Overexpression of *BnWRKY40* Confers Enhanced Resistance in *Brassica napus* Against *Verticillium longisporum* and *Sclerotinia sclerotiorum*

Presented by:

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CANOLA

Brassica napus (AACC: 2×19)

Primarily grown in Alberta, Saskatchewan and Manitoba

Uses of Canola

- Cooking oil
- Industrial uses
- Biofuel
- Animal feed

One of the most widely grown crops in Canada (¼ of all farm crop receipts)

About 90% of the canola grown in Canada is exported

Diseases of Brassica napus: *Verticillium stripe* and *Sclerotinia stem rot*

Verticillium stripe disease

- *Verticillium longisporum* is a soil-borne fungus that causes the vascular disease **Verticillium stripe**
- First reported in Manitoba in 2014
- Yield losses of up to **50–80%**
- The fungus spreads mainly through long-term survival structures known "**microsclerotia**"

Resource: Canola Council of Canada

Sclerotinia stem rot

- *Sclerotinia sclerotiorum* is a fungal pathogen that causes **Sclerotinia stem rot**
- Yield loss depends on environmental conditions - reach **up to 100%**
- Infection occurs when **microscopic airborne spores** called "*ascospores*", produced from apothecia, infect plants during the **flowering stage**

Resource: Canola Council of Canada

Symptoms of Verticillium Stripe and Sclerotinia Stem Rot

Verticillium stripe disease

- **Common symptoms:**
- Leaf chlorosis
- Early ripening
- Stunting
- Vertical yellow to brown stem stripes
- Necrosis
- Shredding of stem tissue

In mature plants, tiny black **microsclerotia** are visible on stem peels and continue to develop on infected plant debris after harvest.

Sclerotinia stem rot

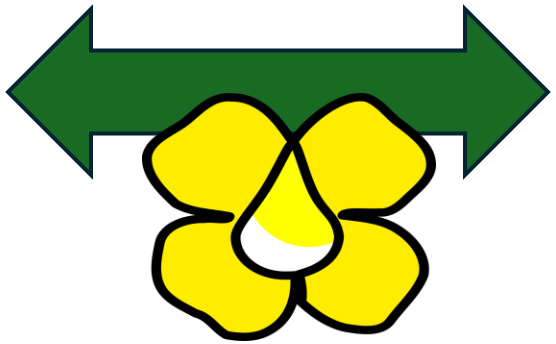
- **Common symptoms:**
- Soft, water-soaked white to grey lesions on leaves and stems
- Stems become brittle; the epidermis may shred
- Bleached and shredded stems at maturity
- Premature ripening of plants
- Wilting and lodging

Objectives

Verticillium stripe disease

Sclerotinia stem rot

To evaluate the *BnRKY40* overexpression lines in *B. napus* (derived from Westar) in response to *V. longisporum* and *S. sclerotiorum* infections



Materials and Methods

Plant material

Two *BnWRKY40* overexpression lines derived from the Westar background:

OEWRKY40-10 and **OEWRKY40-20**

Westar (wild type)

