

Application of genomics research for development of Sclerotinia resistance in *Brassica napus*

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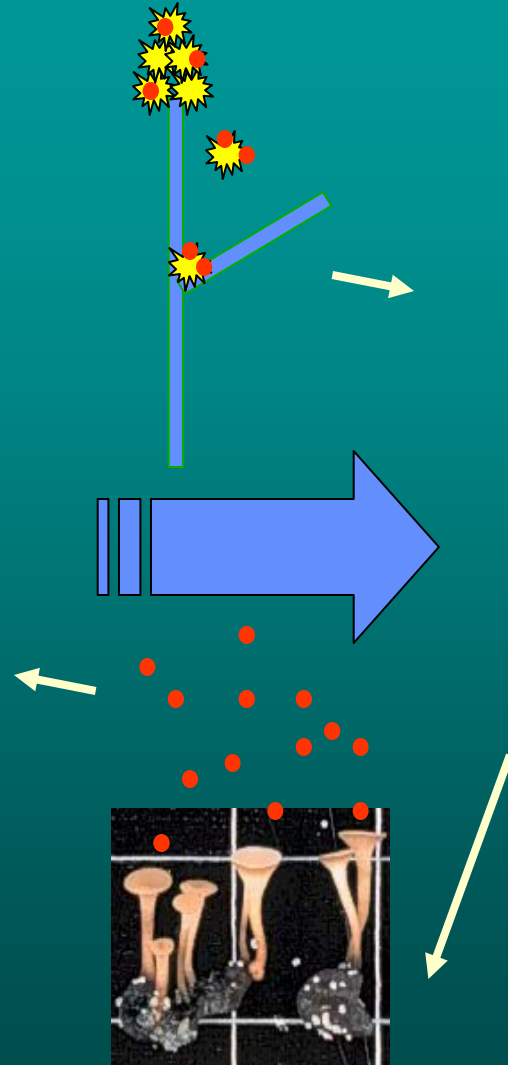
AAFC Saskatoon Research Centre

Applying Genomics to Canola Improvement
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Agriculture and
Agri-Food Canada

Sclerotinia stem rot disease cycle in canola



Resistance to Sclerotinia stem rot

Genomics of Sclerotinia stem rot resistance from Zhong You 821
EST

B. napus PGIP gene family

Mapping of QTL for sclerotinia resistance and marker development
Sclerotinia Consortium

10 steps to make Sclerotinia resistant canola cultivars

1. Identify a source of resistance (Zhong You 821)
2. Establish a method to measure the resistance of a genotype
3. Develop a population of lines segregating for resistance and susceptibility (DH or RIL)
Measure the resistance and susceptibility of each line
4. Make a genetic map of the segregating population
5. Statistically associate the disease phenotype with the genetic map
- Quantitative Trait Loci (QTL) mapping
6. Determine changes in gene expression associated with resistance
7. Identify the genes most important for resistance
8. Determine if these genes are associated with QTL for resistance
9. Test different combinations of QTL and genes to evaluate their role in resistance
10. Use marker assisted breeding to move the desired genes into a suitable genetic background

1. Identify a source of heritable resistance (Zhong You 821)



Zhong You 821

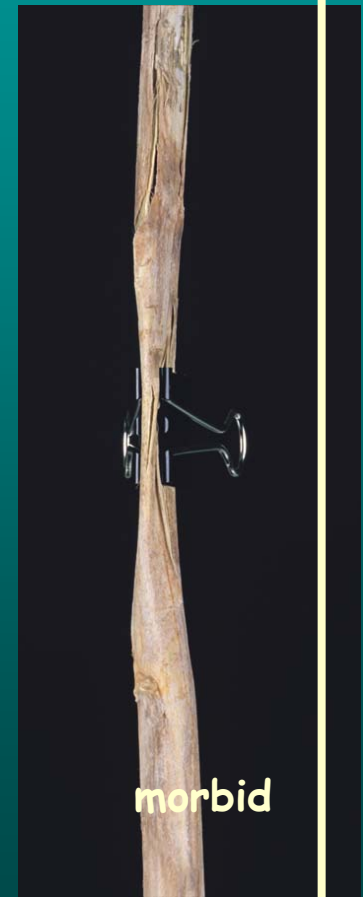
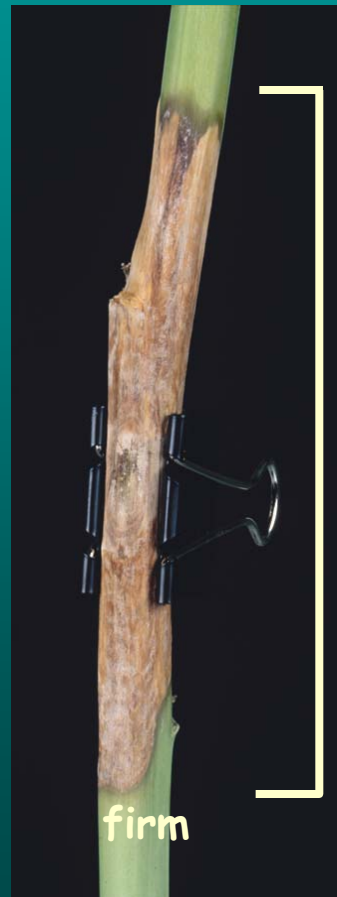
Westar

