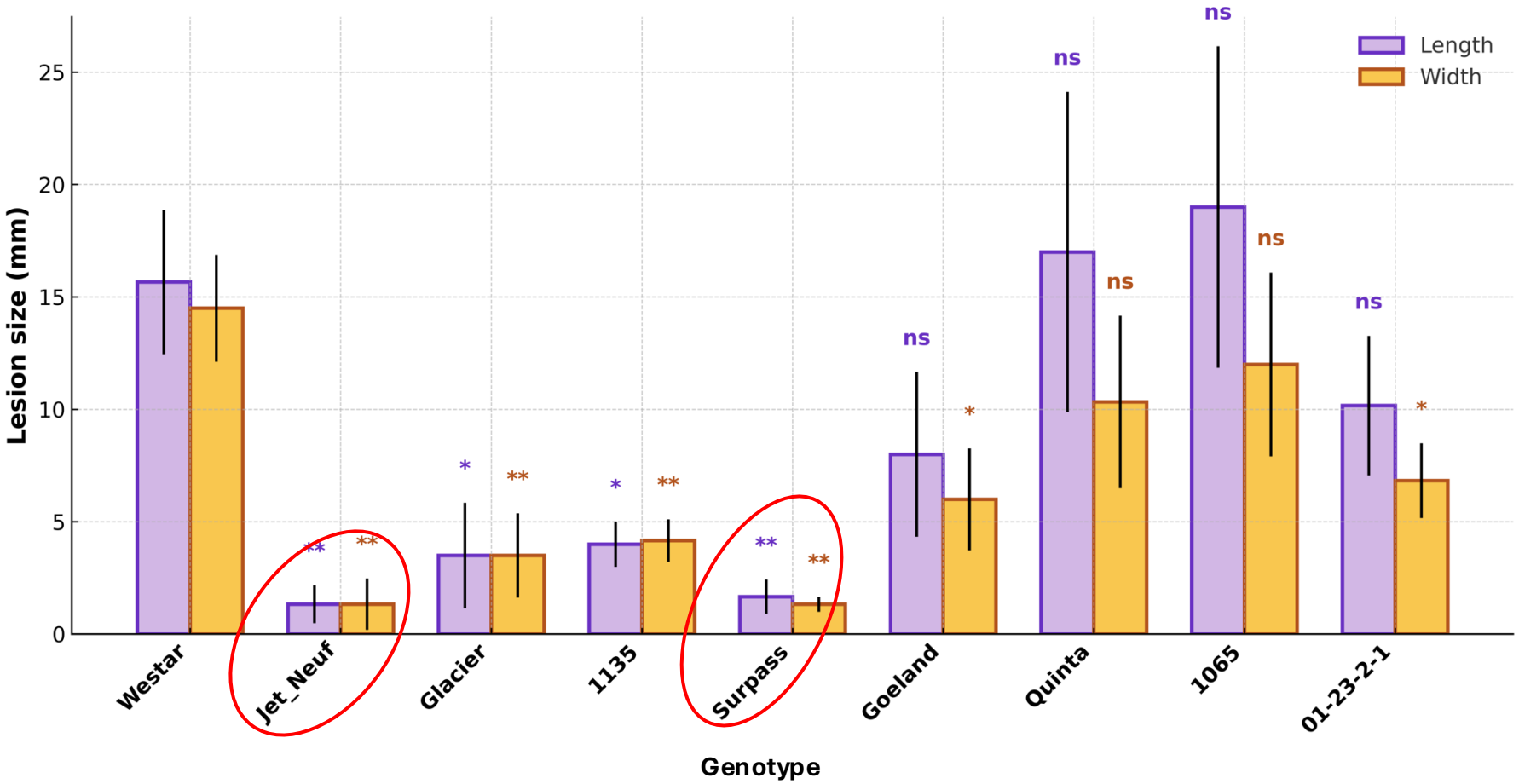


Sampling & RNA Extraction

- *B. napus* lines were inoculated in planta.
- Lesion size were measured, and leaf samples were collected at 24- and 48-hours post-inoculation (hpi).
- Samples were immediately frozen cryogenically and then stored at -80ºC.
- RNA was extracted, and then quality and integrity was checked.
- Samples were sent for RNA sequencing.
- RNA seq raw data was cleaned up and filtered, and the reads were then be mapped onto the *B. napus* genome.
- Differentially Expressed Genes (DEG) were analysed with a focus on *VQ* genes.
- Data validation by qPCR.