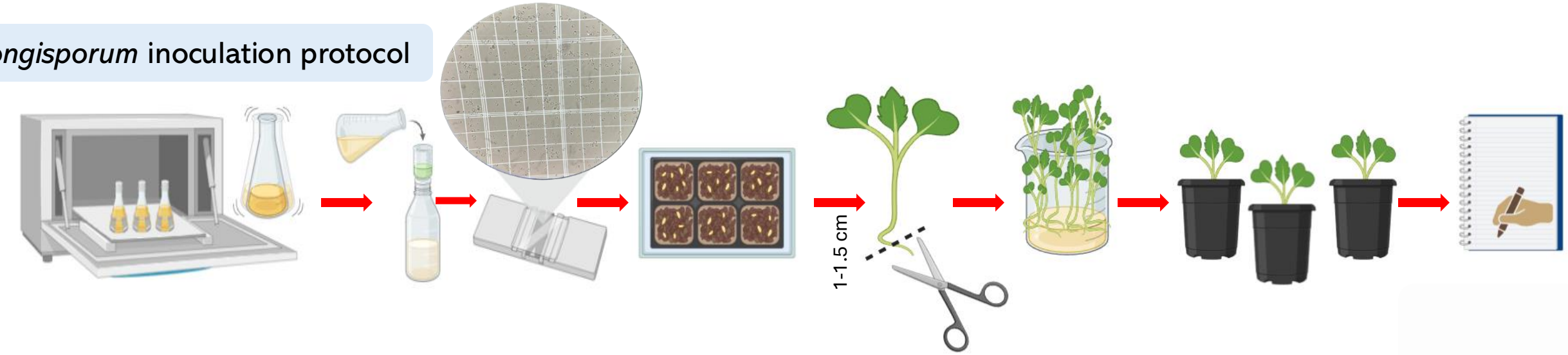
Strains

Verticillium longisporum *and* ***Sclerotinia sclerotiorum*** strains isolated from diseased canola plants in Manitoba

Pathogen inoculation and *V. longisporum* progression analysis by qPCR

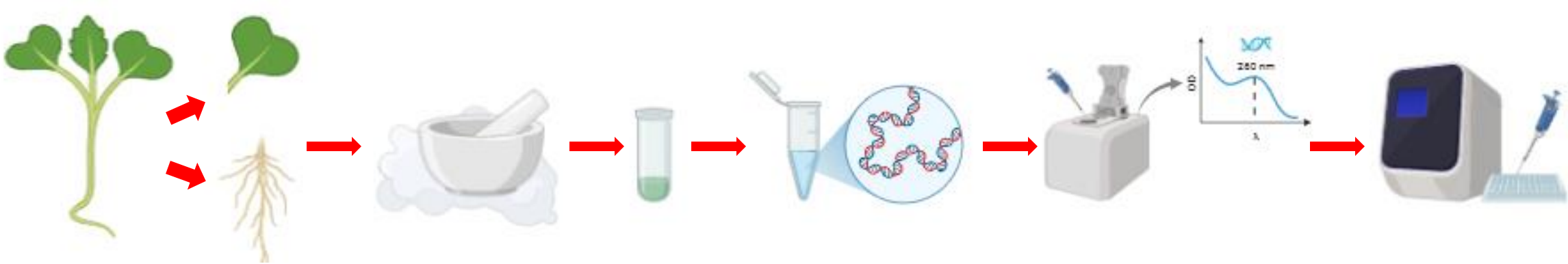
V. longisporum inoculation protocol

1.



V. longisporum DNA quantification

2.

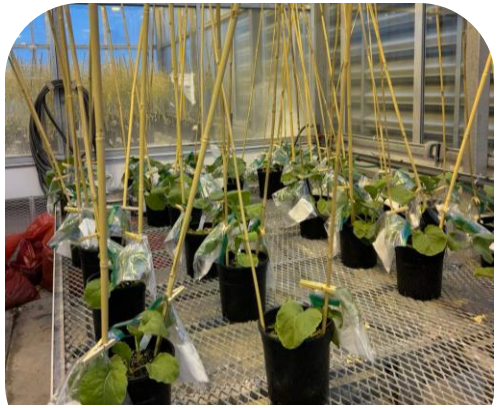


Sclerotinia sclerotiorum inoculation and infection evaluation

Leaf inoculation with *Sclerotinia sclerotiorum*

Edge plugs of *1-week-old S. sclerotiorum* grown on agar

Place agar plugs in adaxial leaf surfaces



Control: PDA plug without fungal mycelium

Measure lesions at 24 and 48 hours post-inoculation (hpi)