**Comparison of
Zhong You 821
and Westar
21 days after inoculation**

2. Establish a reliable protocol to measure the resistance of a genotype

Lesion length (mm) measured: 7, 14 & 21 dai

Appearance: black (bl), brown- necrotic;
firm (f), soft (s), morbid (m)



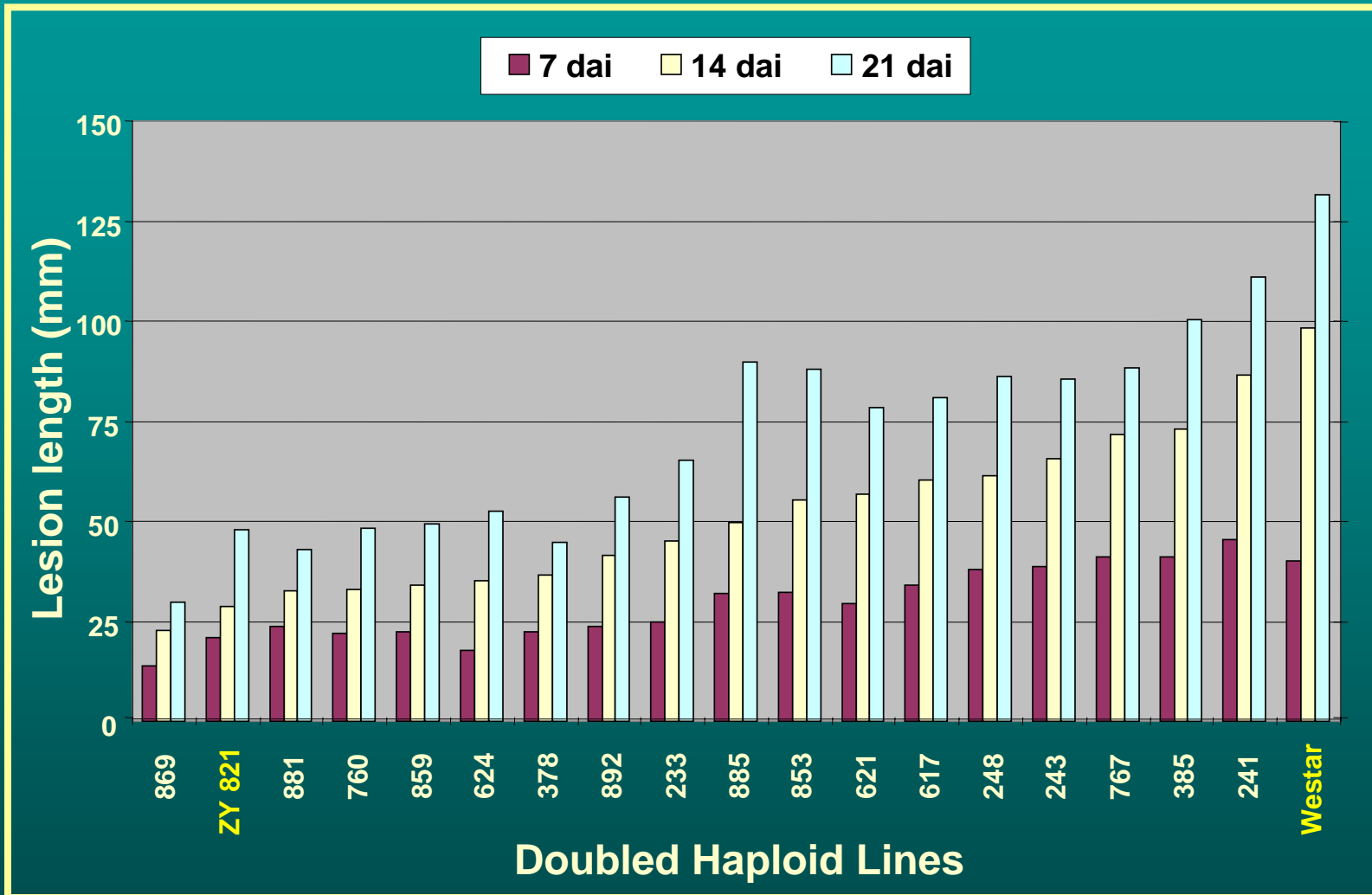
3. Develop mapping populations segregating for resistance and susceptibility (DH or RIL)

