Functional characterization of a *Transcription Factor BnWRKY40* in *Brassica napus* against *Verticillium longisporum*

ONTARIO PEST MANAGEMENT CONFERENCE

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CANOLA

Primarily grown in Saskatchewan, Alberta, and Manitoba

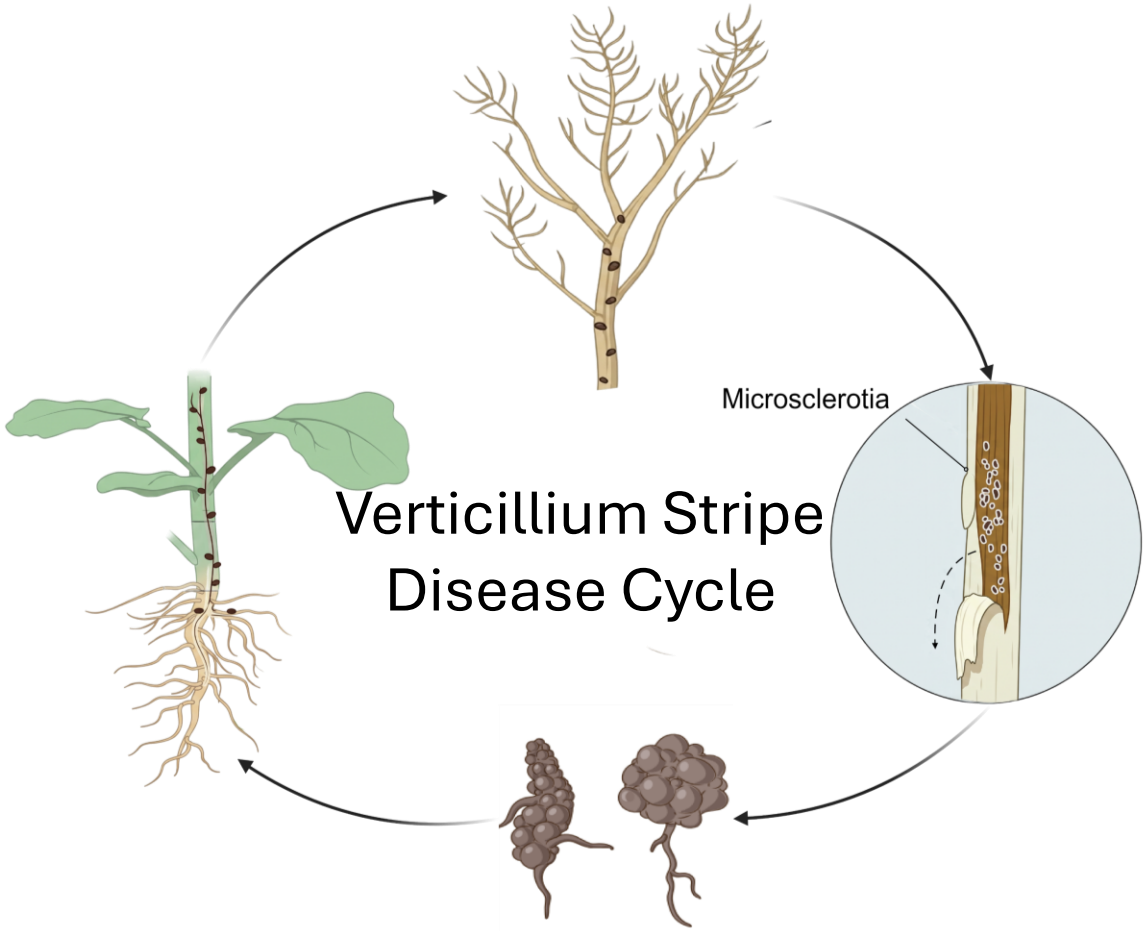
21.8 million metric tonnes produced in 2025, contributing C$43.7 billion to the Canadian economy

Around 90% exported; used for vegetable oil, biofuel and protein-rich livestock feed

Average yield of 44.7 bushels/acre; plant diseases threaten yield and quality

Source: Statistics Canada

Verticillium longisporum: An emerging Challenge for Canadian canola



Adapted from Canola Council of Canada

- *Verticillium longisporum* is a soil-borne fungus causing **Verticillium stripe** in canola
- First detected in Manitoba in 2014; confirmed in **six Canadian provinces**
- Yield losses can reach **50%**, depending on disease severity
- Survives in soil for years as durable structures called **microsclerotia**

Symptoms of Verticillium Stripe

Common symptoms

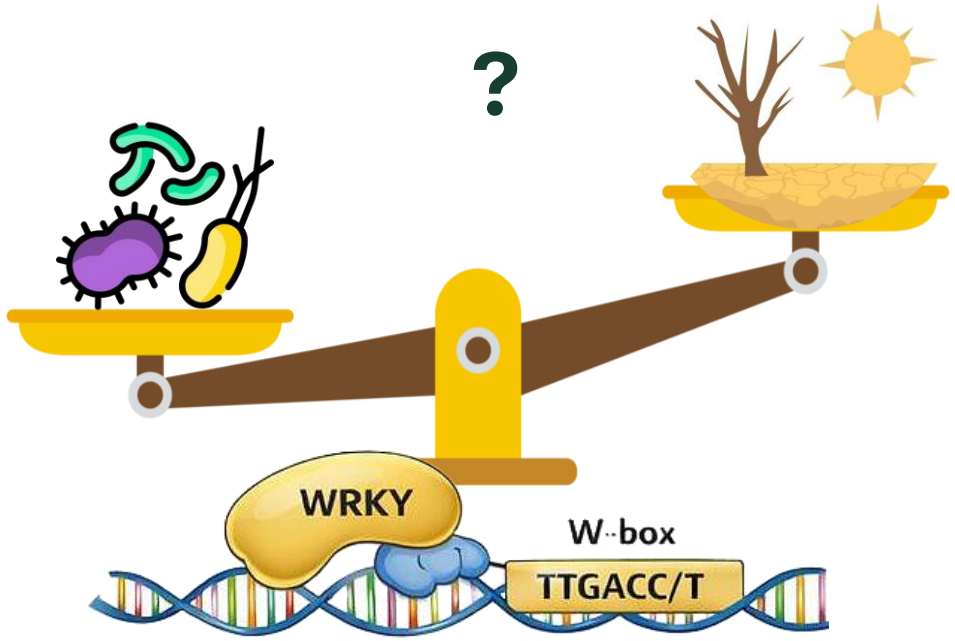
- Leaf chlorosis
- Early ripening and stunting
- Vertical yellow to brown stem stripes
- Necrosis
- Shredding of stem tissue



Source: Canola Council of Canada

Disease management relies on crop rotation and limiting the spread of infested soil

The role of OE*BnWRKY40* in canola defense against *V. longisporum*



- **WRKY** transcription factors are one of the **largest plant TF families**, regulating development and responses to biotic and abiotic stresses
- In *B. napus*, 99 *WRKY* genes **respond** to drought, salinity, temperature stress, or ABA treatment (Chen et al., 2021)
- In *A. thaliana*, ***WRKY* genes** participate in responses to *Pseudomonas syringae* and **SA-** and **JA-mediated signaling** (Dong et al., 2003)
- *AtWRKY40* regulates plant defence through interactions with other WRKY proteins, depending on the pathogen (Xu et al., 2006)
- In *B. napus*, ***WRKY genes*** respond to *fungal* pathogens, including *Sclerotinia sclerotiorum* and *Alternaria brassicae* (Yang et al., 2009)

Materials and methods

Materials

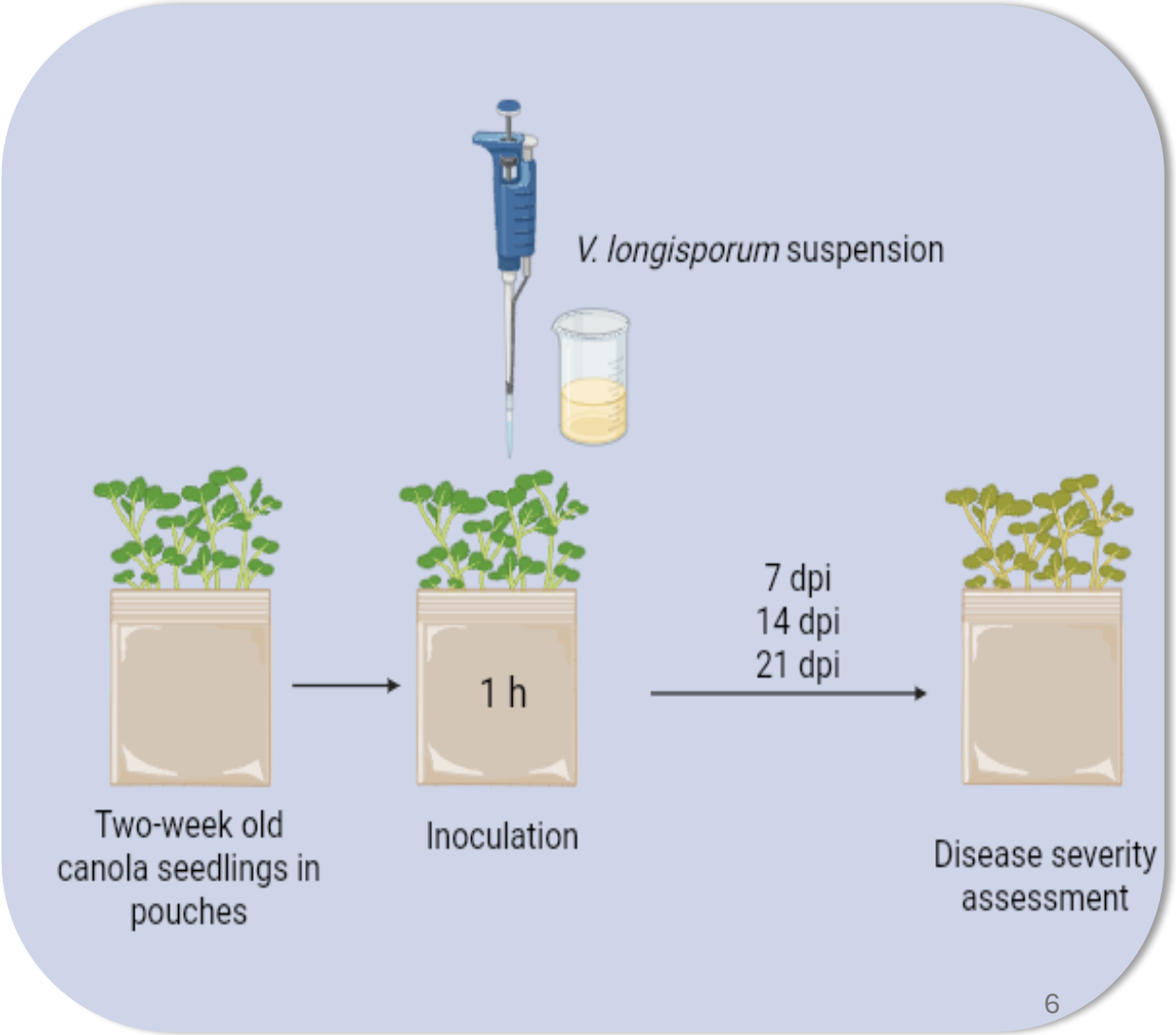
- Westar (wild type)
- *OEBnWRKY40*-10
- *OEBnWRKY40*-20



