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**Lectin genes,
concanavalin, curculin and hevein,
enhance resistance to the fungal pathogen
Sclerotinia sclerotiorum in *Brassica napus***

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Outline

- Sclerotinia phenotyping method
- Discovery of the genes
- Selection of gene copy for cloning and transformation
- Effect on sclerotinia phenotype
- Expression studies for each gene
- Likely function of concanavalin, curculin and hevein proteins

Sclerotinia phenotyping method simulates natural infection



Natural infection occurs when ascospores form mycelium on fallen petals and penetrate into the stem



Mycelium is grown on glucose agar, and 5-7 mm plugs are attached to the stem with Parafilm.



Susceptible phenotype



Resistant phenotype

B. napus germplasm

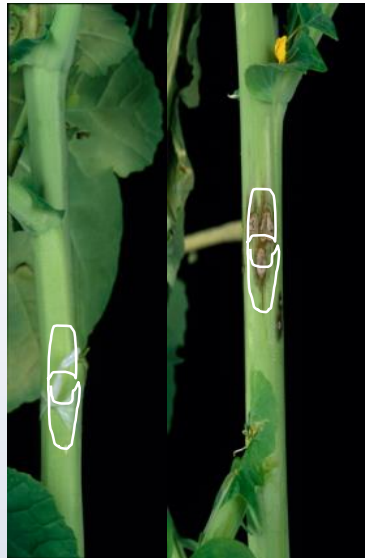


Resistant progenies derived
from variety Zhongyou 821
(China)

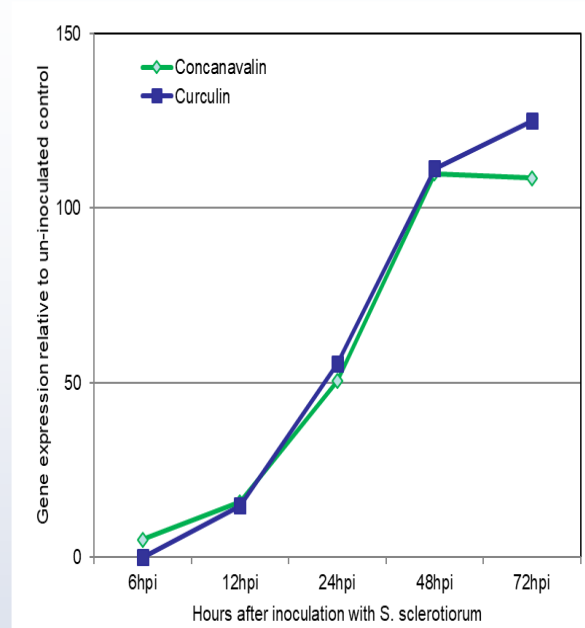


Susceptible variety Westar

Global gene expression study in Zhongyou 821



Zhongyou 821 Westar



Method

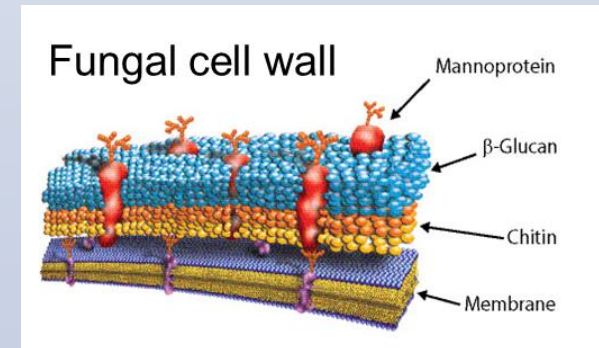
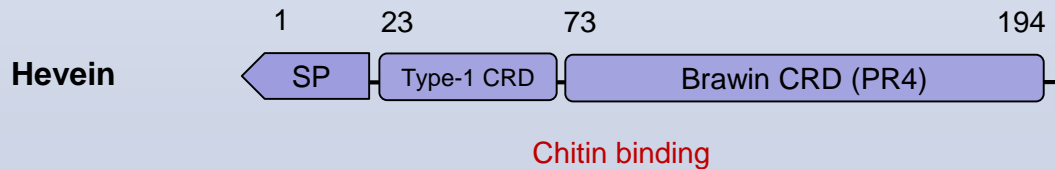
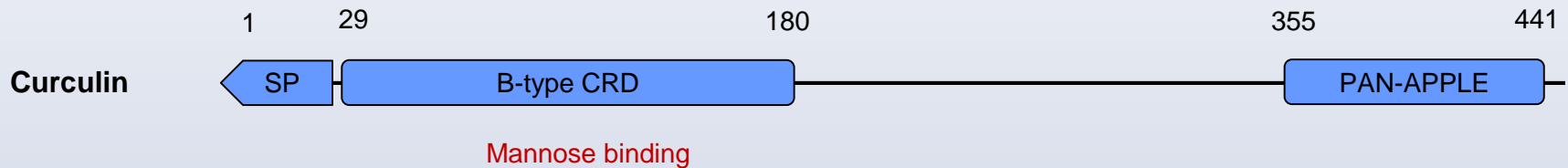
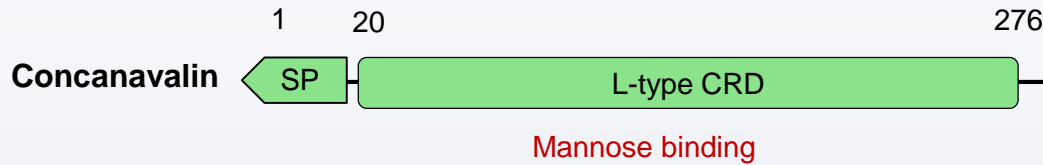
- mRNA isolation
- cDNA libraries
- Hybridized to 15,000 *B. napus* gene array