Five DH populations for genetic mapping and locating Quantitative Trait Loci (QTL) have been developed.

Zhong You 821 X Chinese (winter type)	160 lines	DH1
Zhong You 821 X Chinese (winter type)	110 lines	DH2
Zhong You 821 X Westar (W & S types)	67 lines	DH3
821xW R line X Westar (All S types)	250 lines	DH4
821xW R line X Westar (All S types)	250 lines	DH5

RIL lines to be developed equivalent to DH4 and DH5

Measure the resistance and susceptibility of each line

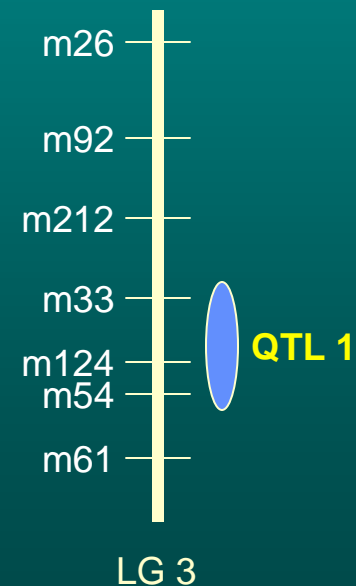


ZY821 X Westar DH3 Mapping Population (Spring types)

4 & 5. Make a genetic map of the segregating population and identify QTL

Five DH populations for genetic mapping and locating Quantitative Trait Loci (QTL) have been developed.

DH1	ZY821 x CW	80 lines	Genetic map in progress
DH2	ZY821 x CW	-	Non polymorphic
DH3	ZY821 x Westar	67 lines	Genetic map completed
DH4 and DH5 are being increased			



6. Determine the changes in gene expression associated with resistance

***B. napus* Defenses**

Subtractive cDNA Libraries

↓
'R' vs 'S' DH lines
Infected vs uninfected ZY821

↓
Resistance Genes

↓
Testing

↘
Mapping to DH populations



ZY821 genes induced during stem infection

Pathogenesis-related proteins

PR1

PR5 (thaumatin)

PR5 (osmotin)

chitinase (5 types)

