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## Promising new sources of sclerotinia resistance in *Brassica napus* from Asia

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Canada

***Brassica napus* germplasm with sclerotinia resistance (tolerance) originates from Pakistan (Kashmir), China, South Korea and Japan**



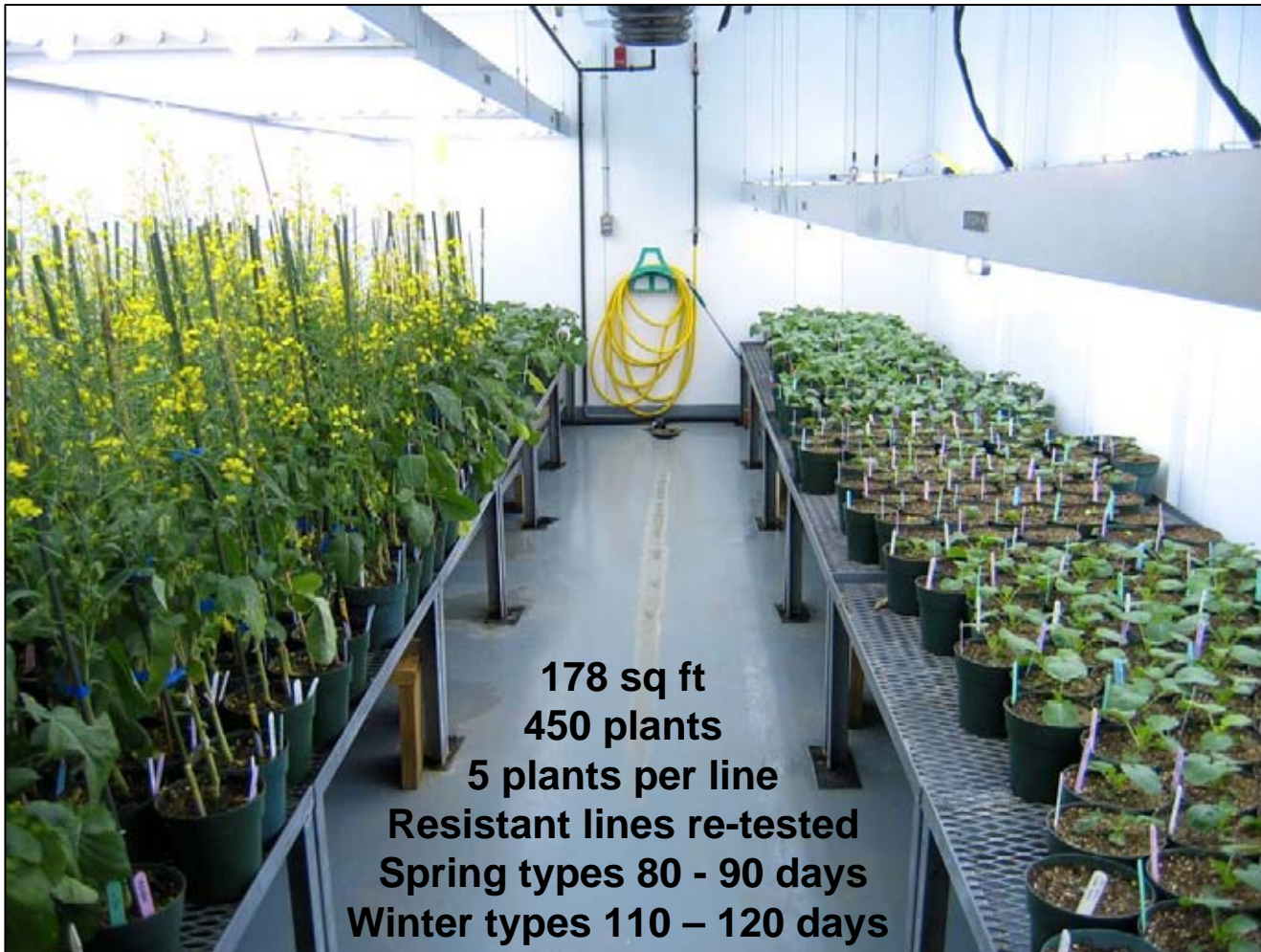
**Funded by SCDC and AAFC's Matching Investment Initiative.**