Stem inoculation with *Sclerotinia sclerotiorum*

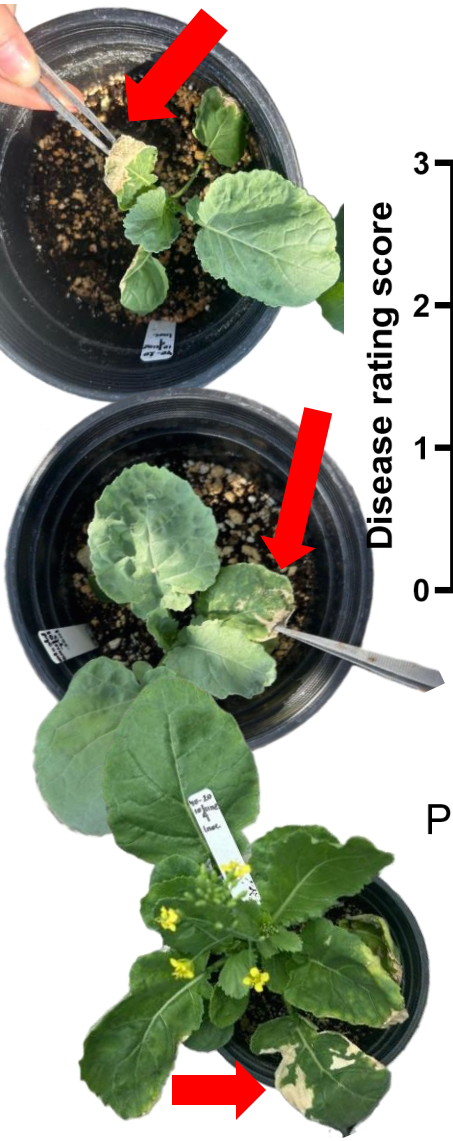
Cut plugs with a 7 mm diameter cork borer

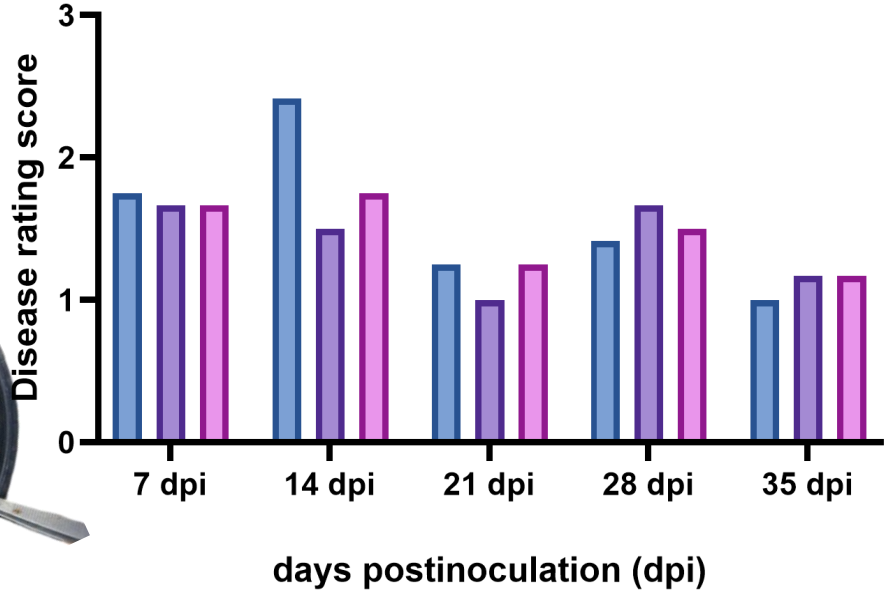
Place one plug, twist two ends of the Parafilm ribbon