glucanase

peroxidase – lignin formation

defensin

Polygalacturonase inhibitor proteins

Regulatory Proteins

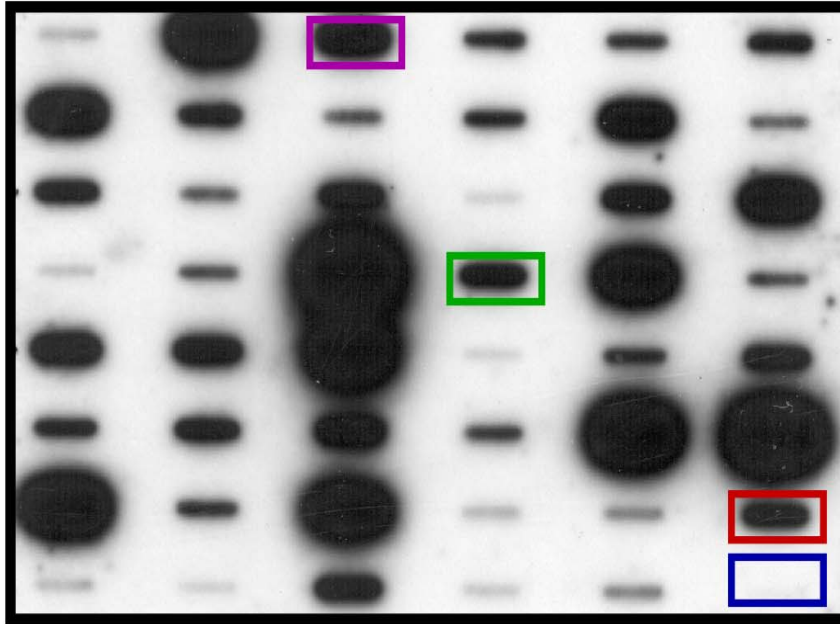
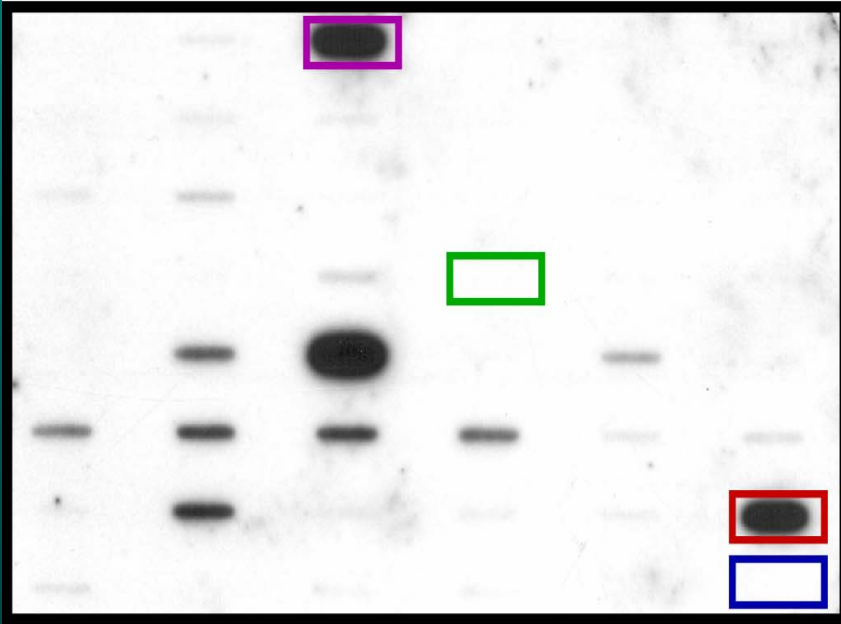
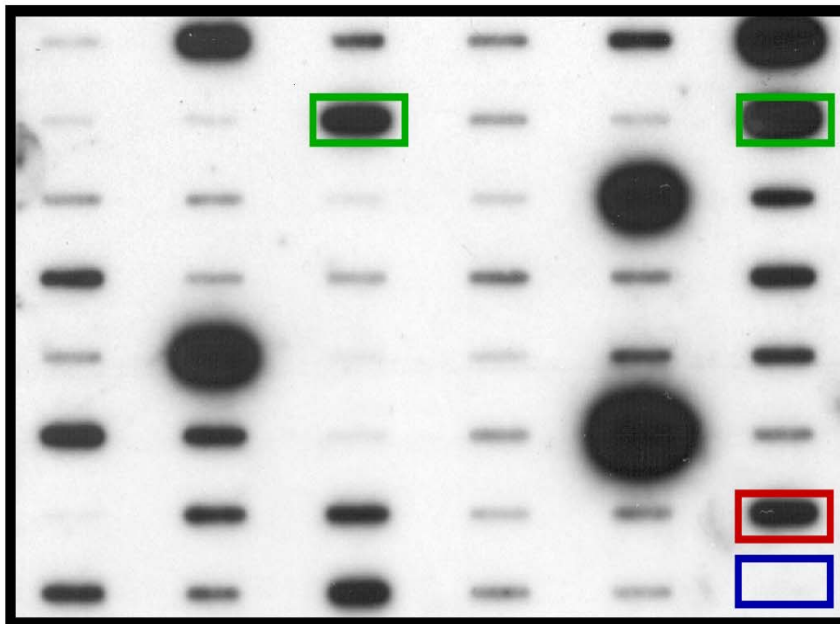
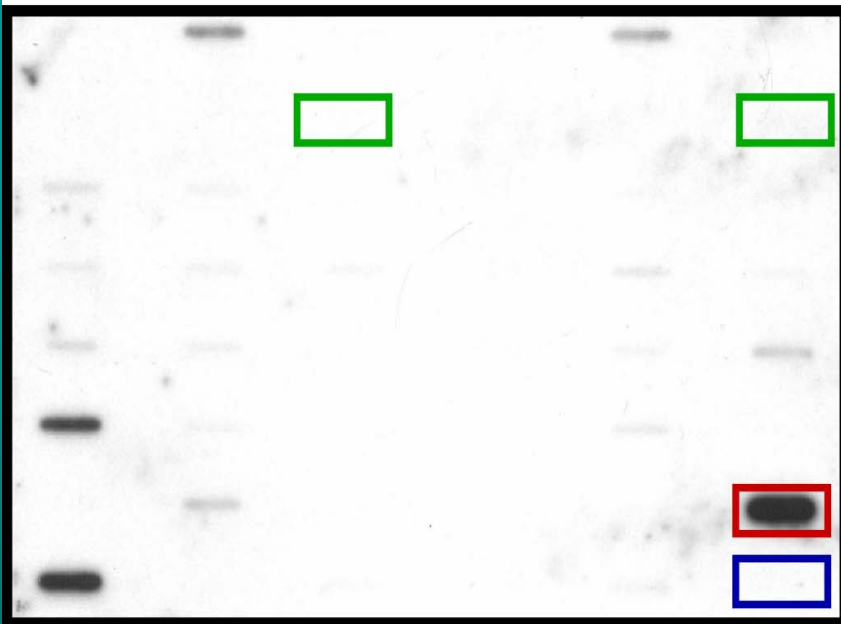
myb