**From April 2006 to March 2010.**

**400 *B. napus* lines and cultivars were screened for sclerotinia resistance.**



## Sclerotinia screening is carried out in a growth cabinet



## Sclerotinia infection under natural conditions



**B) Ascospores colonizes petals collecting in leaf axils and proceeds to infect the stem**



**C) Stem lesions causes the greatest yield loss**



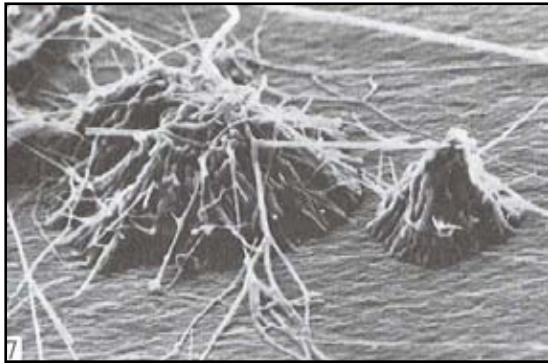
**A) Apothecia germinate from resting structures in the soil. Ascospores are spread by wind.**



**D) Sclerotia (resting structures) develop inside stems and survives in the soil for several years**



## Mycelium is used as inoculum which simulates colonization of petals



'Infection cushions' on a petal consisting of mycelium

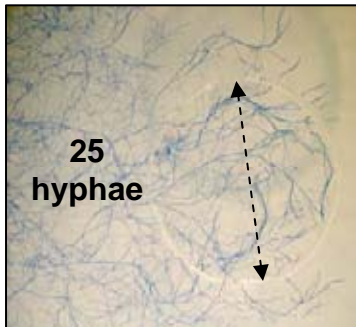


Bottom of an infection cushion showing hyphae tips

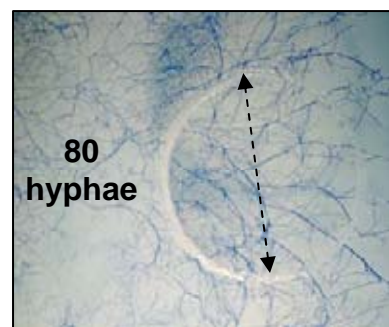


Mycelium grown on nutrient agar in a Petri dish

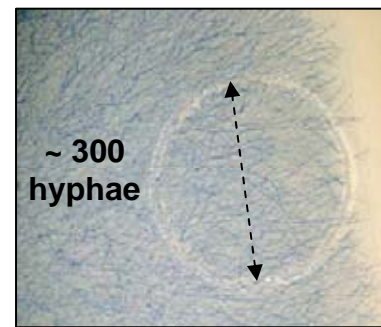
## Hyphae density depends on nutrients



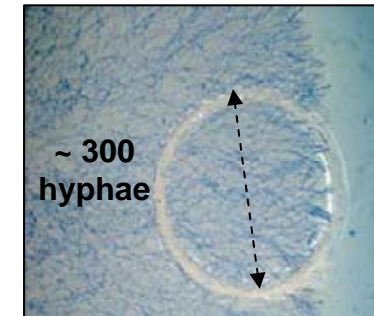
Glucose minus potassium



Glucose plus potassium



Potato-dextrose



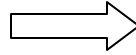
V8 juice



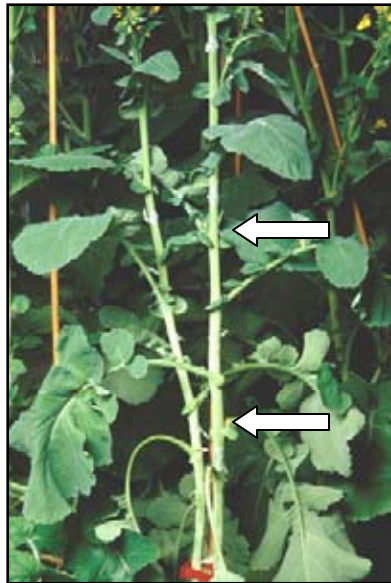
## Inoculation and disease rating methods



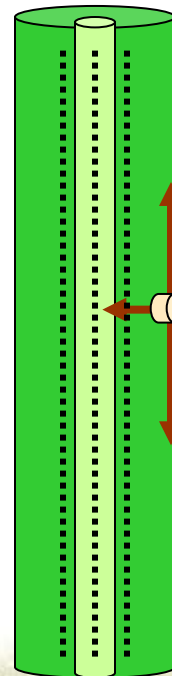
**Mycelium plugs removed from the growing margin**