Result

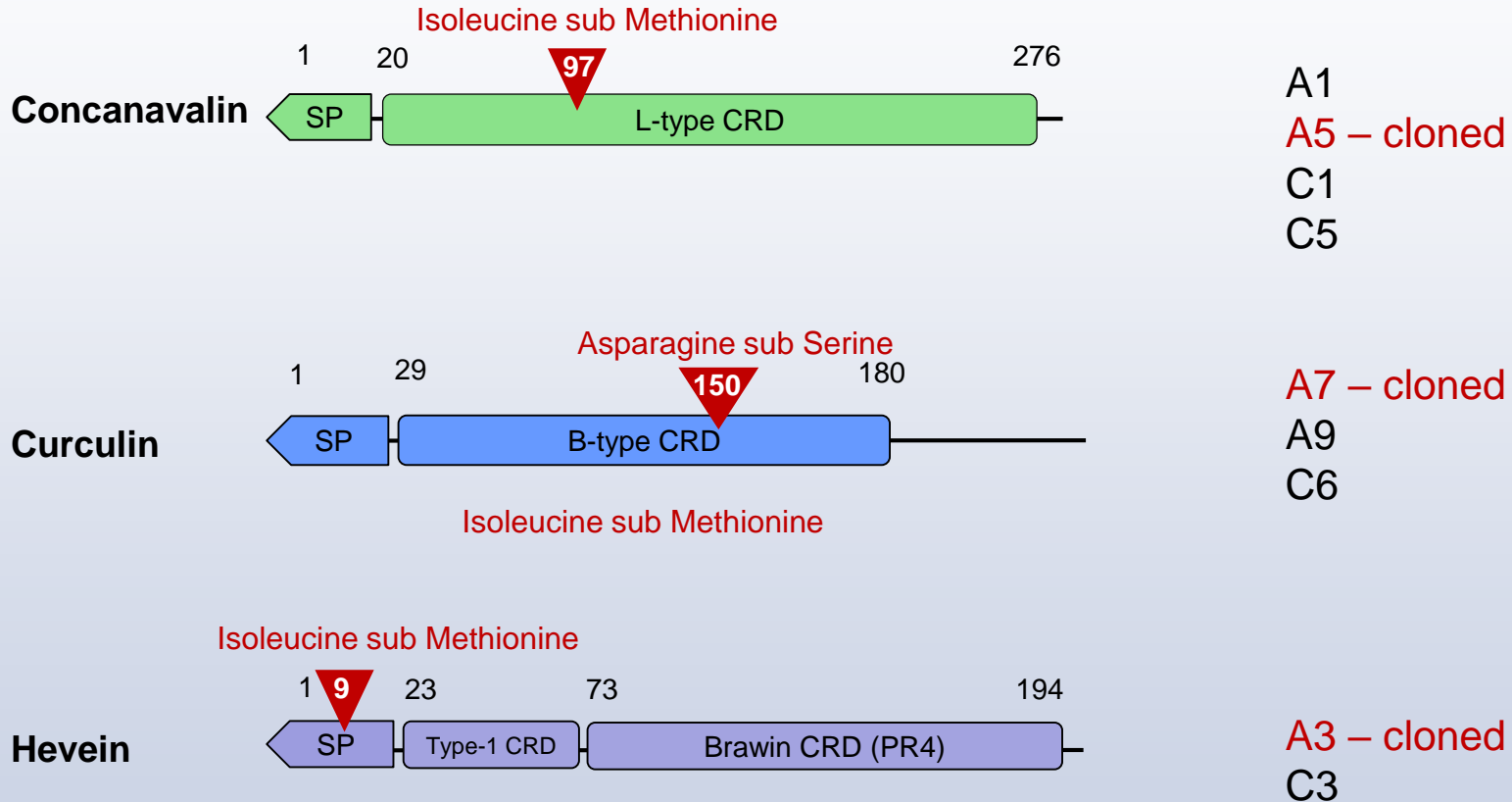
Lectin genes, *Concanavalin* and *Curculin*, were highly upregulated during sclerotinia infection