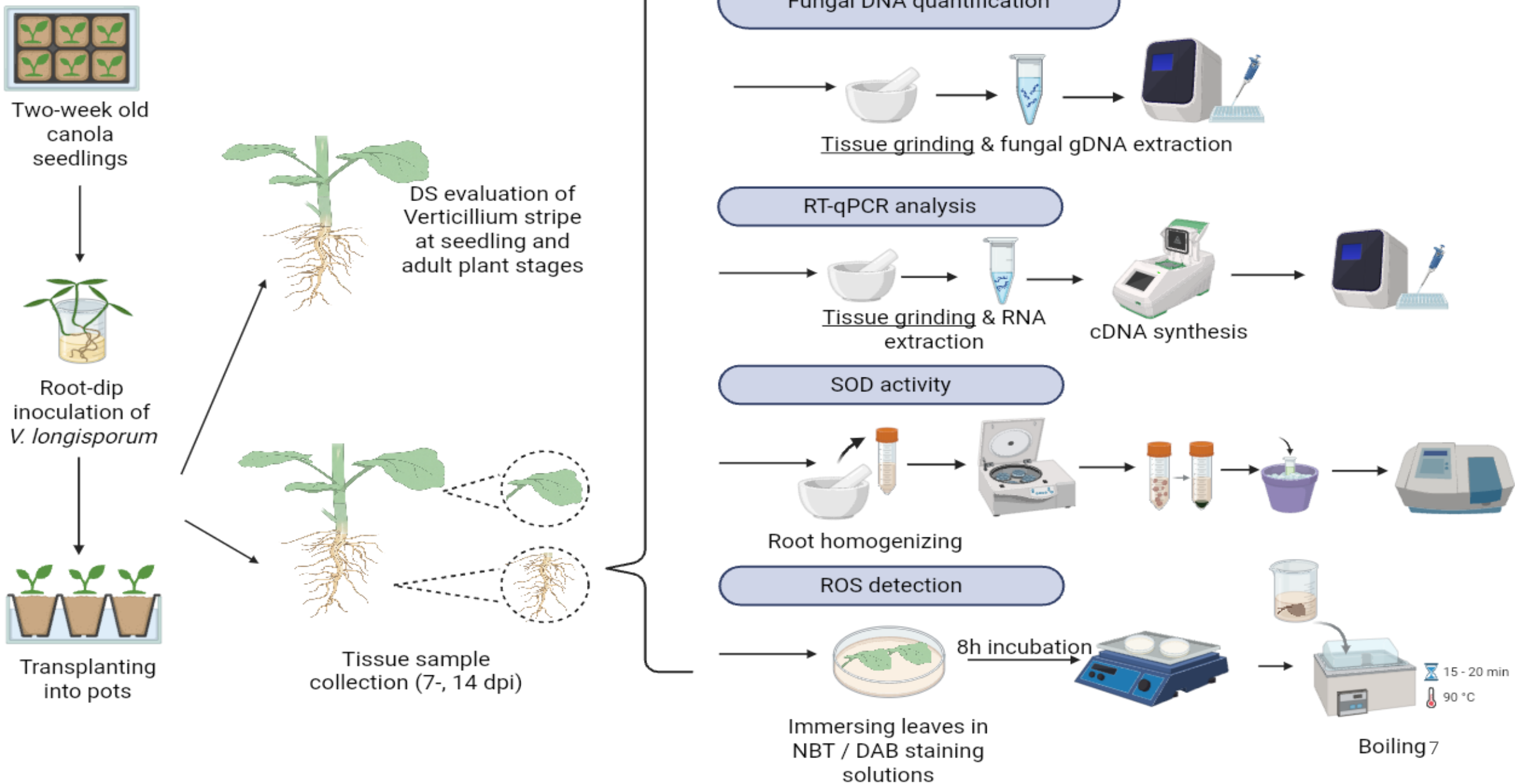


V. longisporum isolate from diseased canola in Manitoba

Germination-pouch inoculation

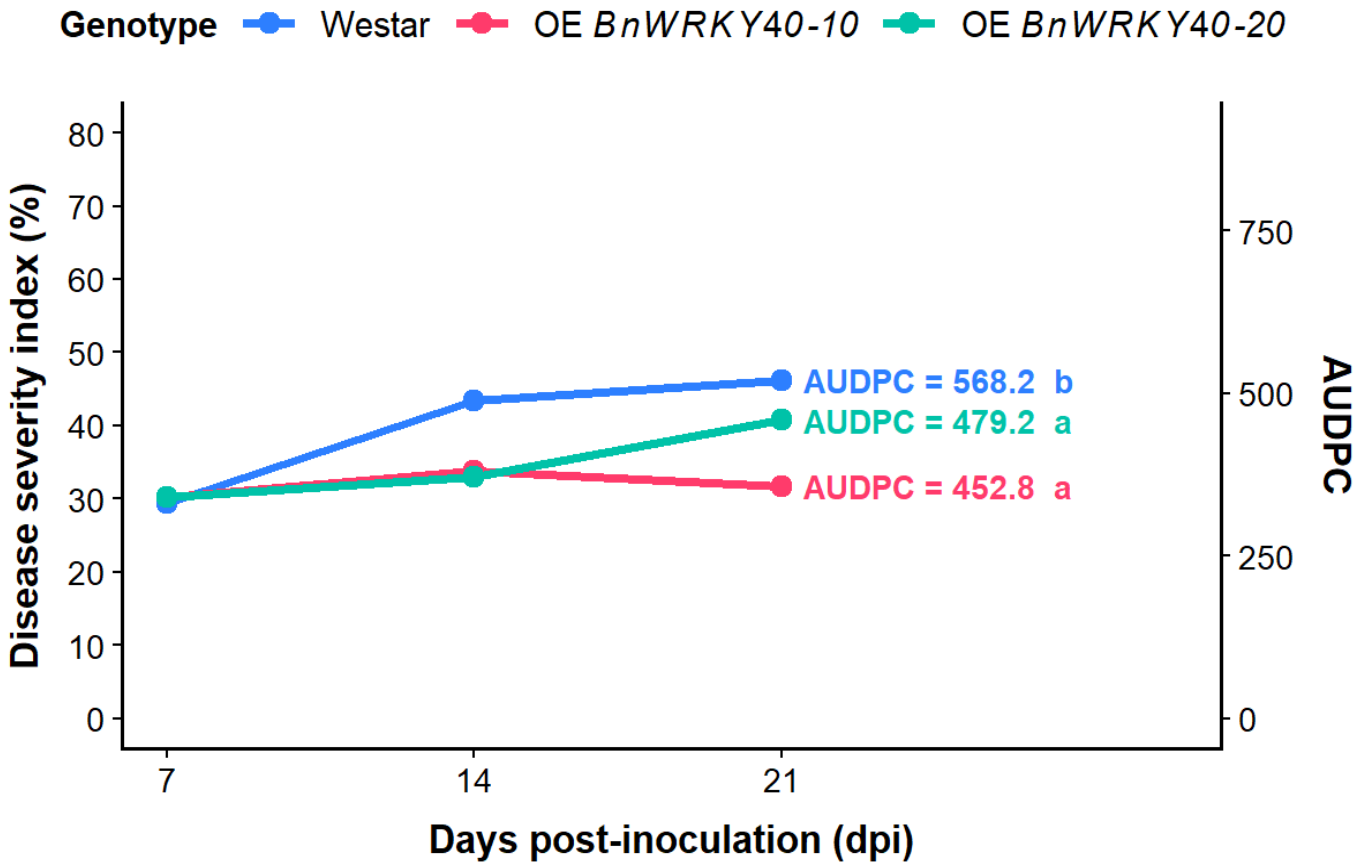


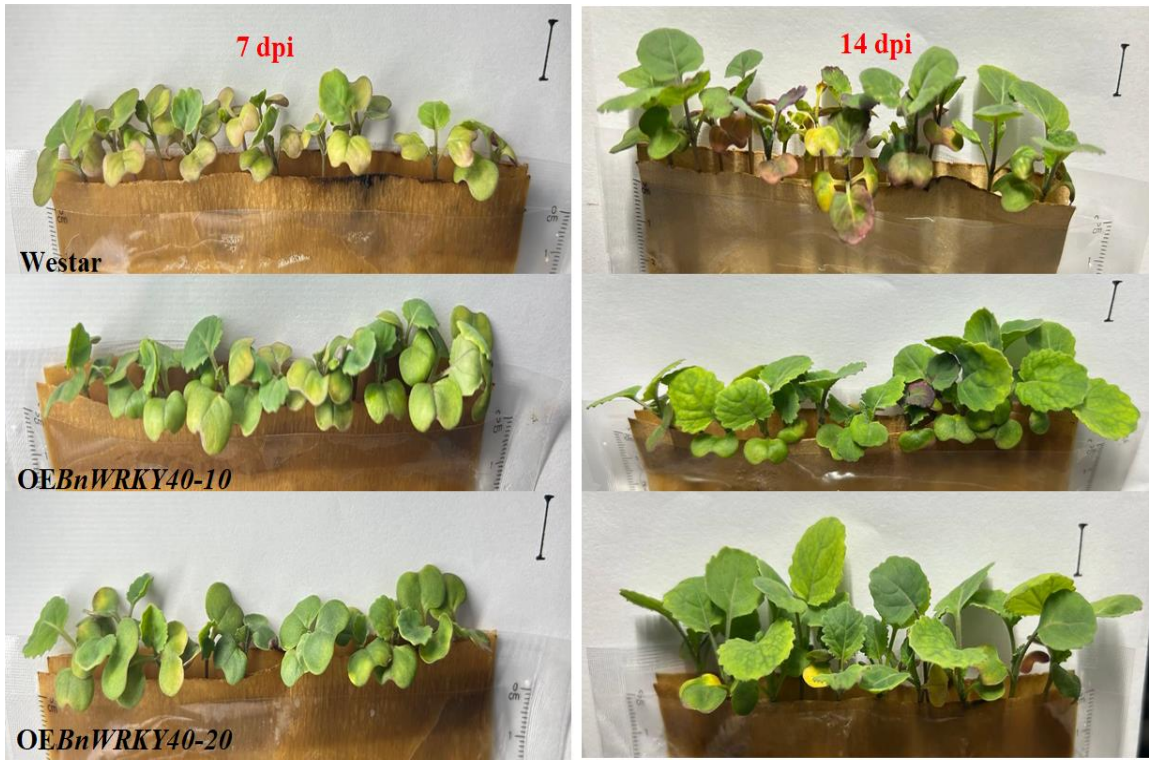
Experimental design




Results