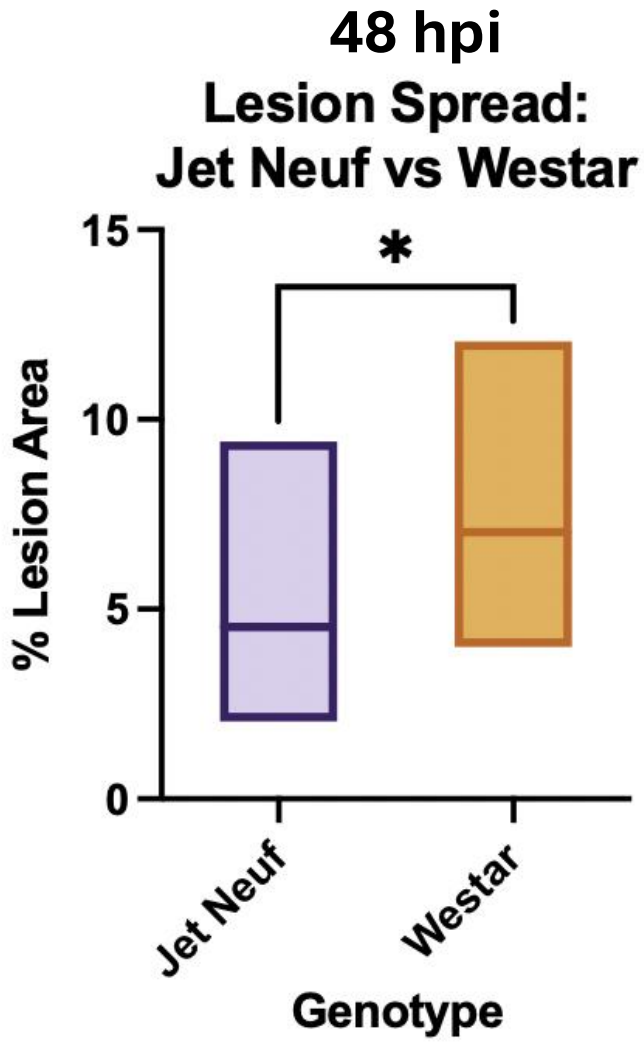


Screening Results

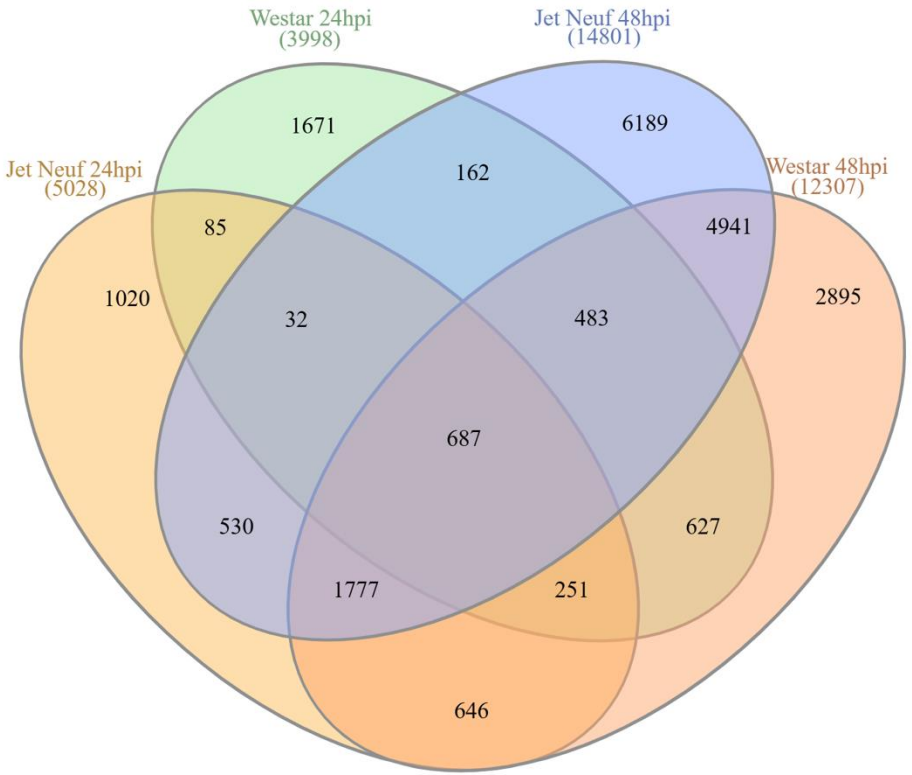


Westar – Jet Neuf

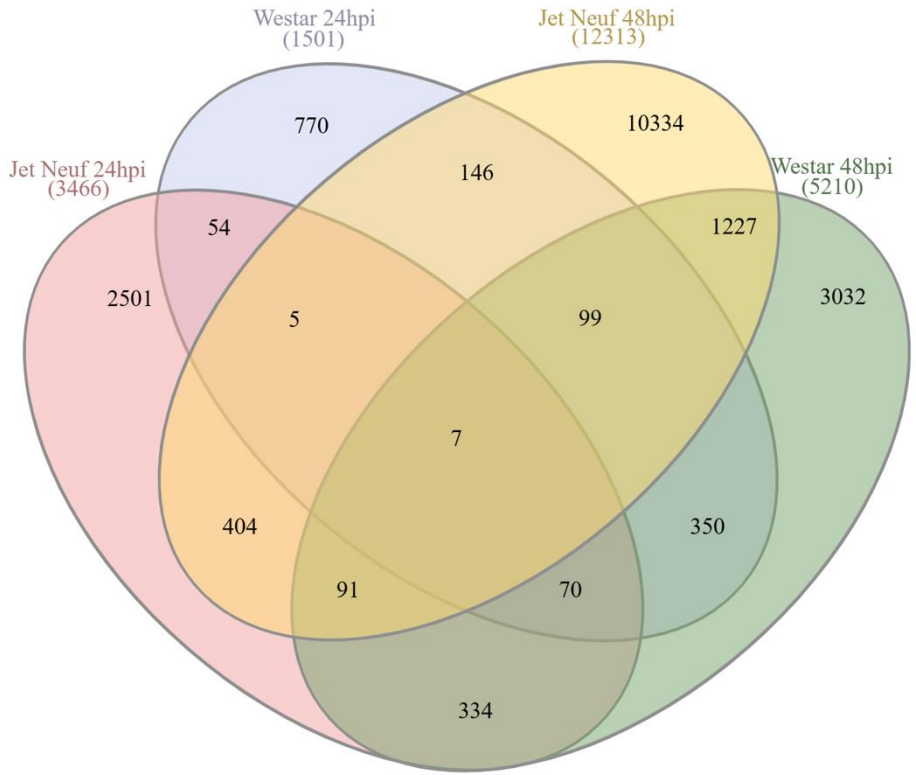
	Westar	Jet Neuf
24 hpi		
48 hpi		



Differential Gene Expression

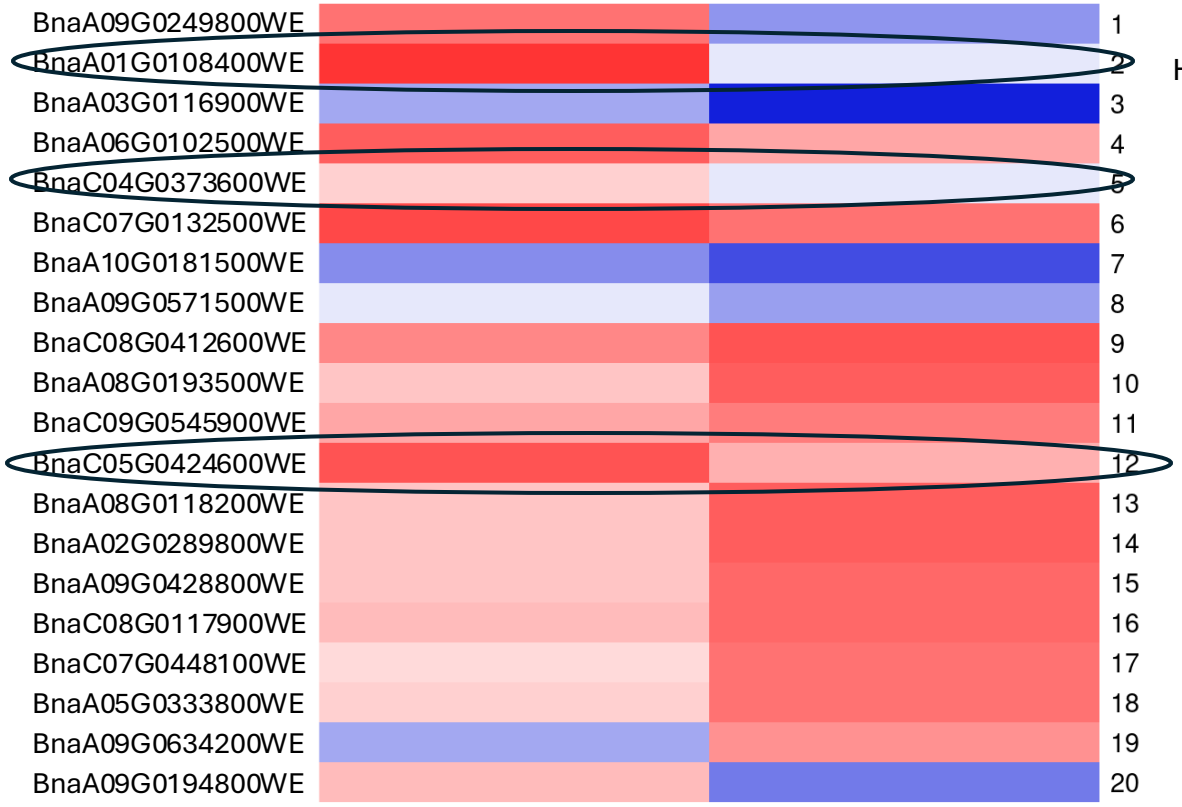


Upregulated Genes

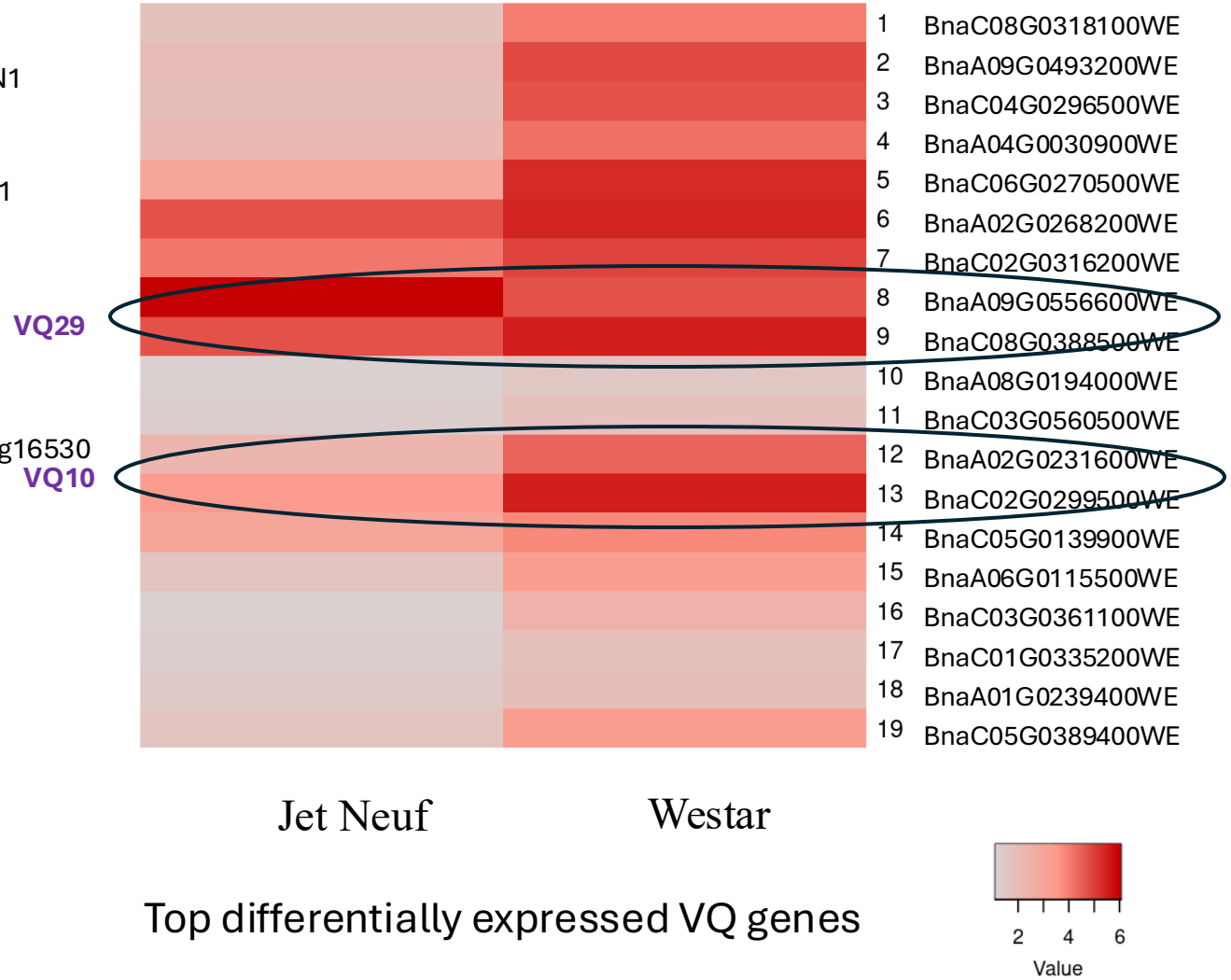