Lectin gene structure and binding specificities

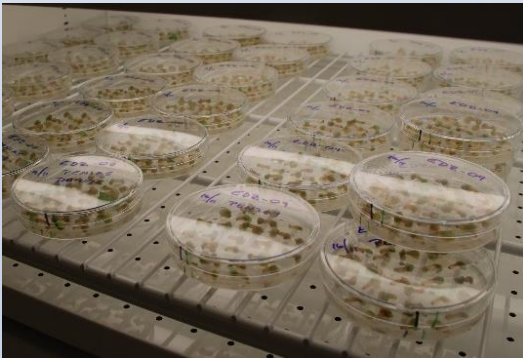
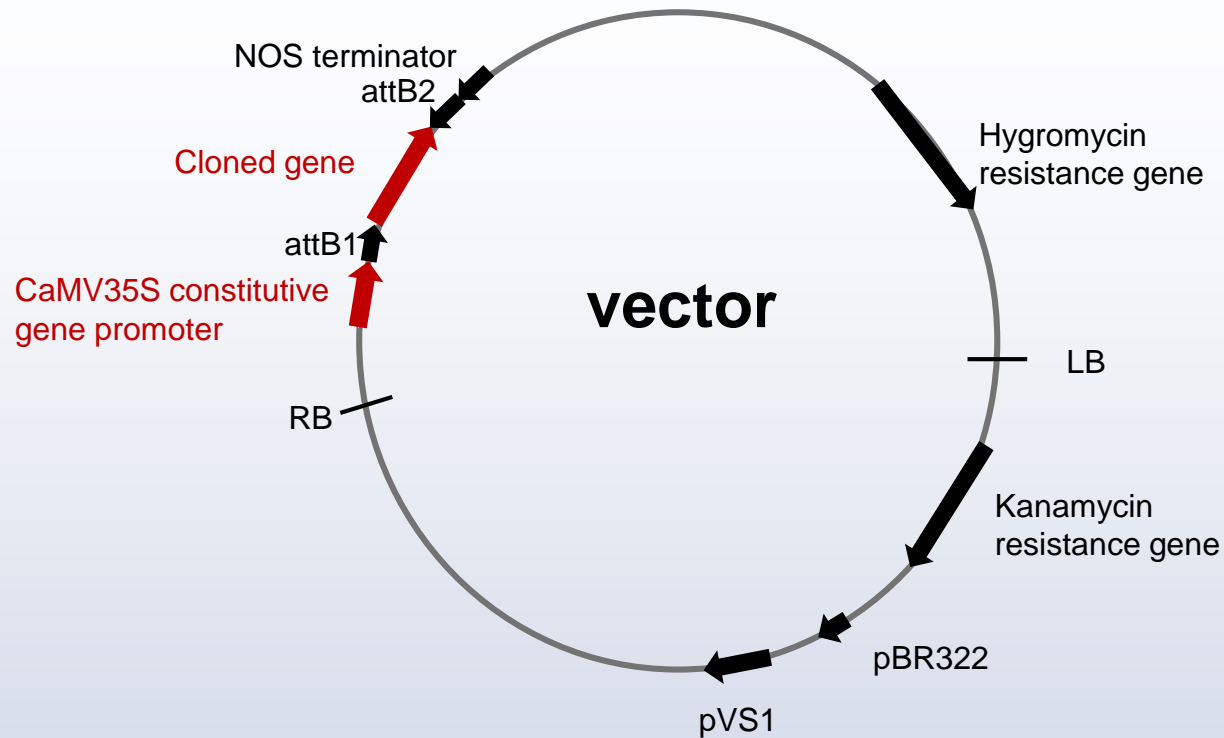
- SP = signal peptide guides the protein to vacuoles and the cell's secretory system
- CRD = carbohydrate recognition domain which is highly substrate specific
- No kinase domains



SNP resulting in amino acid changes between the susceptible DH12075 and resistant Zhongyou 821