Verticillium Stripe Disease Assessment by Germination Pouches Assays



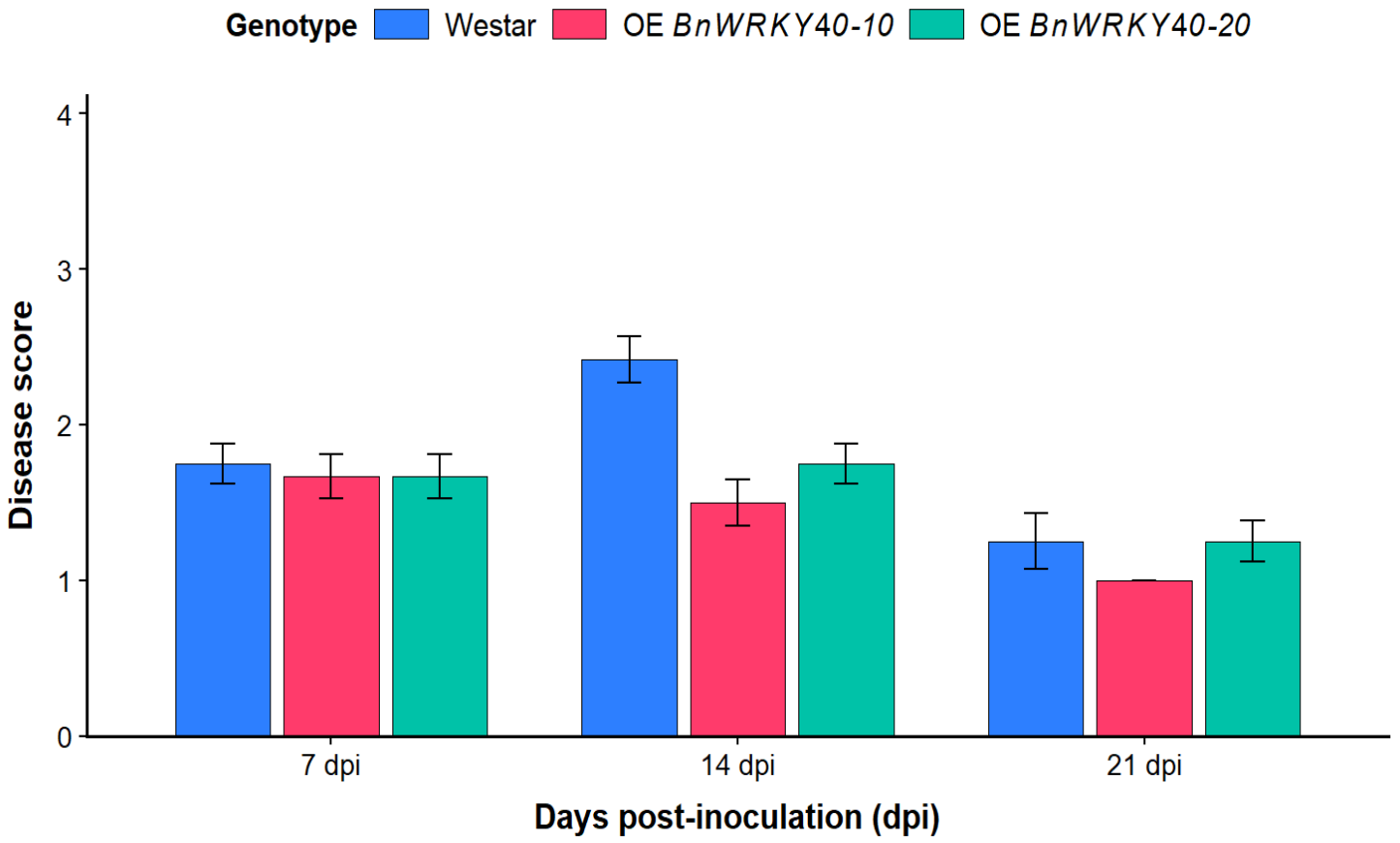


Disease Assessment at Young Seedling by Root Dipping











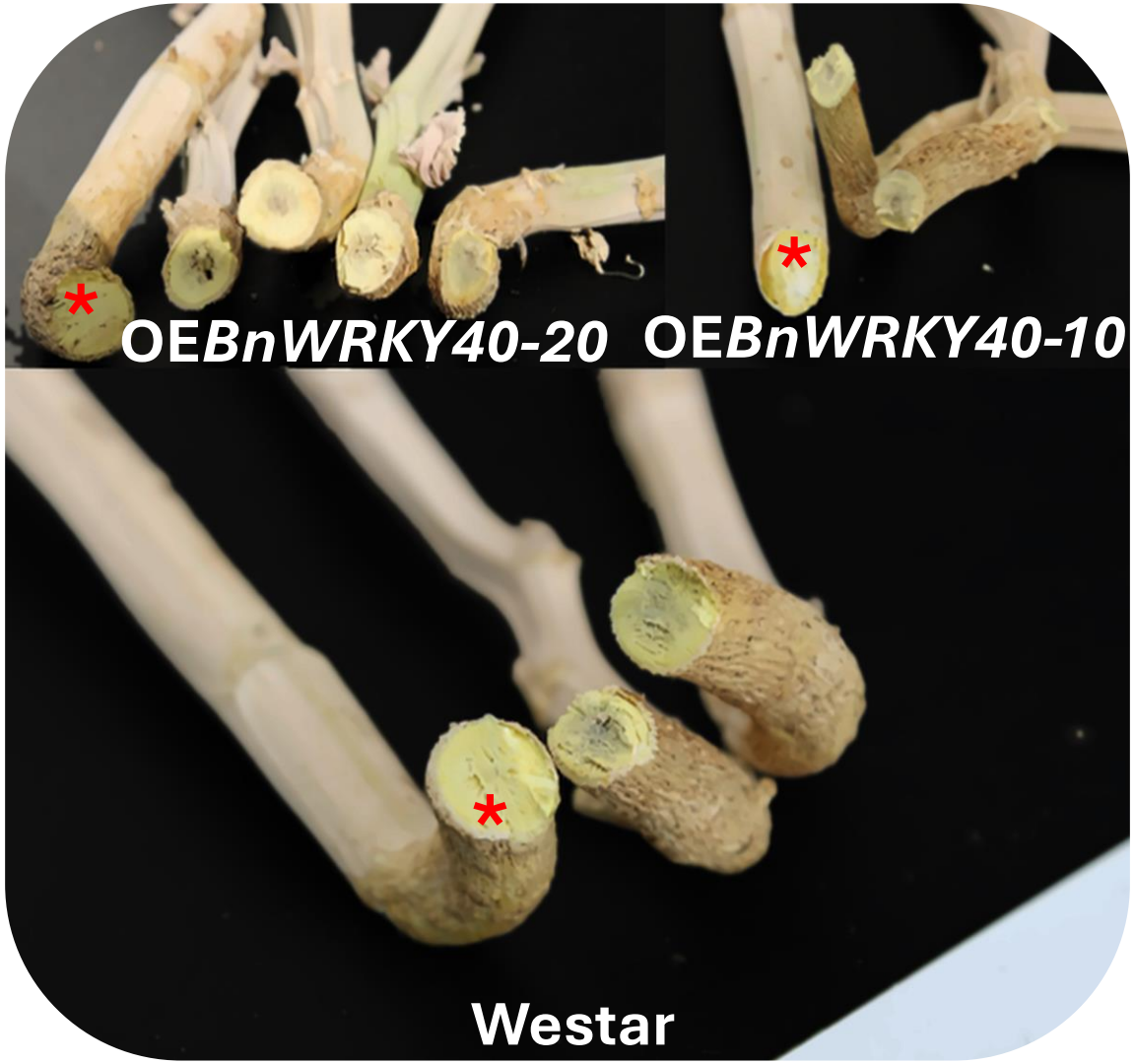




Disease Assessment at Adult Plant Stage by Root Dipping



Microsclerotia

