

# Investigating the Function of *BnNAC19* as a Negative Regulator in *Brassica napus* and *Verticillium longisporum* Interaction

## Introduction

- *Verticillium longisporum* is the causal agent for Verticillium stripe disease in *Brassica napus* (Canola), resulting in yield losses of up to 50% (Depotter *et al.* 2016).
- *BnNAC19*, a transcription factor, has previously been identified to enhance resistance against another important fungal pathogen in canola, blackleg (Zou & Fernando. 2024).
- My previous research has indicated that the *BnNAC19* is a negative regulator of the Salicylic (SA) and Jasmonic (JA) acid defense signalling pathways in canola against Verticillium stripe.
- Pathogenesis-related gene 1 (*PR1*) and *PR2* have been frequently studied to participate the SA and JA signalling pathways.

## Objectives

The objective of this study aims to further investigate and confirm the function of *BnNAC19* in response to *V. longisporum* infection.

## Materials and Methods

- “Westar” is a common variety of canola which is susceptible to most diseases including Verticillium stripe.
- Two overexpressed lines of *BnNAC19* will be used
  1. OEBnNAC19-10
  2. OEBnNAC19-05
- A conidial suspension ( $2 \times 10^6$ ) will be prepared. Roots will then be wounded using sterile scissors, and the Root-dipping method will be used to inoculate two-week-old seedlings (Cui *et al.* 2022).
- Phenotypic analysis will be conducted at 1, 3, 7, 14, 90 and 105 days post inoculation (dpi). A score will be given based on a scale of 0-6 for seedlings and 0-4 for adult plants, respectively (Cui *et al.* 2022).
- Leaves and roots will be sampled at 7 and 14 dpi to further assess the expression of several pathogenesis-related (*PR*) genes by qPCR.

## Results

- *Verticillium longisporum* causes disease in canola seedlings and adult plants (Fig 1&3).



Fig 1. Scores are assigned based on the severity of symptoms, with 0 indicating a healthy plant and 6 representing a plant that has died.

- Overexpression of *BnNAC19* in canola resulted in greater stunting, necrosis, and yellowing of the cotyledons and leaves in seedlings. More serious disease symptoms were observed in overexpressed *BnNAC19* transgenic lines (Fig 2&3).