two-component response regulator

zinc finger

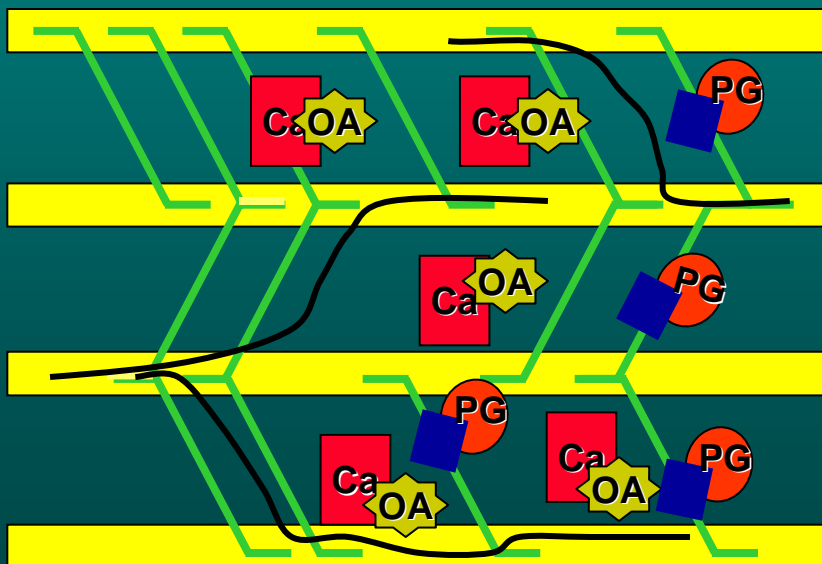
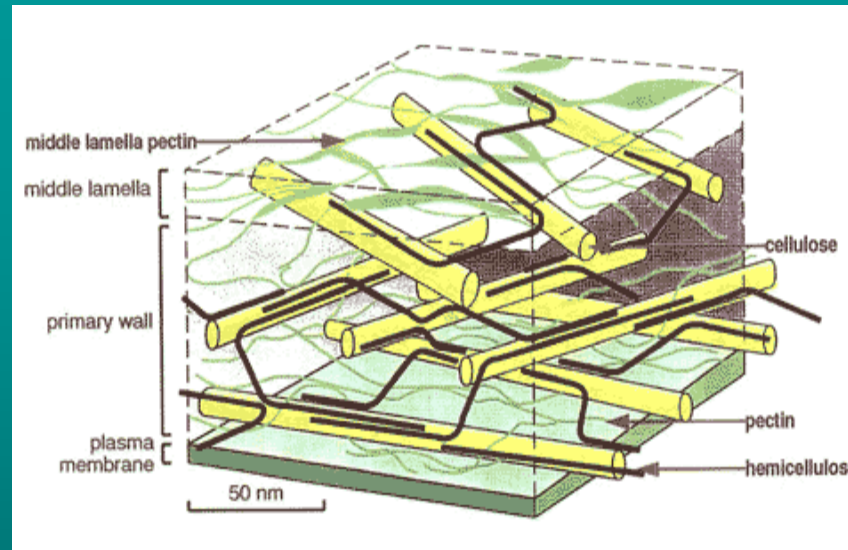
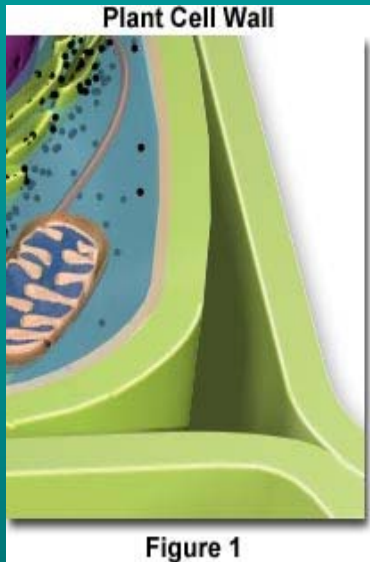
Non-infected Stems