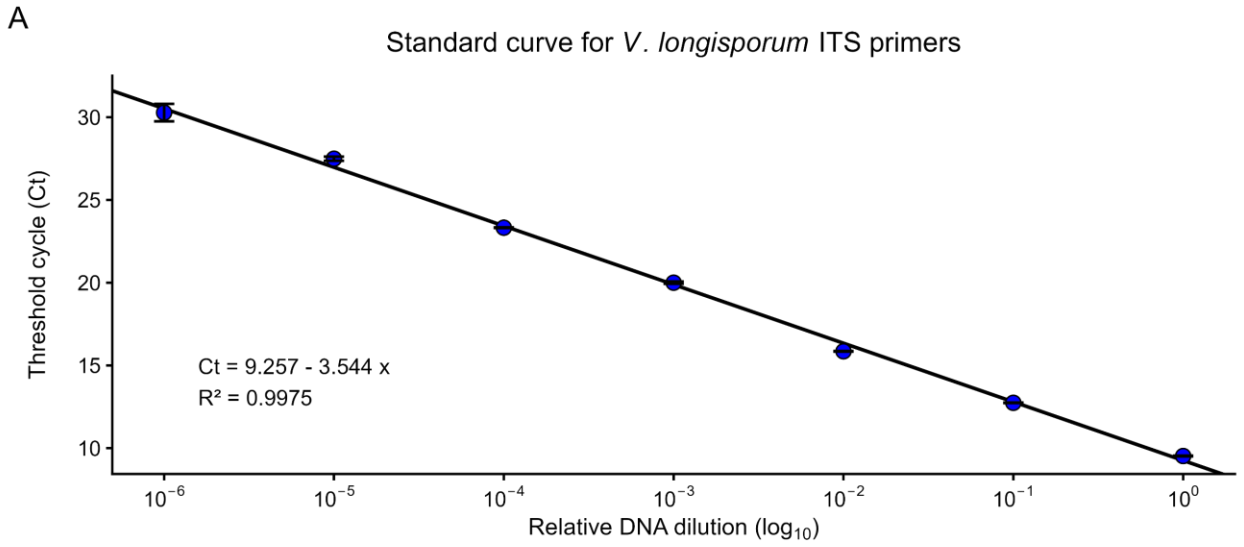


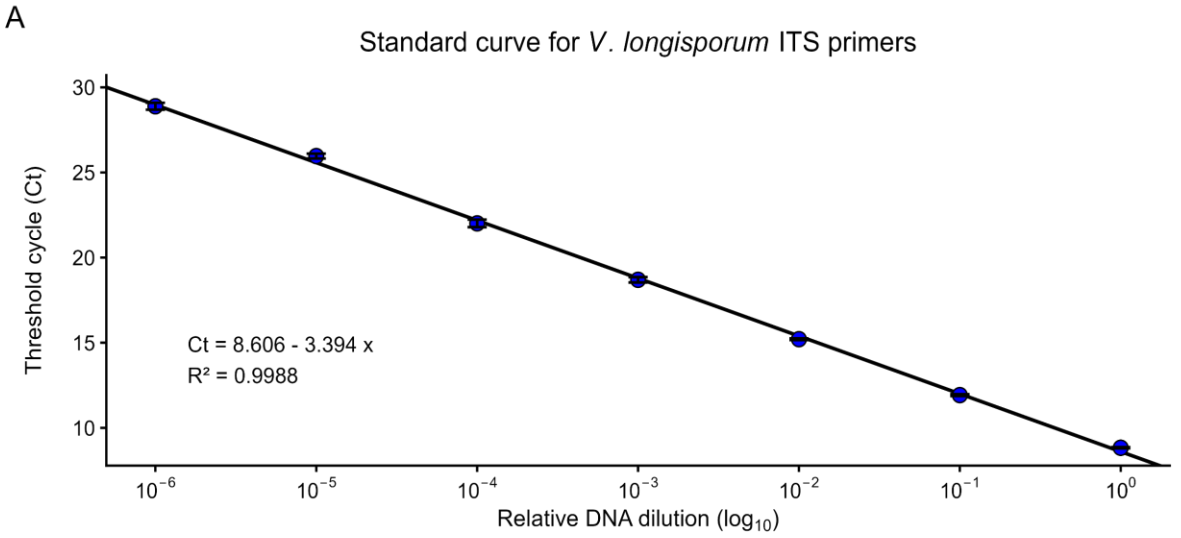


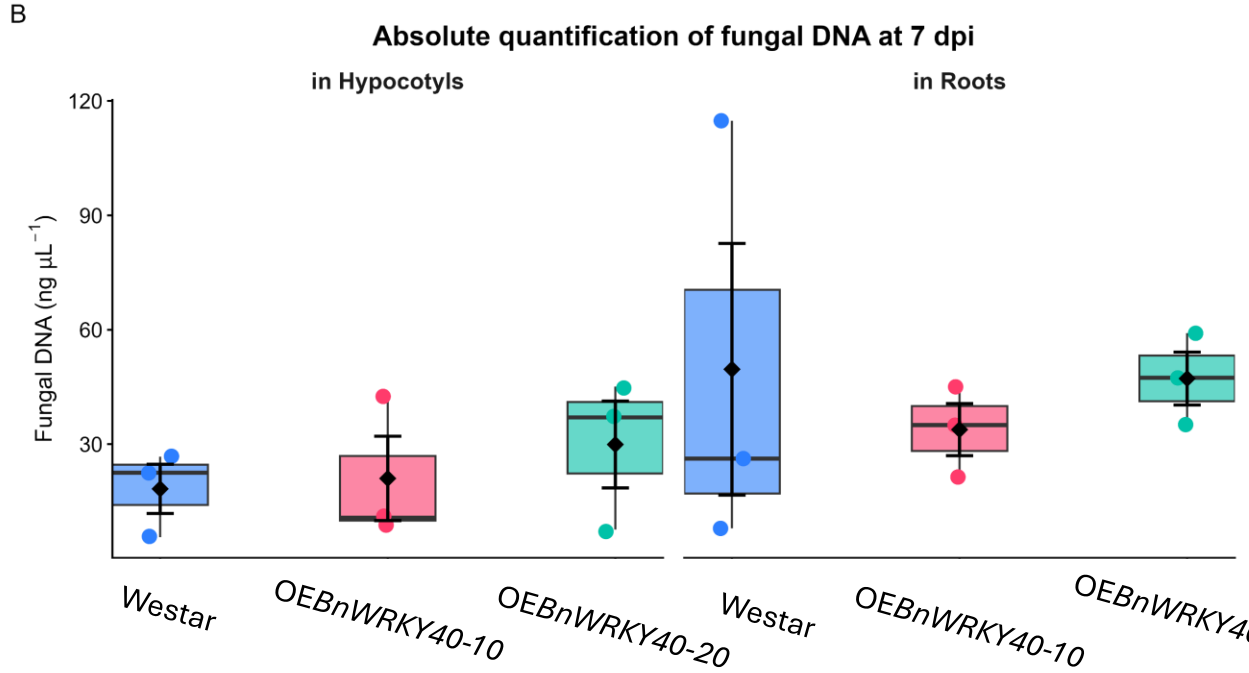
Inoculated canola plants

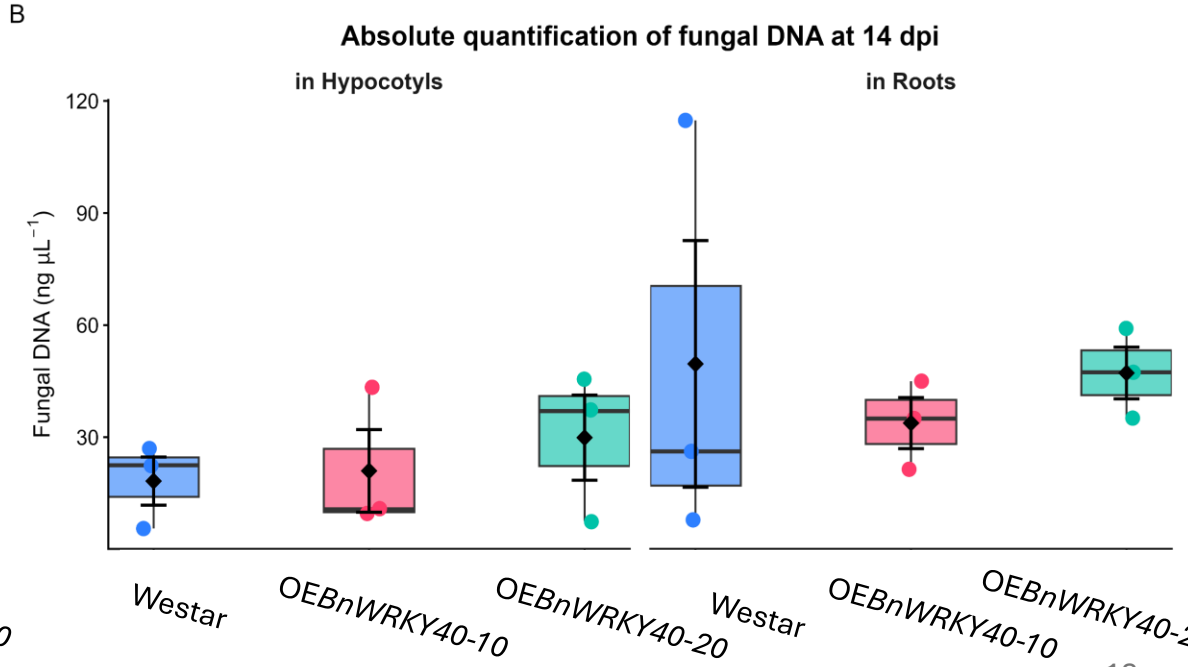
* Control Plant

V. longisporum DNA Absolute Quantification by qPCR

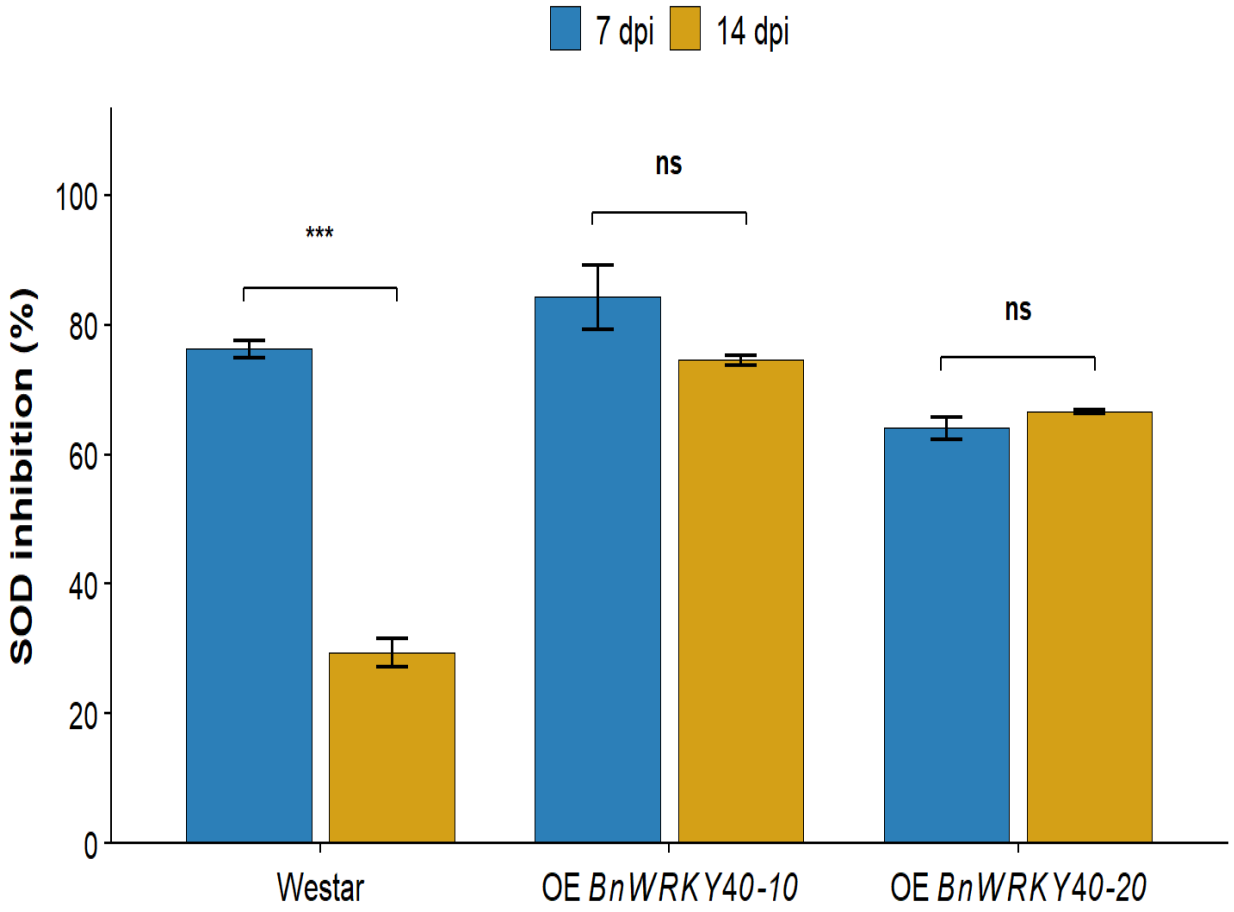