**Plugs placed on plastic film (Parafilm)**



**Plugs attached with Parafilm to the stem at two internodes**

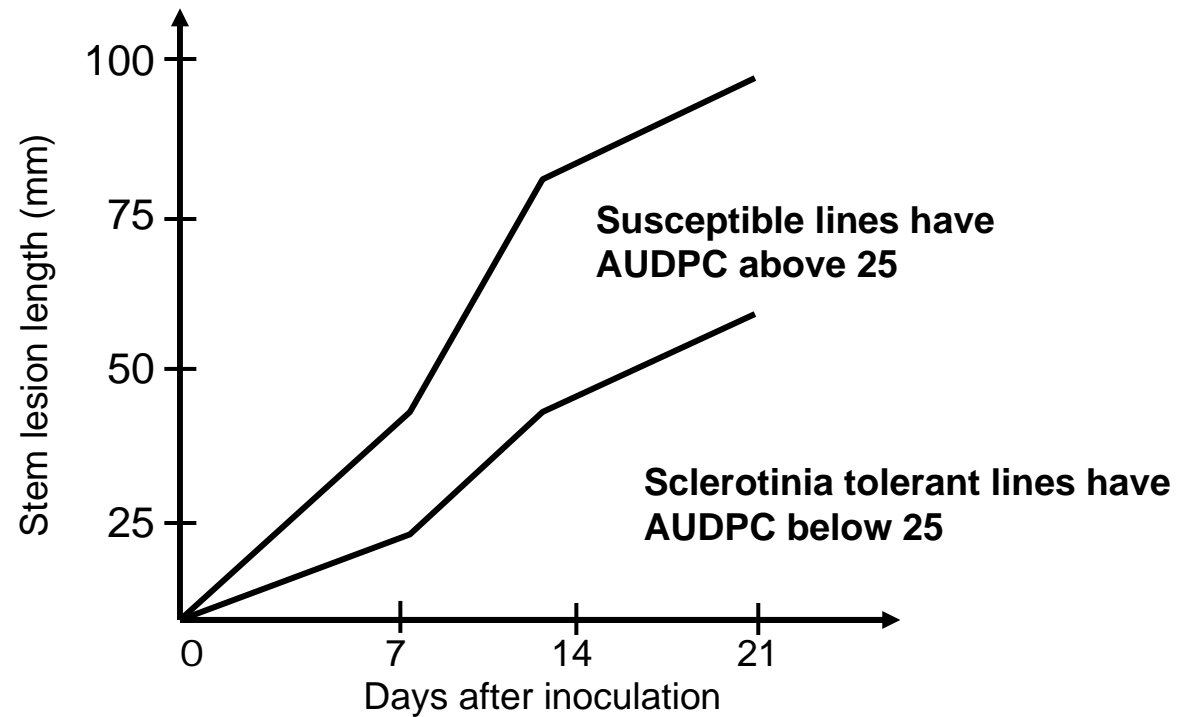


### Disease measurements

1) Stem lesion length 7, 14, 21 dai is used to calculate the area under the disease progress curve (AUDPC).

2) Depth of penetration (firm, soft, collapsed) is used to calculate % soft + collapsed lesions