Infected Stems



 Rubisco  tubulin  pSPORT  Fungal

How *S. sclerotiorum* dissolves plant cell walls

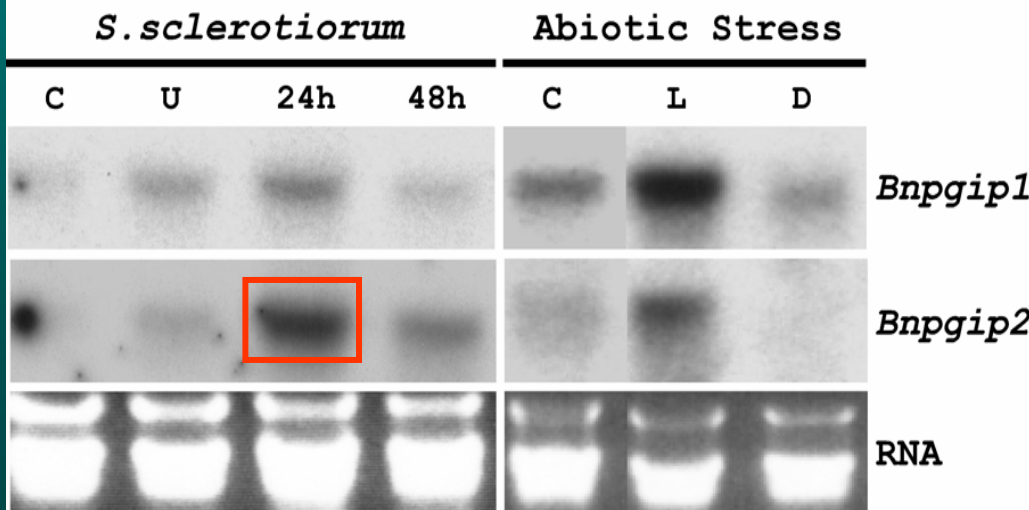
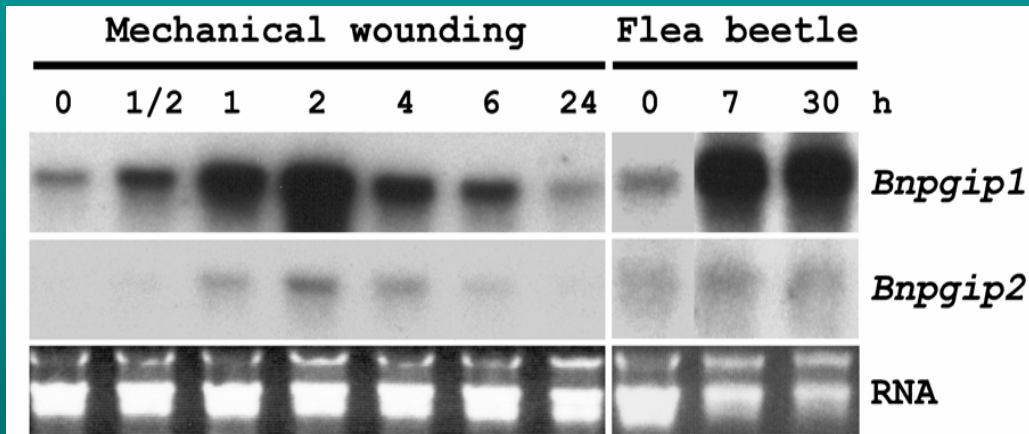