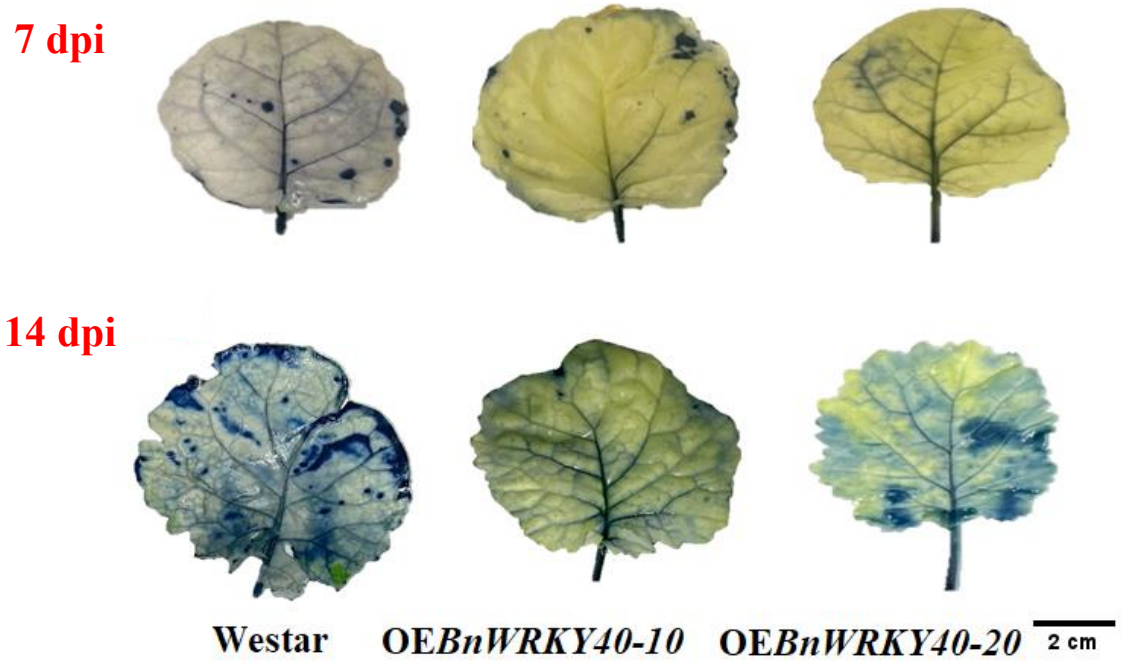




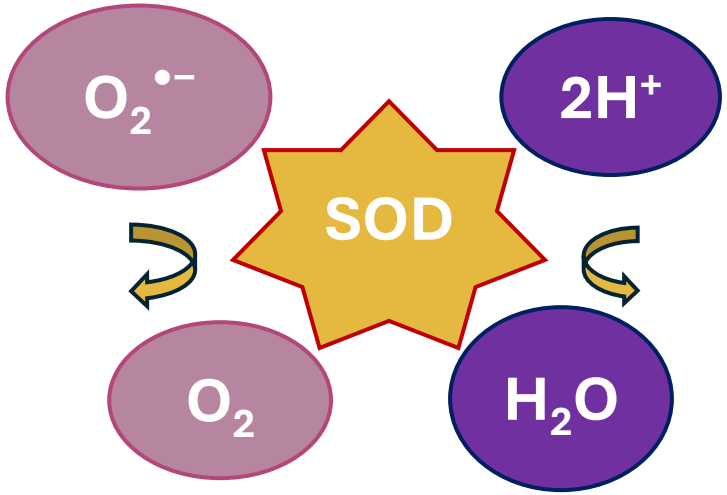
Root Superoxide Dismutase Activity and ROS Detection



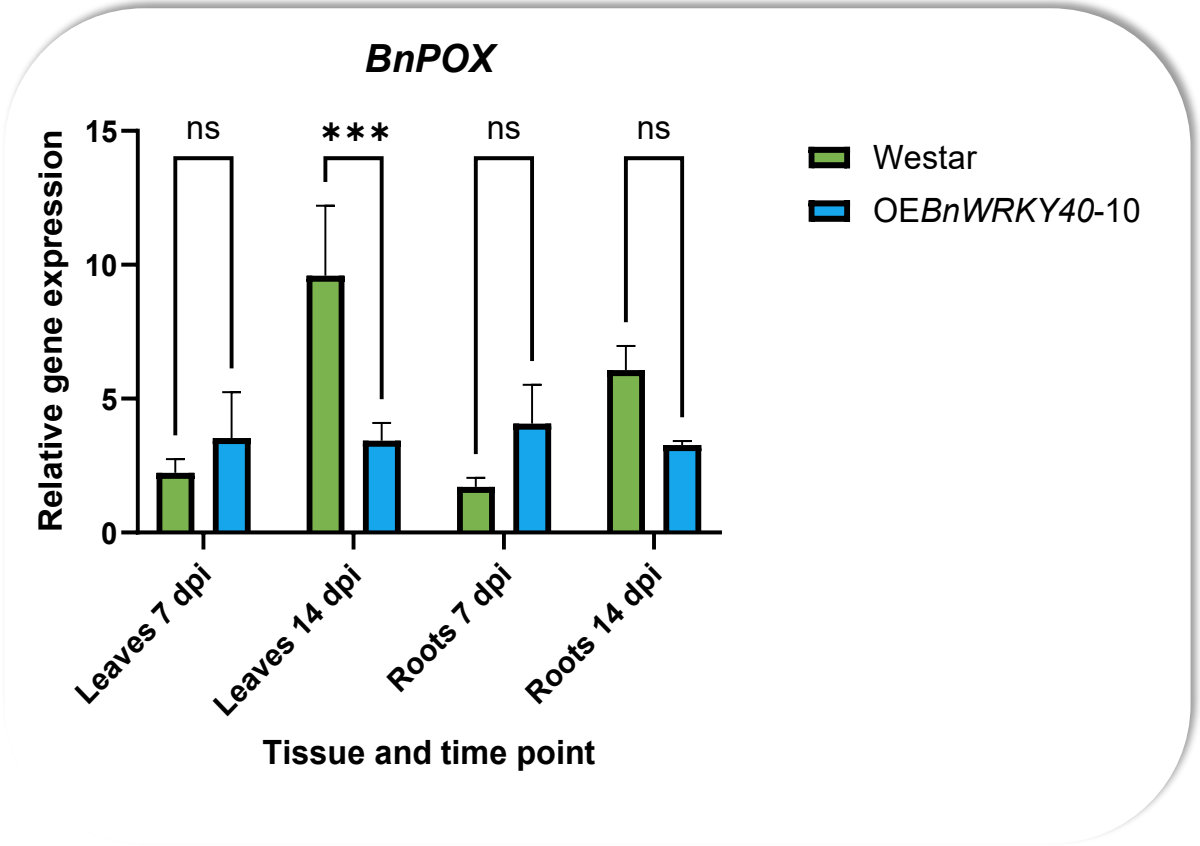
Root SOD activity in Westar and OEBn*WRKY40* overexpression lines after root-dipping inoculation