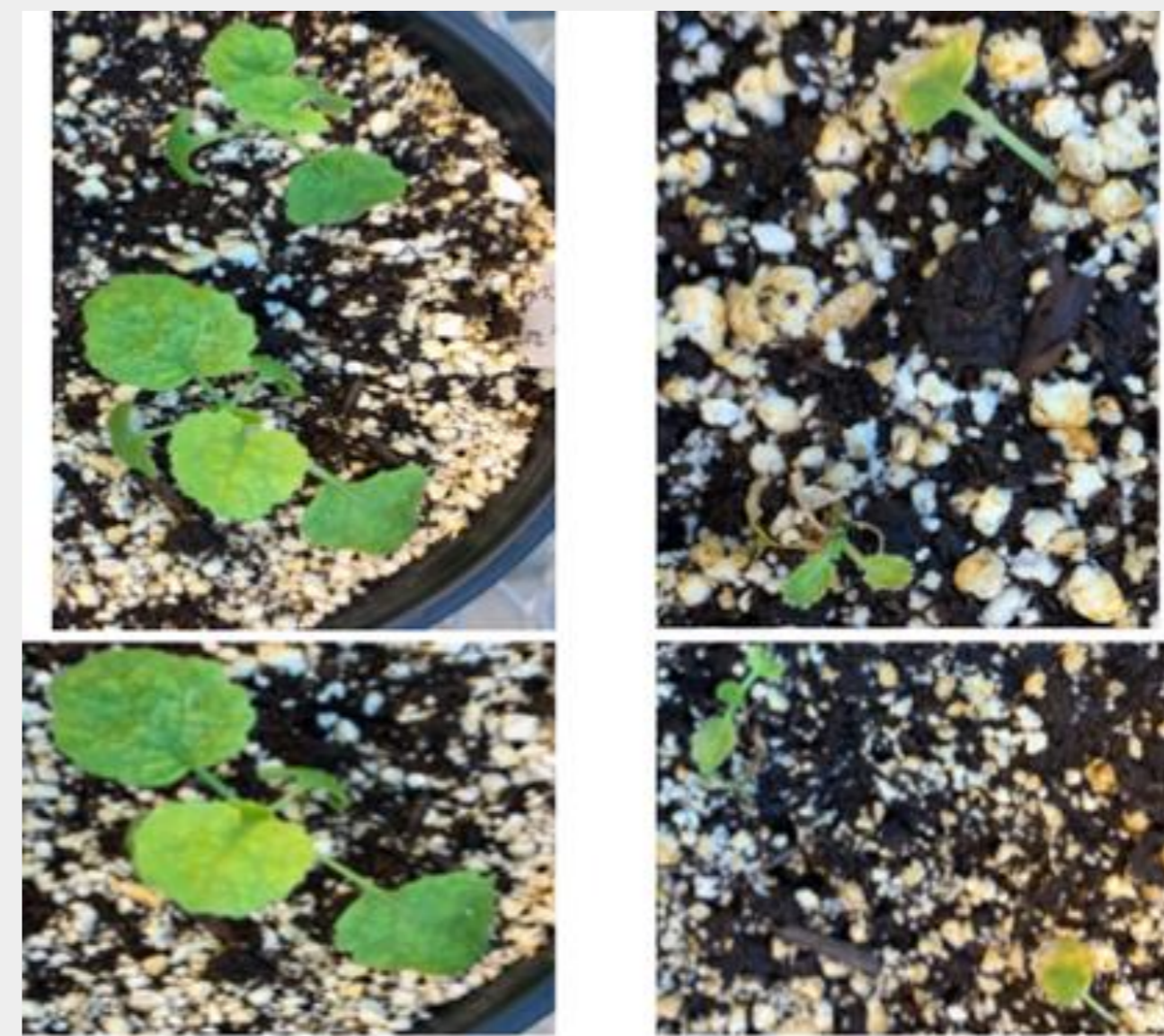


Fig 2. Westar seedlings (left) and OEBnNAC19 seedlings (right) both at 14 dpi (days post-inoculation).



Fig 3. Inoculated OEBnNAC19 adult plant (left) and water control OEBnNAC19 plant (right).

- Gene expression analysis indicated that *PR1* gene was highly induced by *V. longisporum* infection at 7 and 14 dpi in overexpression of *BnNAC19* canola transgenic plants. However, *PR2* gene displayed suppressed expression at 7 and 14 dpi in response to *V. longisporum* infection (Fig 4).

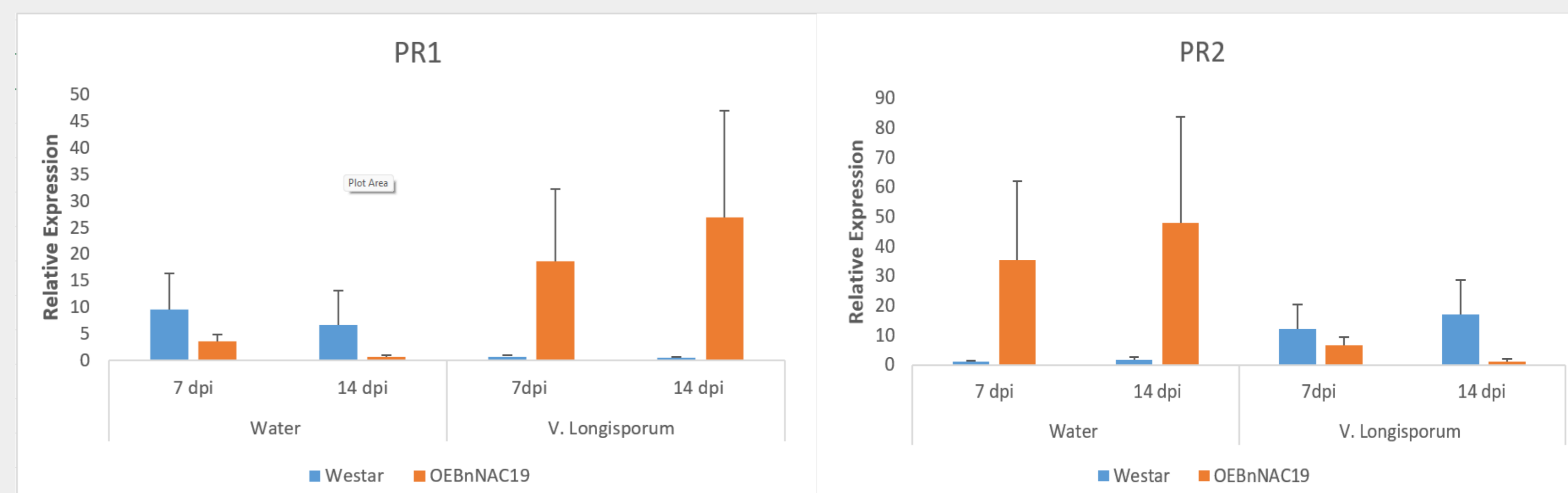


Fig 4. Relative expression of *PR1* and *PR2* in both OEBnNAC19 and Westar. Data was collected from both water control and inoculated plants at 7 and 14 dpi.

## Future work

- Confirm negative regulatory function of *BnNAC19* transcription factor.
- Gain knowledge on gene expression and antioxidant activity in OEBnNAC19 transgenic lines.

## References

- Cui, J. *et al.* (2022). *Canadian Journal of Plant Pathology*, 45, 92-102.  
Depotter, J, *et al.* (2016). *Molecular Plant Pathology*, 17, 1004-1016.  
Zou, Z., & Fernando, W. G. D. (2024). *Plant Pathology*, 73, 104-114.

## Acknowledgments