Downregulated Genes

Gene Expression at 24 hpi

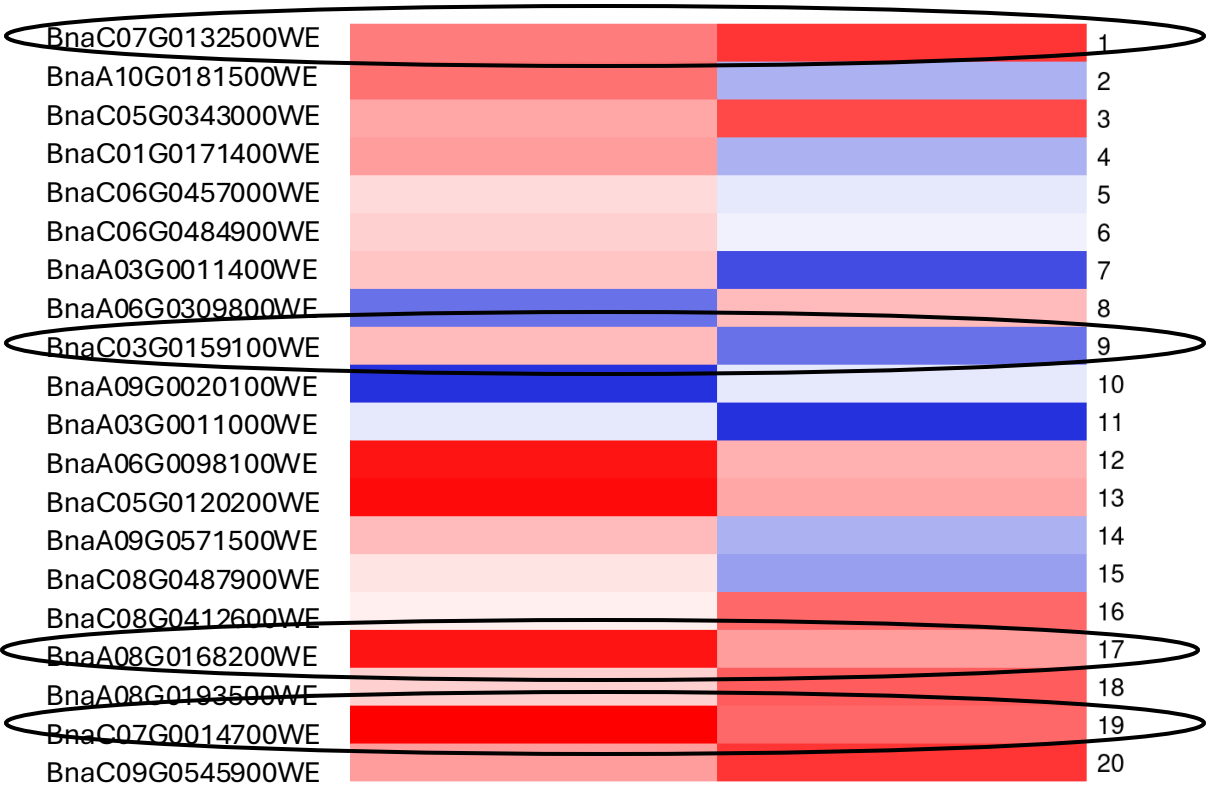


Top Differentially Expressed Genes

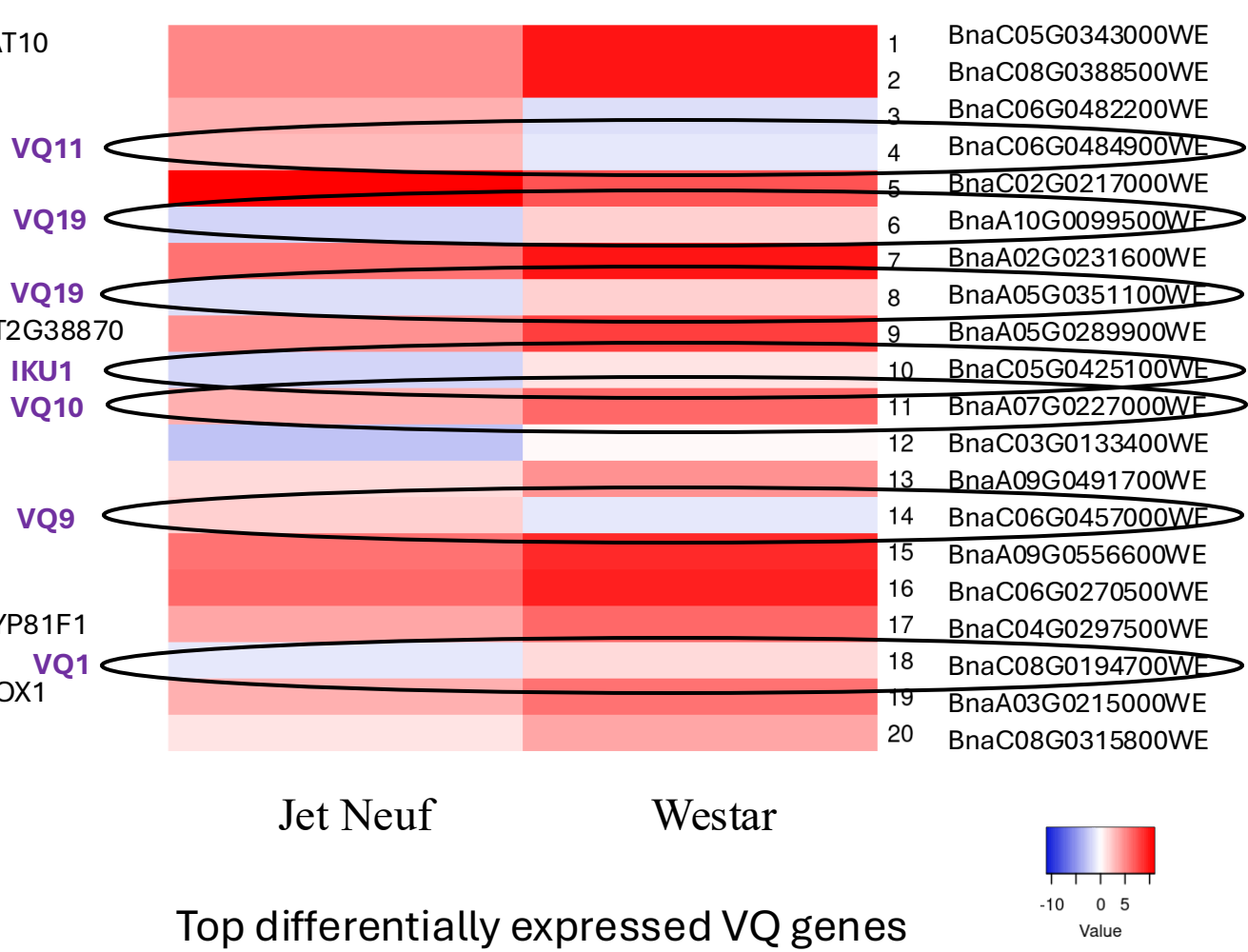


Top differentially expressed VQ genes

Gene Expression at 48hpi



Top Differentially Expressed Genes



Top differentially expressed VQ genes

Future Directions

- Further explore and validate candidate genes that can be used for improving *B. napus* resistance
- Apply multiplex CRISPR genome editing to knock out selected down-regulated *BnVQ*s (from Jet Neuf) to assess their roles in disease susceptibility
- Develop DNA-free mutant lines from genome editing to create pre-breeding lines with enhanced disease resistance suitable for canola breeding programs
- Investigate the molecular mechanisms underlying the interaction between B. napus and S. sclerotiorum to guide future disease resistance breeding strategies

Significance

- Provide insight into candidate genes that can potentially be targeted to develop *B. napus* pre-breeding lines with improved resistance to SSR.
- Cultivars with improved SSR resistance.
- Reduce yield loss from SSR.
- Help ensure the sustainability and security of canola production.
- Reduce the need for fungicides.
- Improve profitability of the canola industry.

Acknowledgements

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