Agrobacterium-mediated gene transformation of wt DH12075



- Each plant is a separate gene transformation event

Similar morphology of wt DH12075 and lectin transformants

1- 2 years

Cycles of selfing to obtain plants

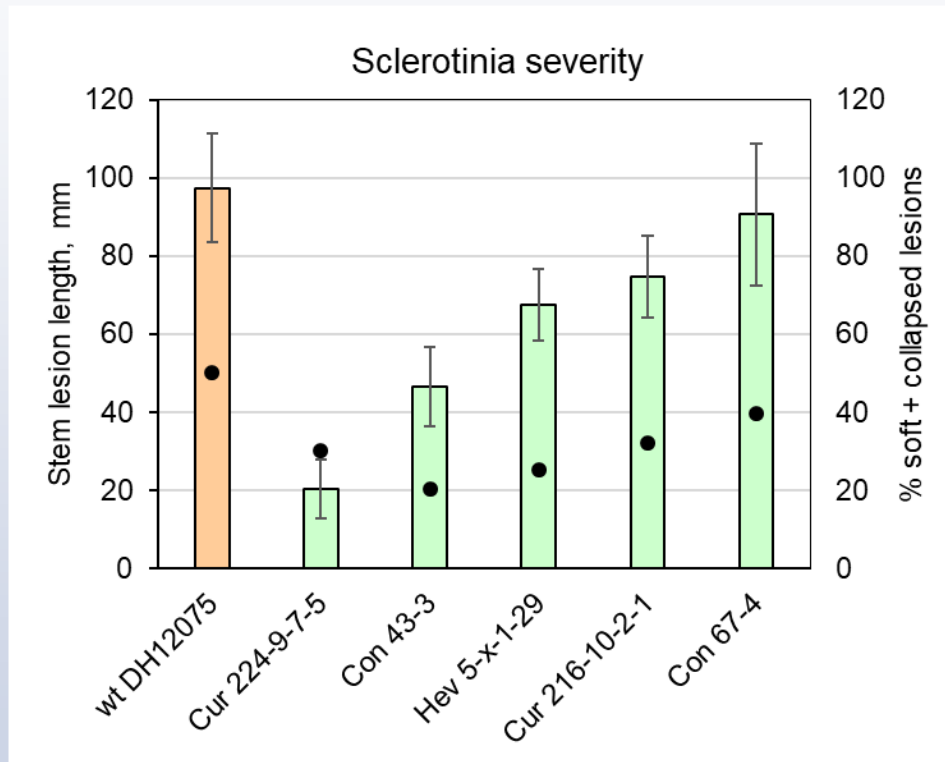
- a single gene copy
- homozygous state
- expressing (mRNA)

Results

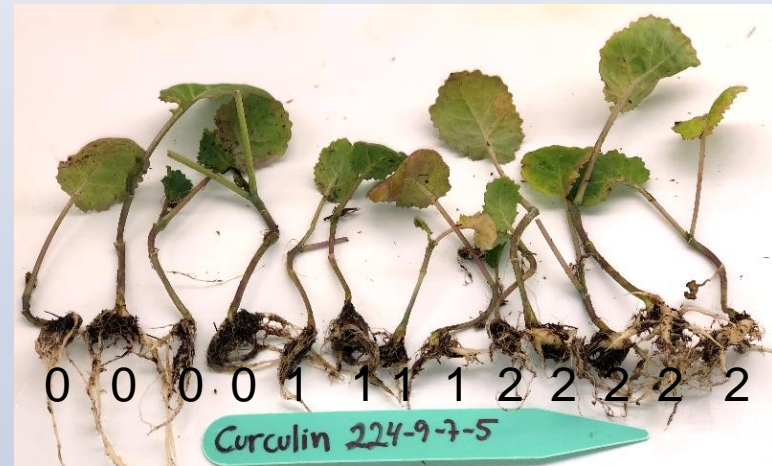
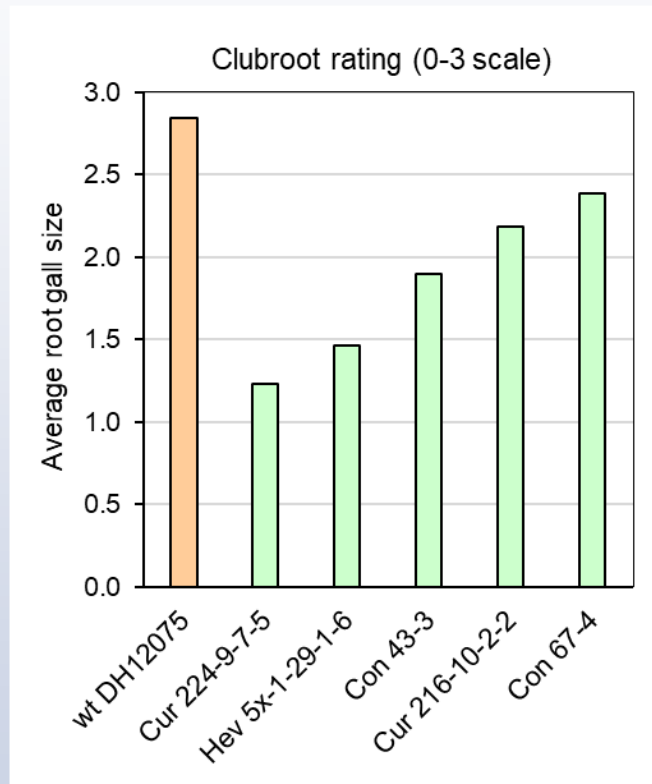
- Concanavalin 11 lines
- Curculin 10 lines
- Hevein 4 lines