Measurement of stem lesions at 5-7 dpi

RESULTS

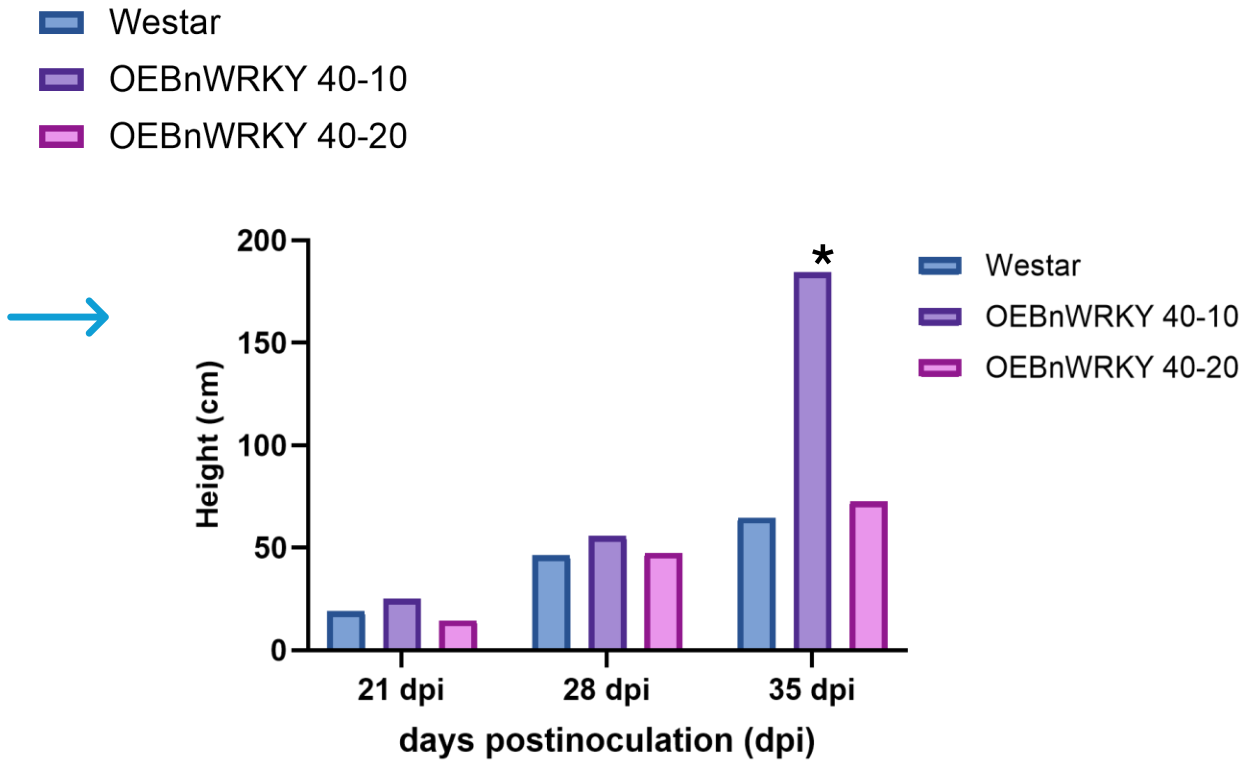
Verticillium longisporum disease progression