Pathogenicity factors of Sclerotinia

Oxalic acid (OA) binds Ca^{2+} to form insoluble oxalate.
Oxalic acid reduces pH to around 4.
Polygalacturonase (PG) digests pectin in the middle lamella and plant cell wall.

7. Identify which genes are most important

The role of putative resistance genes (PGIP) may be revealed by response to external stimuli



- 20 BnPGIP genes cloned

- *BnPGIP2* induced strongly by *Sclerotinia* infection

Further Project Objectives:

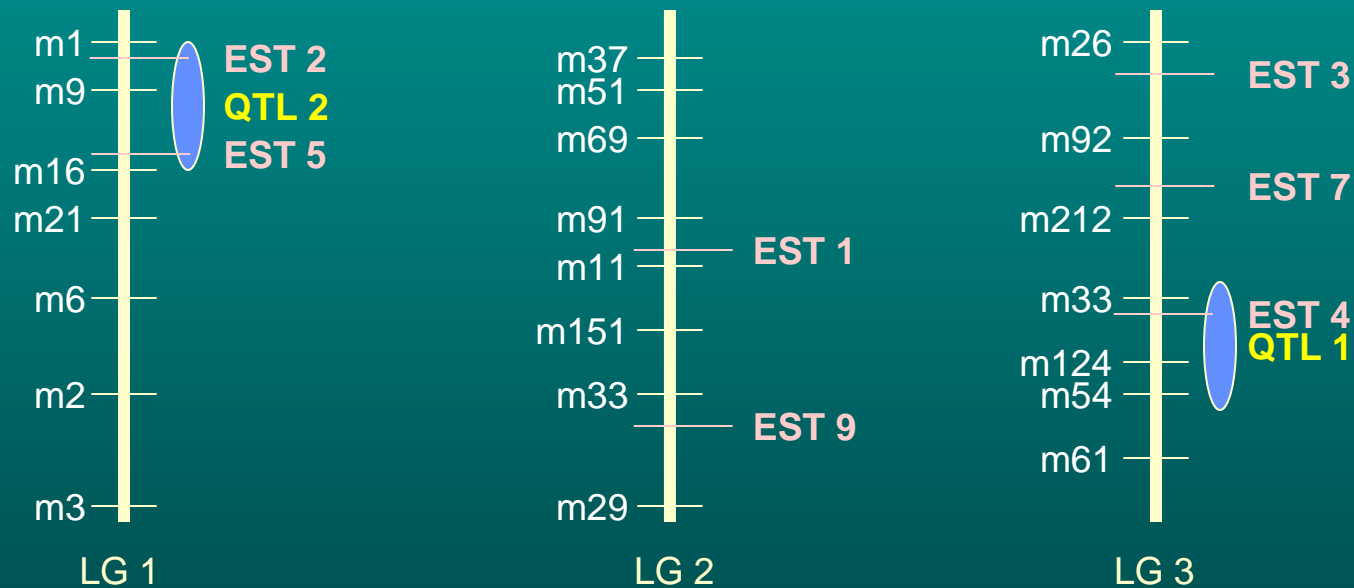
7. Testing candidate genes

- overexpression in susceptible DH line**
- RNAi or VIGS knockout in resistant DH lines**

8. Map the resistance genes onto the QTL maps

8. Determine if the resistant response genes are associated with QTL for resistance

ESTs (cDNA sequences) used as molecular markers to map genes to ZY821 DH population genetic maps



Further Project Objectives:

9. Test combinations of genes for role in resistance

10. Marker assisted breeding

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