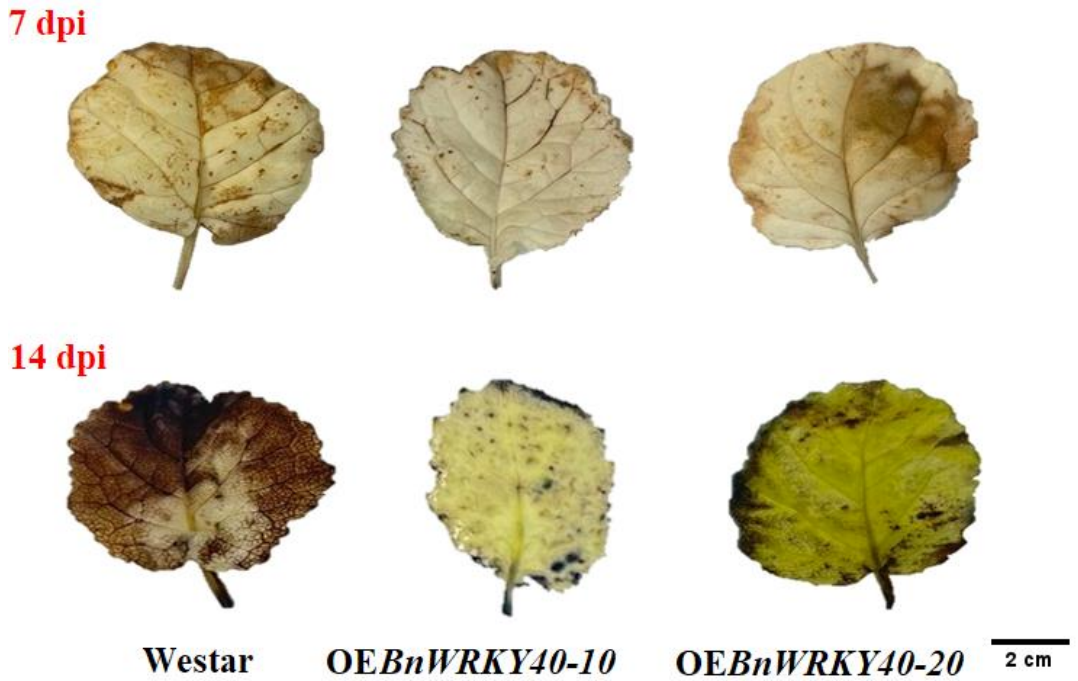


Leaf **NBT** staining following root-dipping inoculation

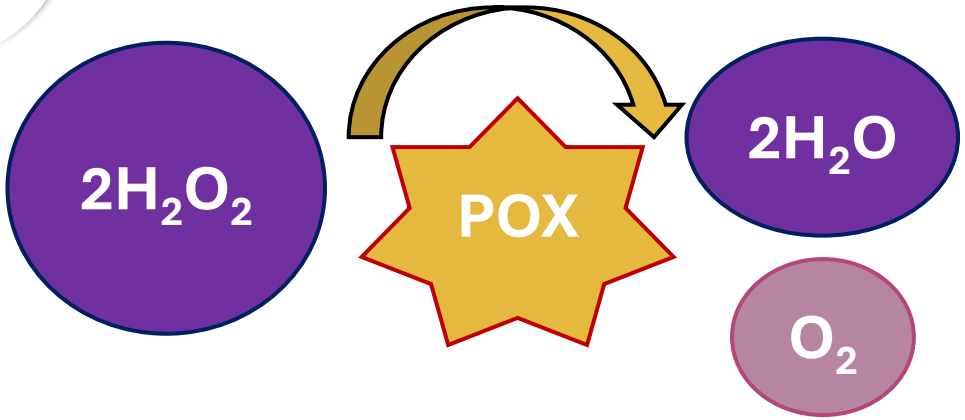


Oxidative Stress and H_2O_2 Detoxification



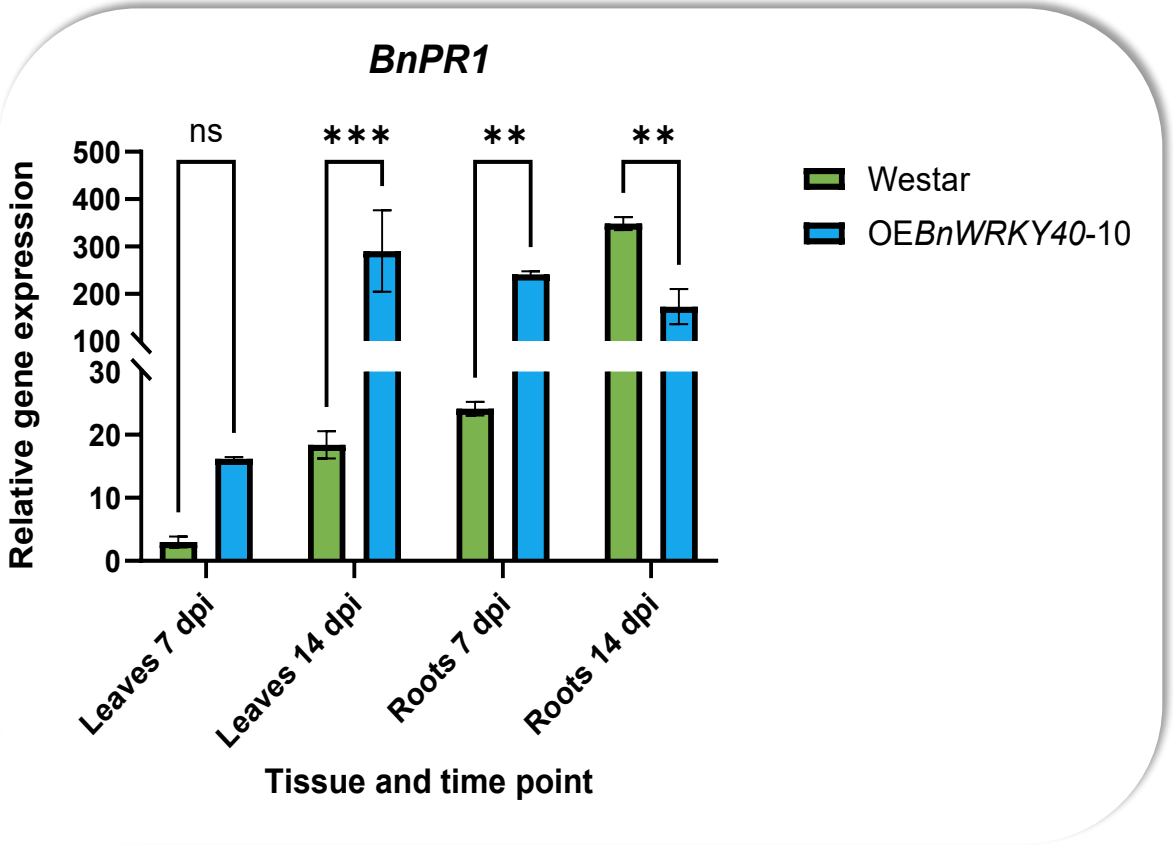


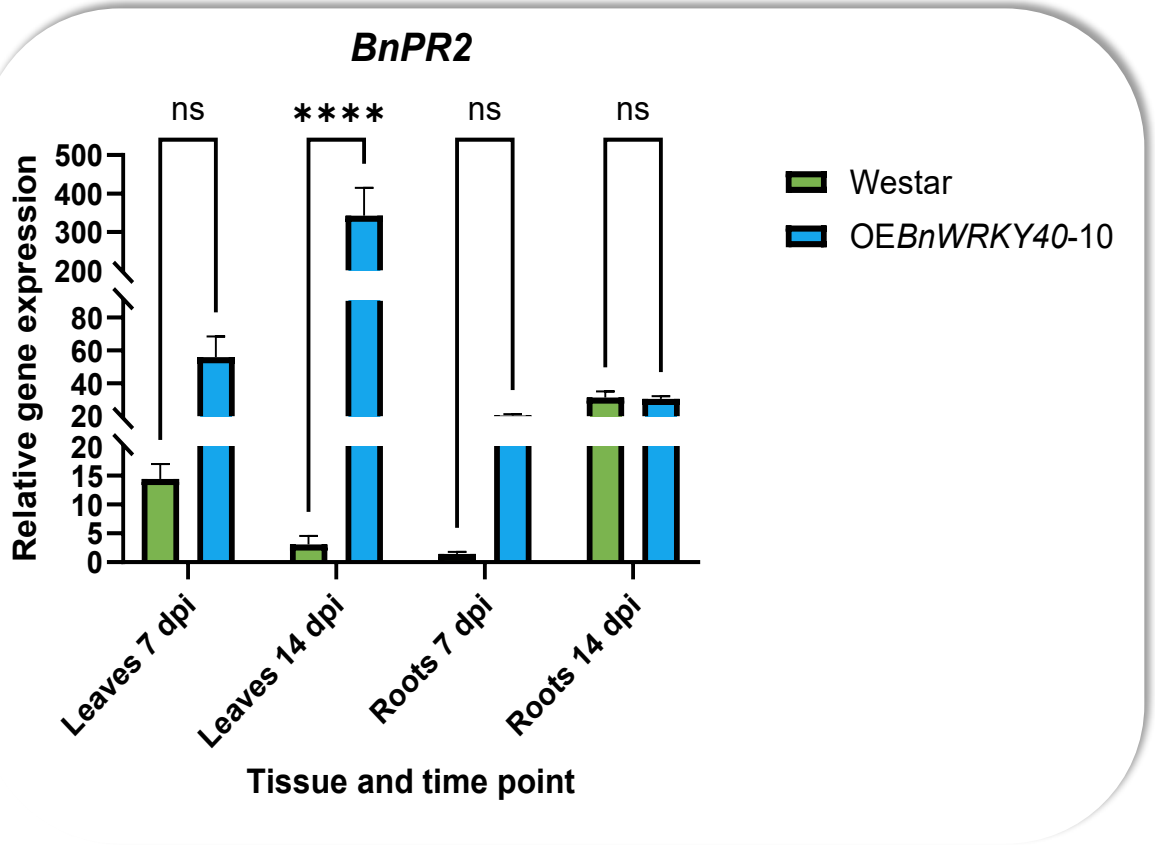
Leaf **DAB** staining following root-dip inoculation