Plant disease evaluation based on a 1–9 scale

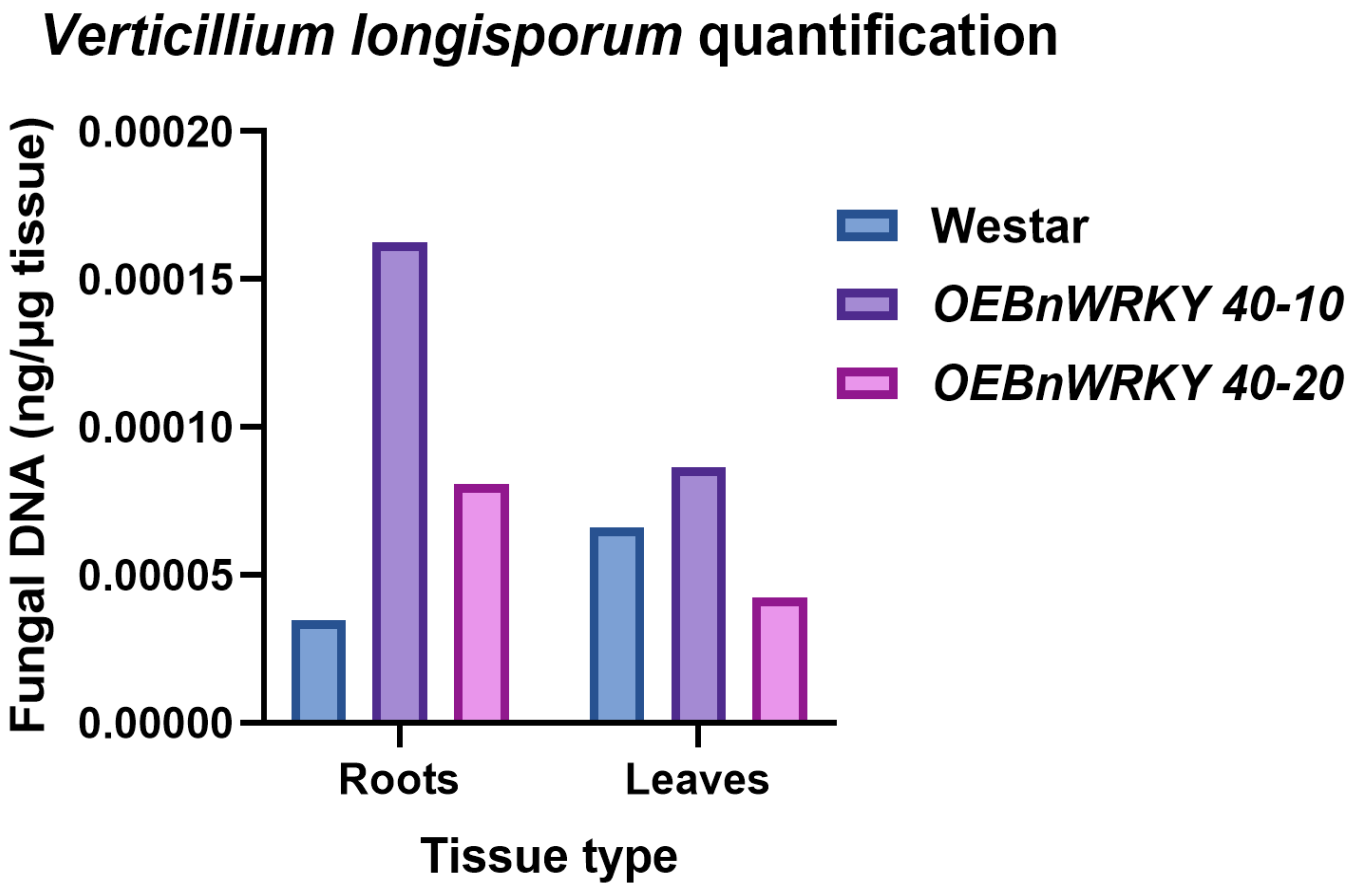
Fig. 1 Disease rating score



Height comparison of plants over time

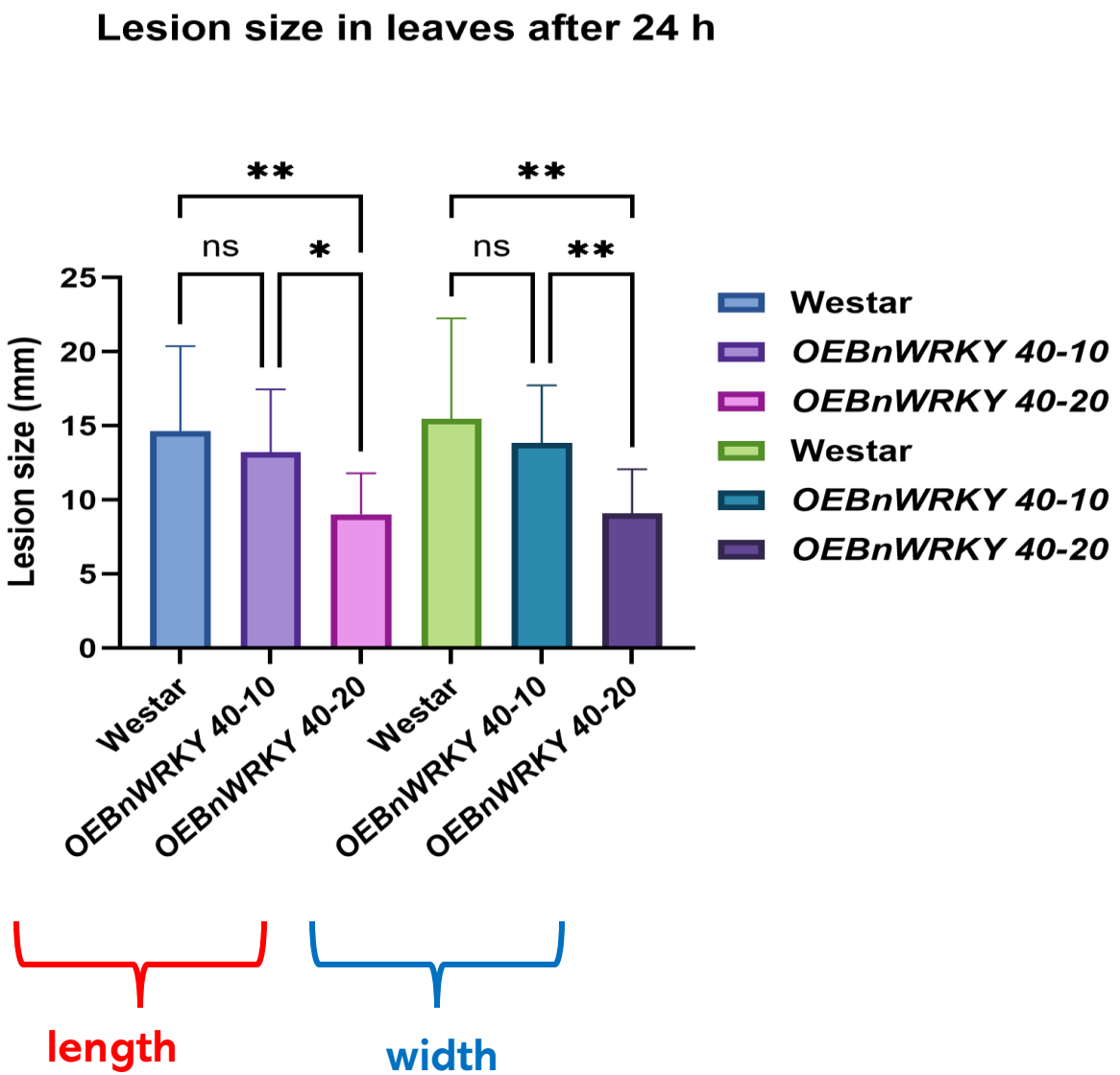