B. napus* lines transformed with concanavalin, curculin and hevein that confer quantitative resistance to *S. sclerotiorum



Concanavalin, curculin and hevein confer quantitative resistance to oomycete (*Plasmodiophora brassicae*) causing club root in *B. napus*



Concanavalin expressed in *A. thaliana* confer resistance to oomycete downy mildew (Hok et al. 2011)
Hevein expressed in *N. tabacum* confer resistance to oomycete *Phytophthora parasitica* (Koo et al. 2002)

Gene expression study

Lines

- wt DH12075
- Con 43-3 and Con 67-4
- Cur 216-10-2-1 and Cur 224-9-7-5
- Hev 5x-1-29-1-6

Stem tissue collection

- 24hpi, 72hpi, 7dpi
- mRNA isolation
- cDNA libraries

Quantification of mRNA by ddPCR using gene-specific primers

- *Concanavalin* on chr A5
- *Curculin* on chr A7
- *Hevein* on chr A3
- No amplification of sclerotinia lectin genes
- *TIP41* internal standard - Single copy in *B. napus*, expression unaffected by sclerotinia infection

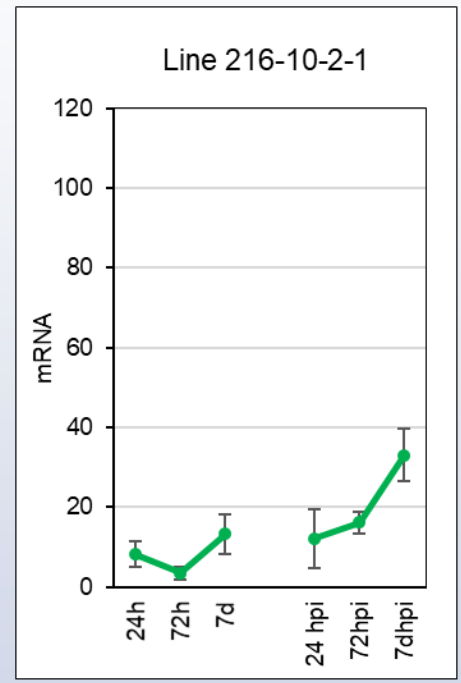
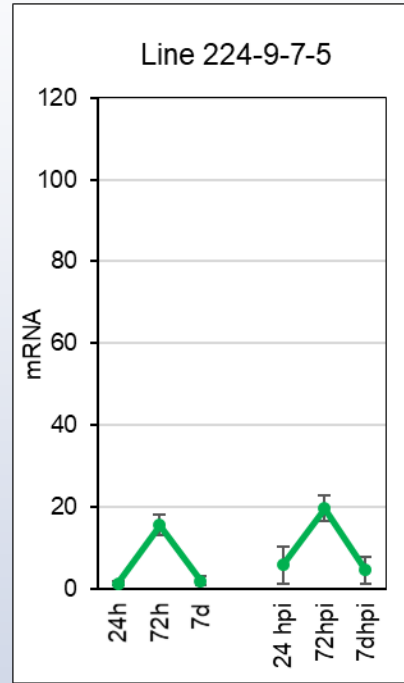
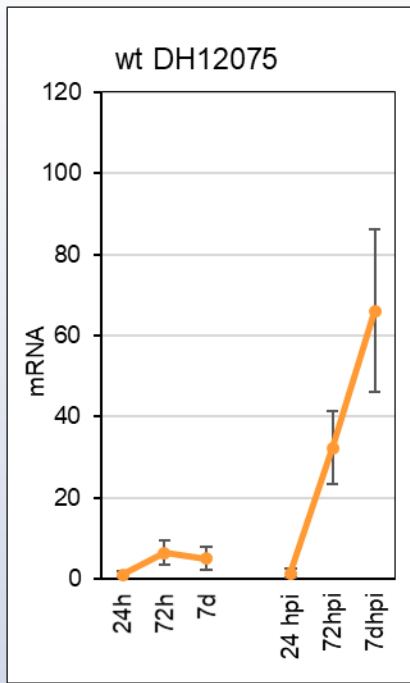
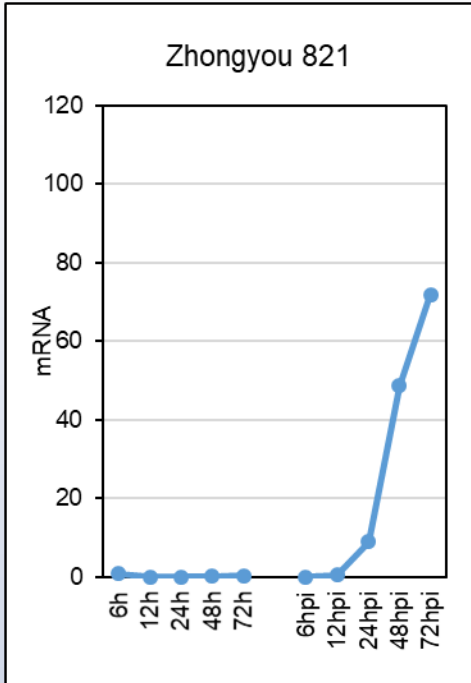