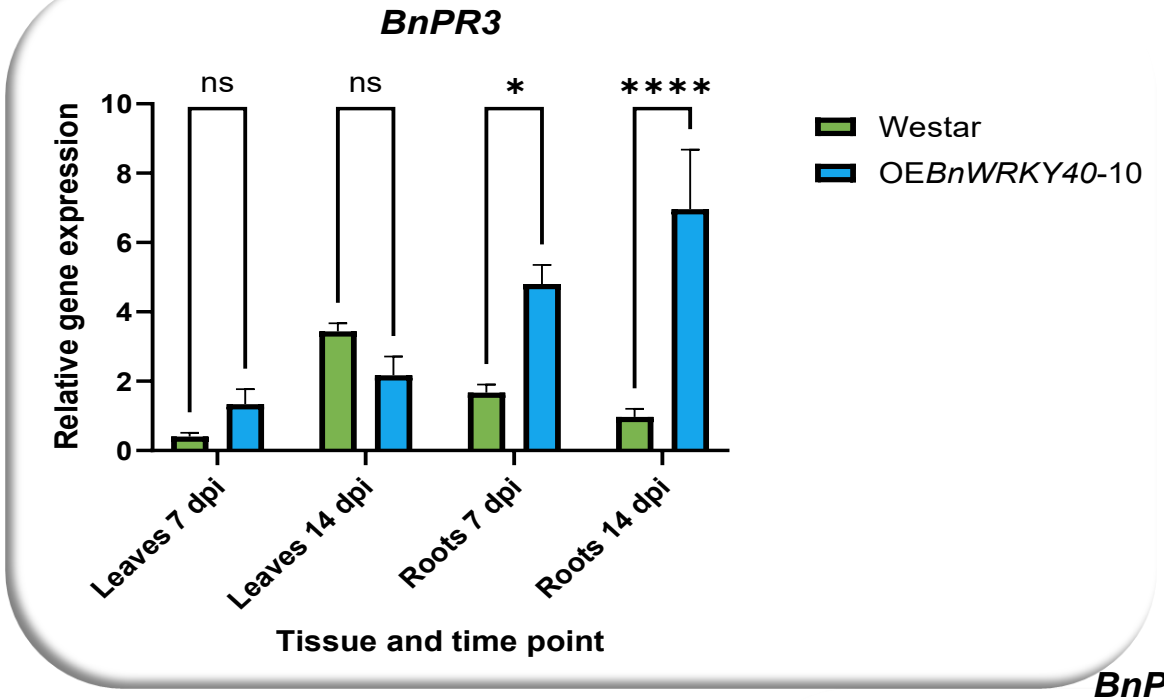
Defense-Related Gene Expression

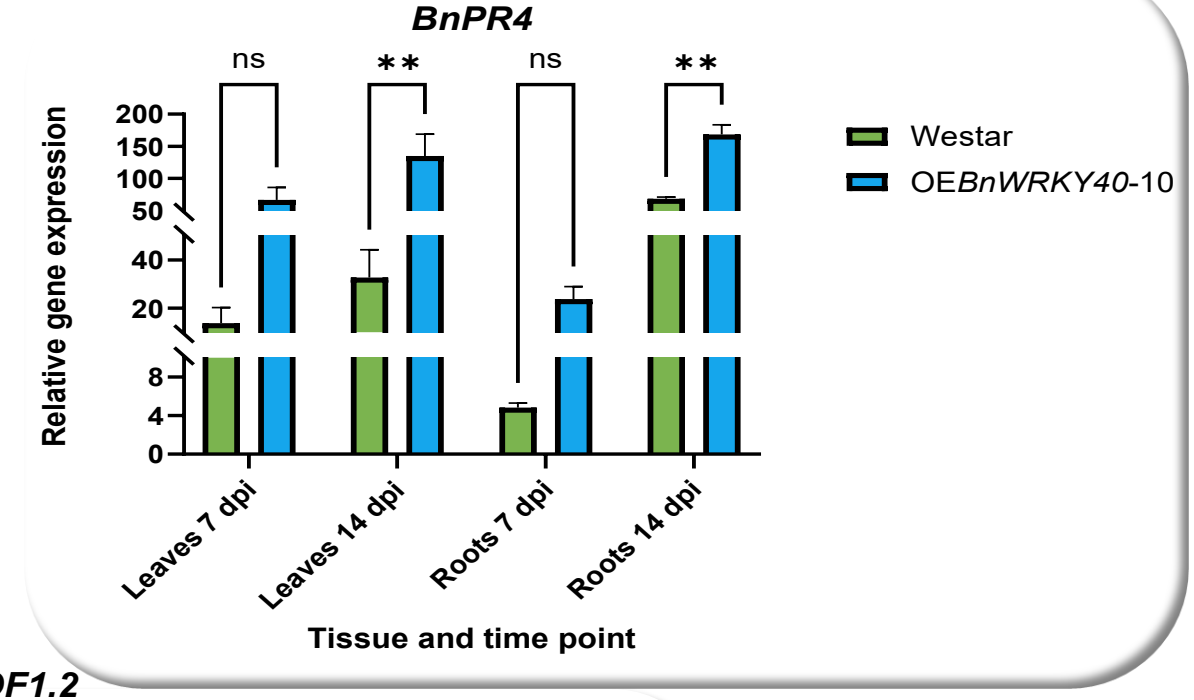
Salicylic acid (SA) mediated genes

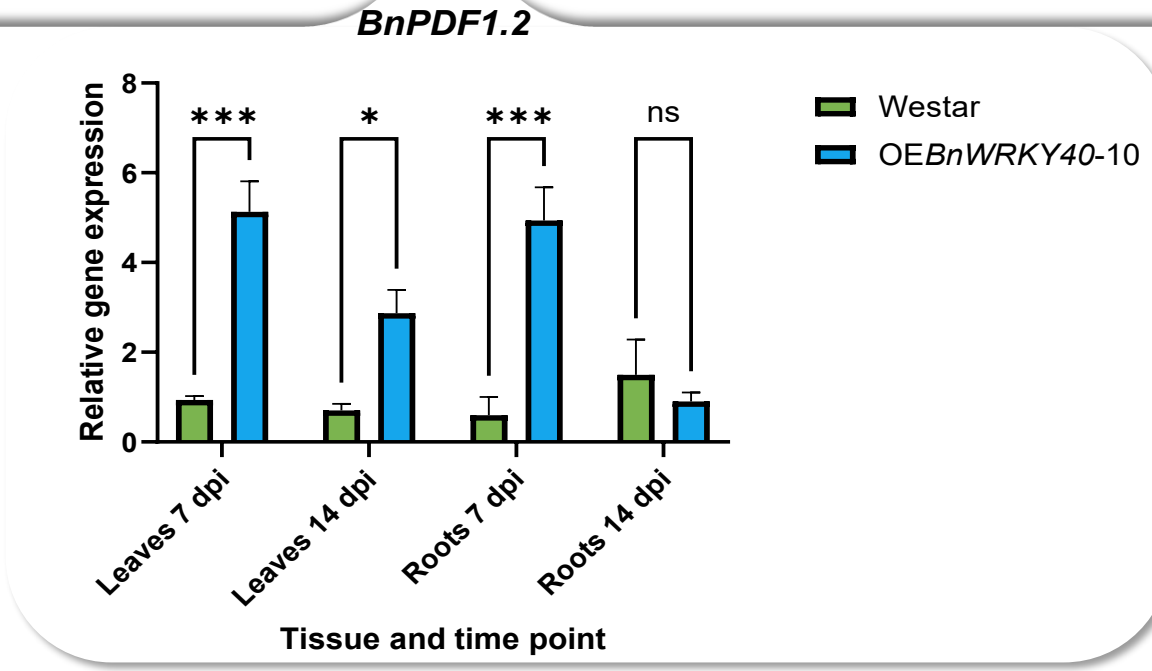




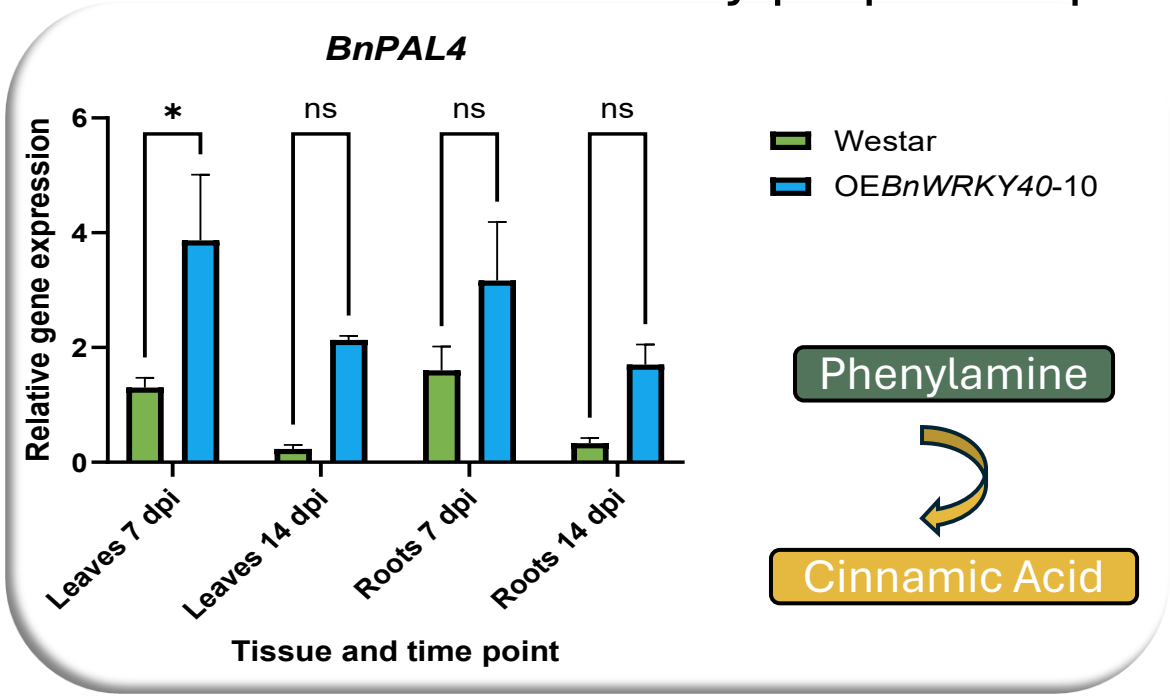
Jasmonic acid (JA) mediated genes

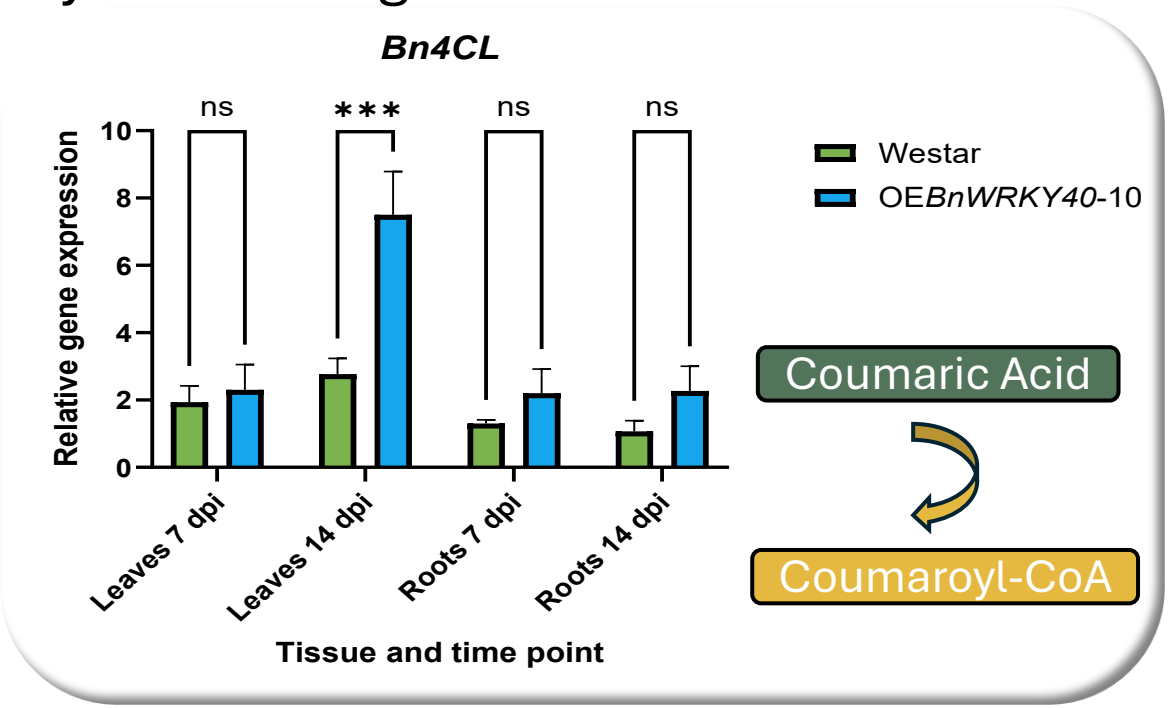


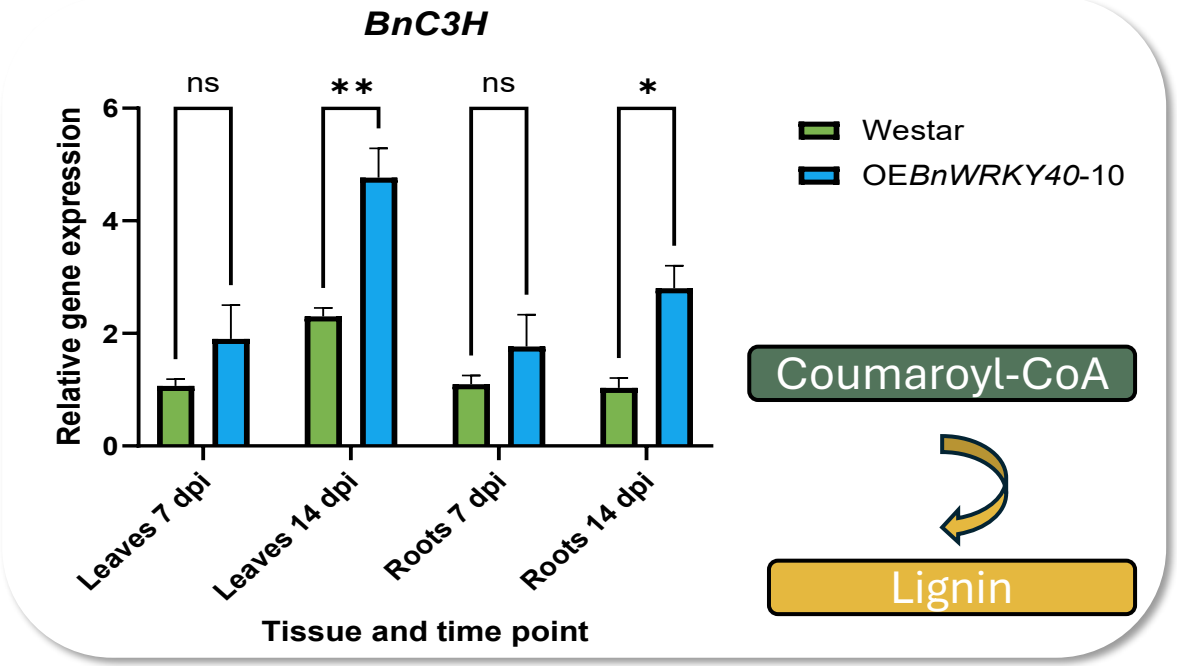


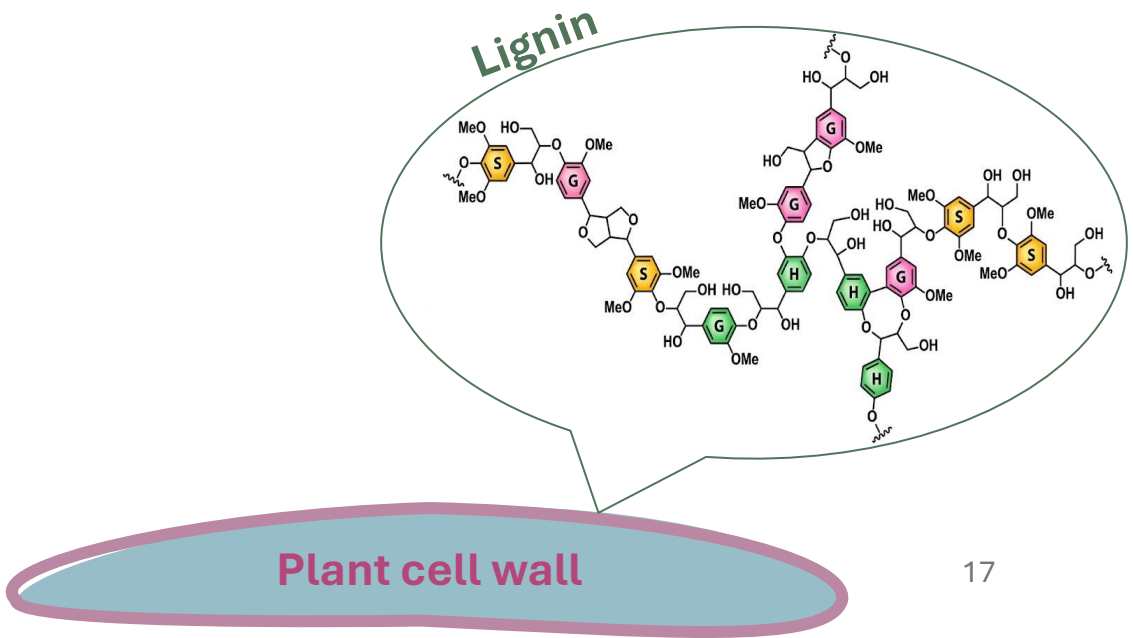


Phenylpropanoid pathway mediated genes











Take-Home Messages

Reduced disease severity: Both OE*BnWRKY40* lines showed trends toward reduced disease severity

Lower fungal DNA: Westar roots tended to contain more *V. longisporum* DNA, but differences were not significant

Enhanced defense gene expression: OE lines showed increased expression of genes involved in *SA/JA signaling* and *phenylpropanoid biosynthesis*

Different SOD patterns: SOD activity remained stable in both OE lines but declined in Westar by 14 dpi

Higher ROS in Westar: DAB and NBT staining showed greater ROS accumulation at 7 and 14 dpi

BnWRKY40
Defense responses

Acknowledgments

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Dr. Matthew Smith (WLU)
Dr. Yang Xu (University of Guelph)

Collaborator: Dr. Fei Liu, Henan University



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