Fig. 2 Plant height

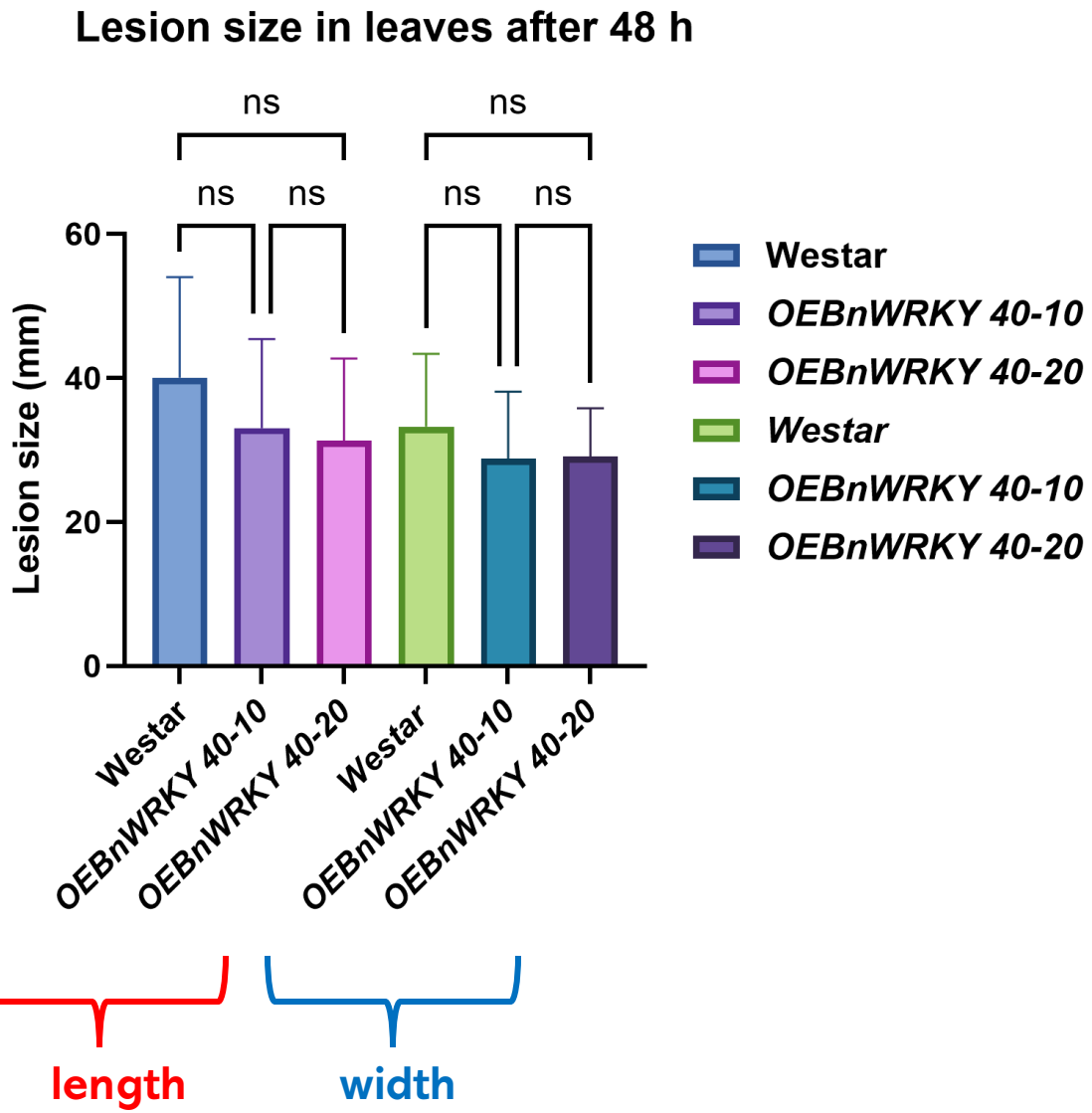
Quantification of *Verticillium longisporum* DNA

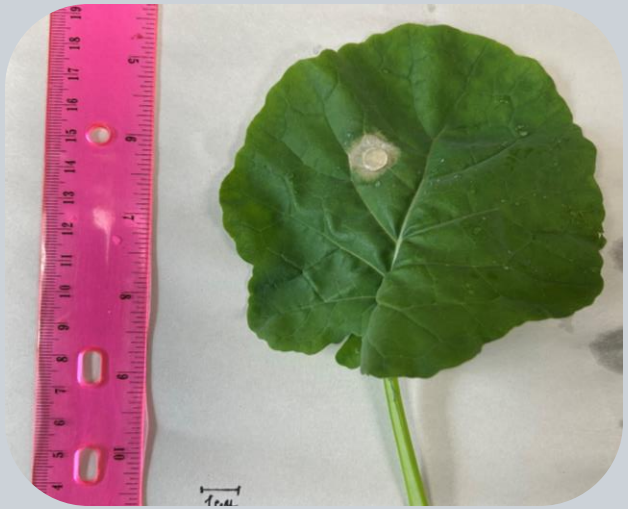


Overexpression lines showed higher fungal gDNA levels than Westar, supporting earlier phenotypic results and indicating that their immune response is likely stronger at more early infection stages rather than at 35 dpi.