Curculin gene expression

Resistant gene donor

Susceptible wild type

Transformants

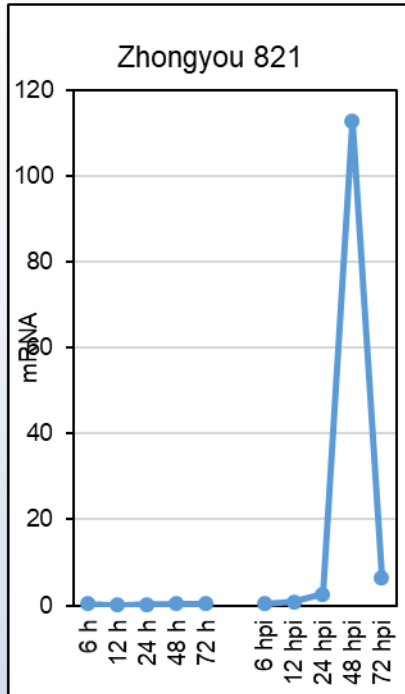


Curculin is upregulated during sclerotinia infection in both R and S

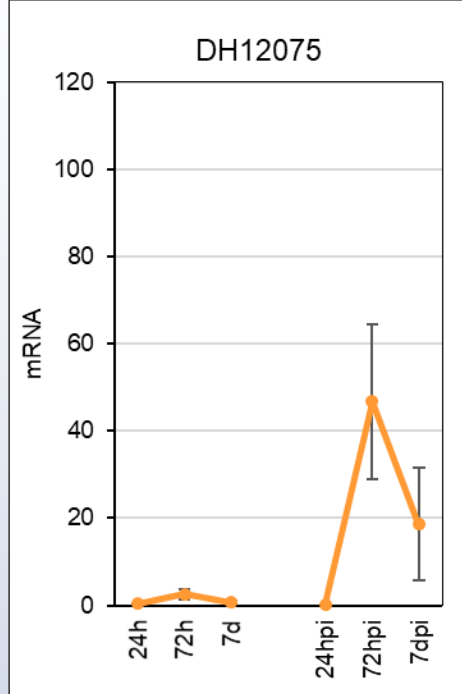
Curculin is constitutively expressed in both un-inoculated and inoculated stem tissue.