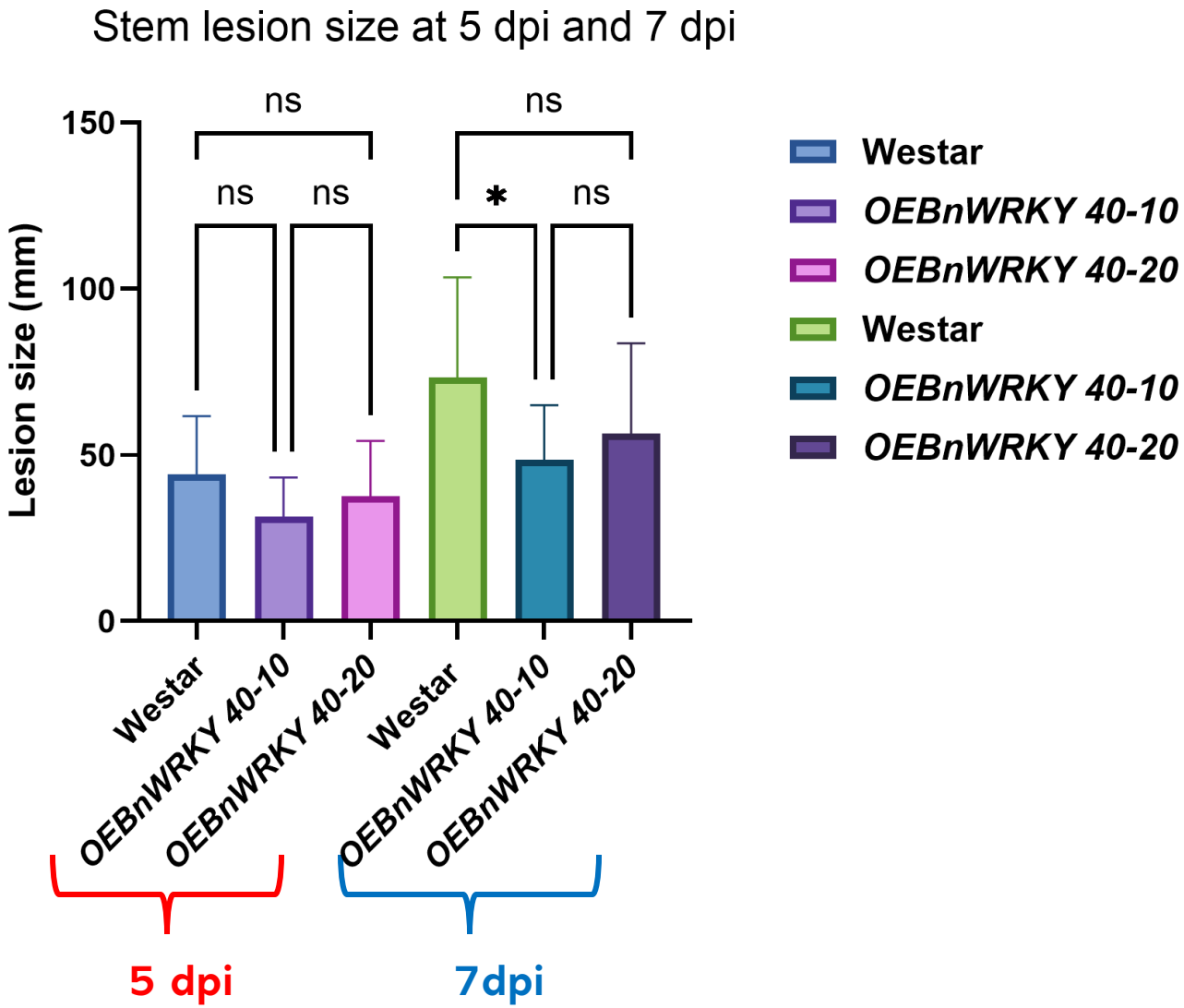
Disease evaluation of *Sclerotinia sclerotiorum* in leaves





	Westar	*OEBnWRKY 40-10*	*OEBnWRKY 40-20*
24 h			
48 h			

Disease evaluation of *Sclerotinia sclerotiorum* on stems



Future research

Defense-related gene expression in *B.napus* during infection by *V. longisporum* and *S. sclerotiorum*

Investigating the impact of rising CO_2 on the cultural growth and pathogenicity of *S. sclerotiorum* against *B. napus*

Western blot analysis of WRKY40 family protein expression in transgenic canola lines in response to pathogen infections

Phenotypic investigation of canola wild-type and overexpression lines (mono- and polyunsaturated fatty acids) under disease pressure

Acknowledgments

Advisory Committee:

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The ZOU LAB
MOLECULAR PLANT PATHOLOGY

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