Concanavalin gene expression

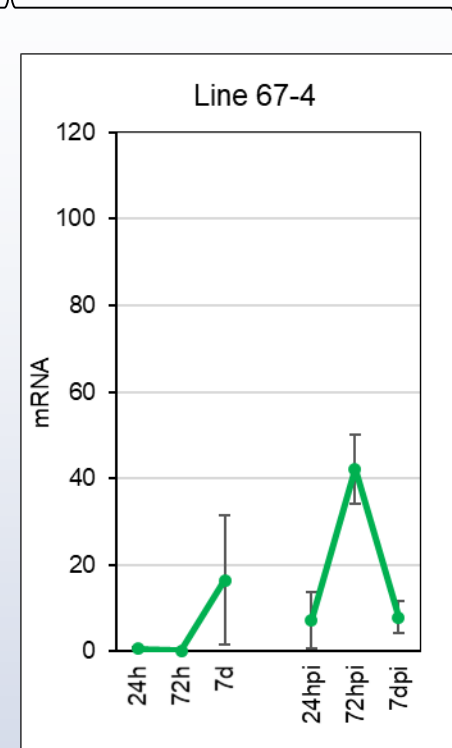
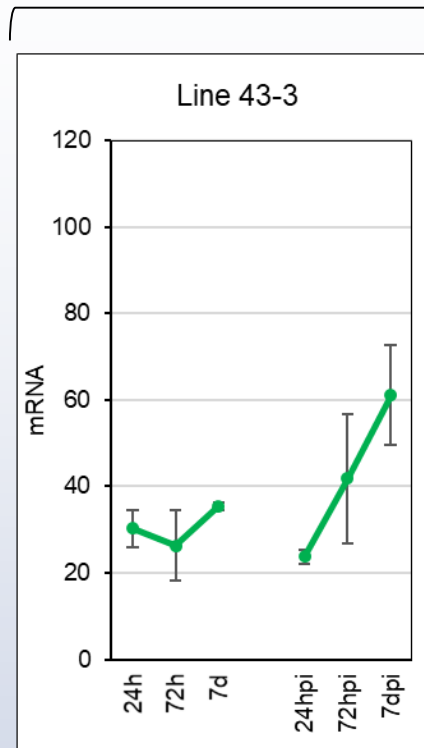
Resistant gene donor



Susceptible wild type



Transformants



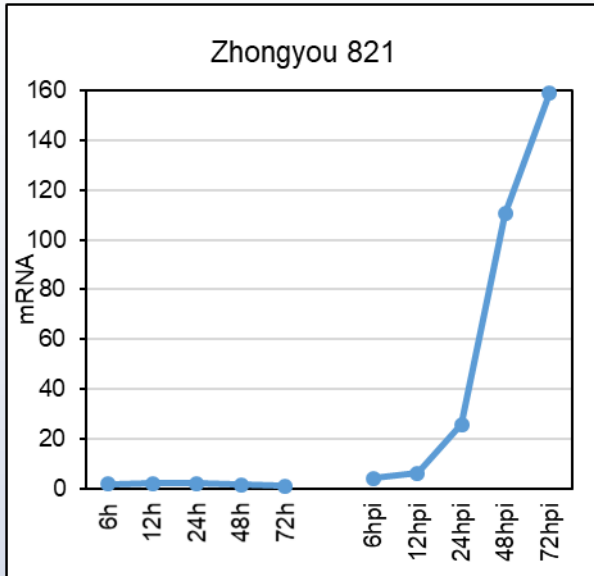
Concanavalin in both R and S spikes 48-72hpi.

Concanavalin is constitutively expressed with additional upregulation 7dpi.

Concanavalin spikes 72hpi similar to R and S lines

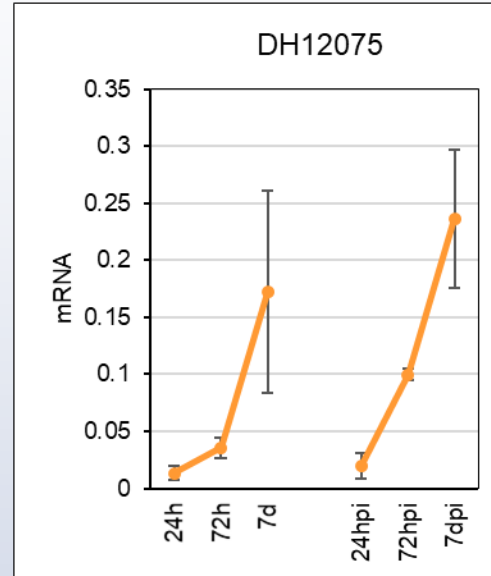
Hevein gene expression

Resistant gene donor



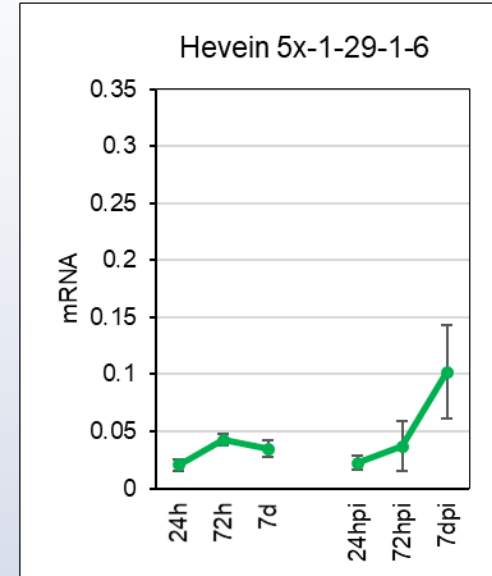
Hevein is highly upregulated in the R line during infection

Susceptible wild type



Hevein were extremely low in DH12075 - more investigation is needed to explain this result

Transformant



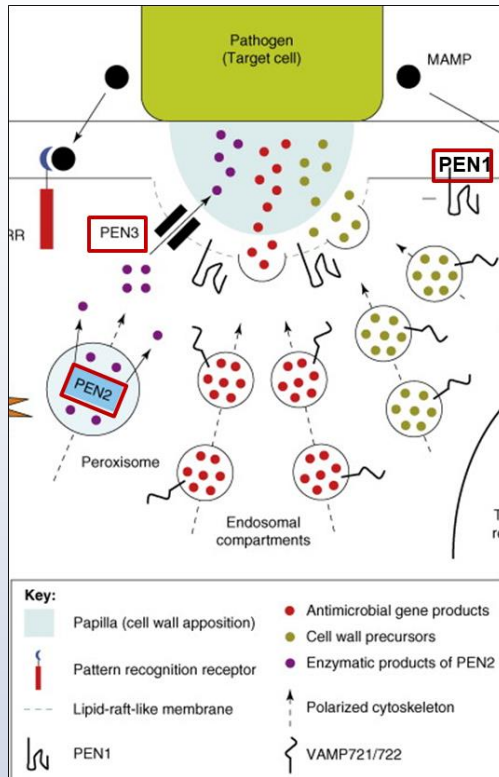
Homolog lectin genes in *B. napus* and *Arabidopsis*

Protein	<i>B. napus</i> gene	<i>Arabidopsis</i> gene	DNA similarity
Concanavalin	BnaA05g24230D A5	AT3g16530	85%
Curculin	BnaA07g34440D A7	AT1g78850	90%
Hevein	BnaA03g28780D A3	AT3g04720	87%

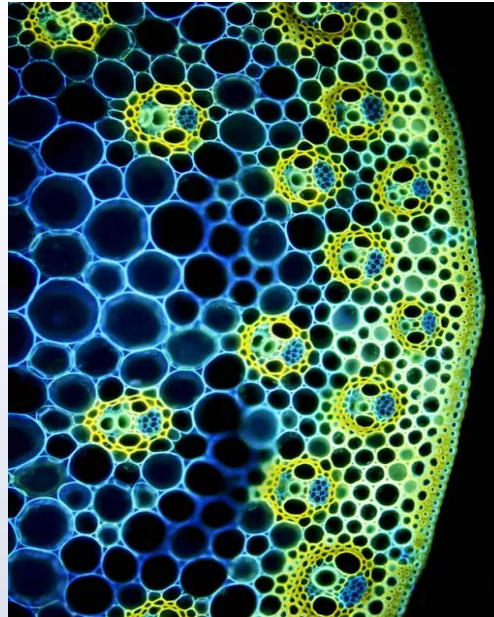
Location of lectin proteins in plant tissue

- Concanavalin and curculin in apoplastic fluid of *A. thaliana* (Boudart et al. 2005)
- Curculin is present in xylem sap of *B. oleracea* (Ligat et al. 2011)
- Concanavalin and curculin are present in xylem sap of *B. napus* (Kehr et al. 2005)

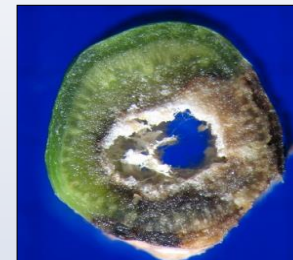
Possible function of concanavalin and curculin



(Kwon et al. 2008)



Resistant



Susceptible



Resistant phenotype

- The signal peptide guides lectins into vacuoles which in turn are secreted into the apoplast
- Lectins are transported into the xylem
- The CRDs of curculin and concanavalin bind mannoproteins and CRD of hevein bind chitin in the fungal cell wall thereby reducing colonization of the plant

Summary

- The signal peptide of curculin, concanavalin and hevein ensures the proteins are secreted out of the cell into the apoplast
- It has been shown the proteins are present in plant's apoplast and xylem
- Curculin, concanavalin and hevein were shown to enhance resistance to both sclerotinia and clubroot
- The inhibition of fungal growth is likely due to CRD in concanavalin and curculin which bind mannose moieties and hevein which binds chitin in the fungal cell wall

Acknowledgements



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