## Stem lesion length is used to calculate the AUDPC - area under the disease pressure curve



# Depth of stem penetration is used to calculate % soft + collapsed lesions

Sclerotinia tolerant lines have less than 25% soft + collapsed lesions

Resistant or tolerant



firm



firm



firm

Susceptible



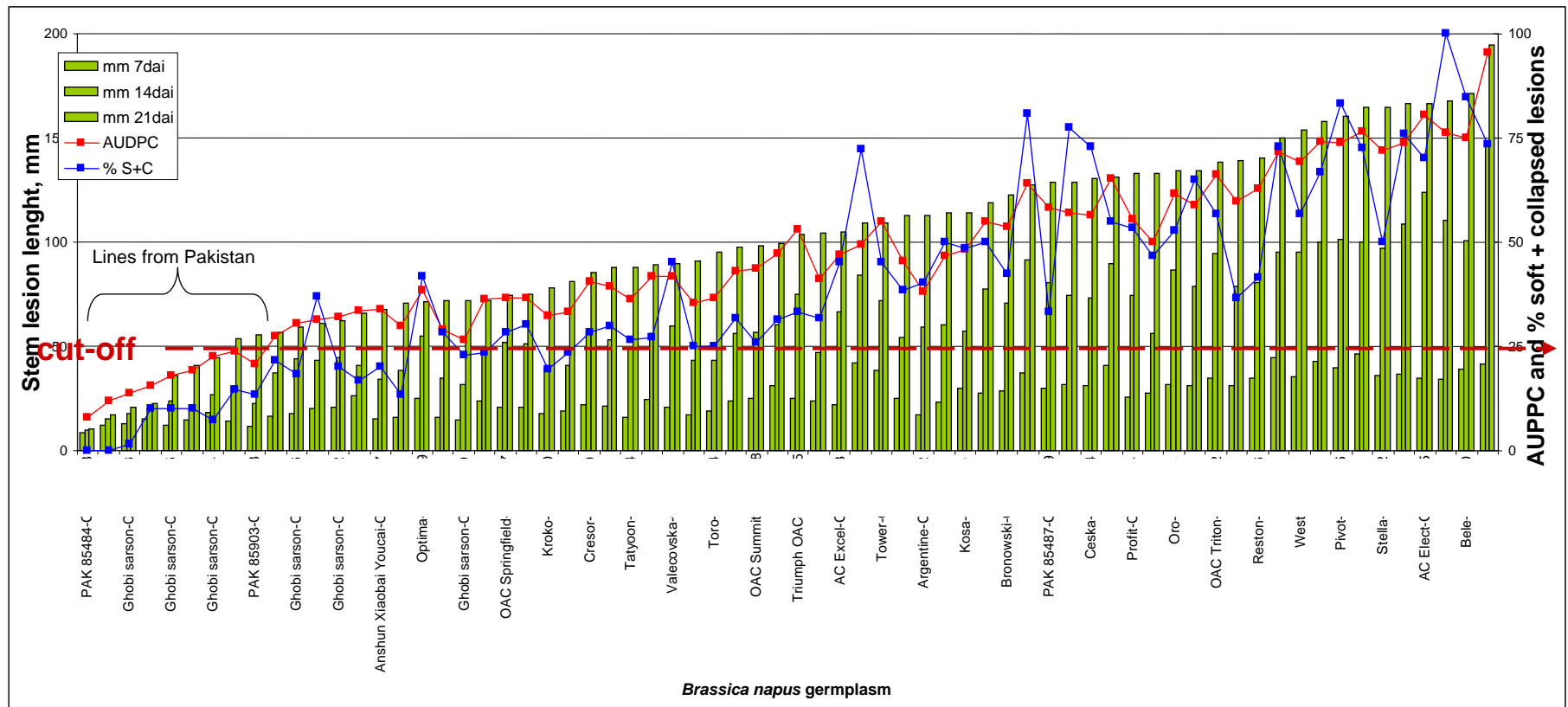
soft



collapsed



# Comparison of 68 *B. napus* lines for sclerotinia resistance

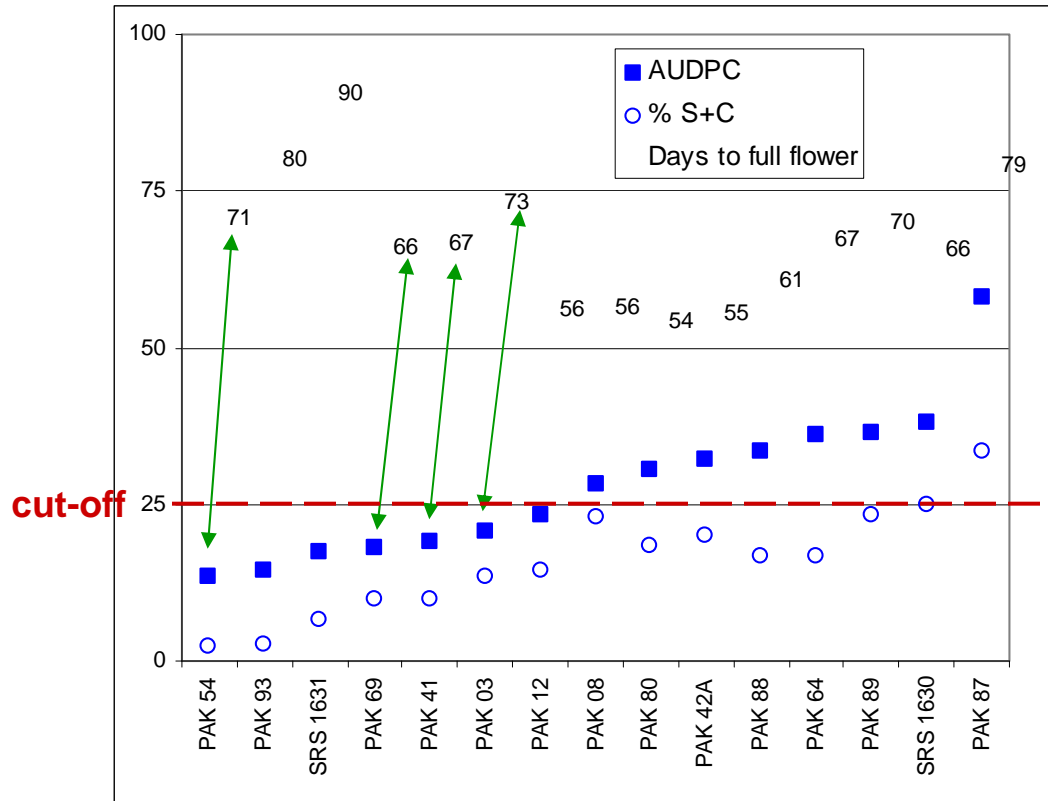


This graph shows 6 lines from Pakistan with useful level of sclerotinia resistance

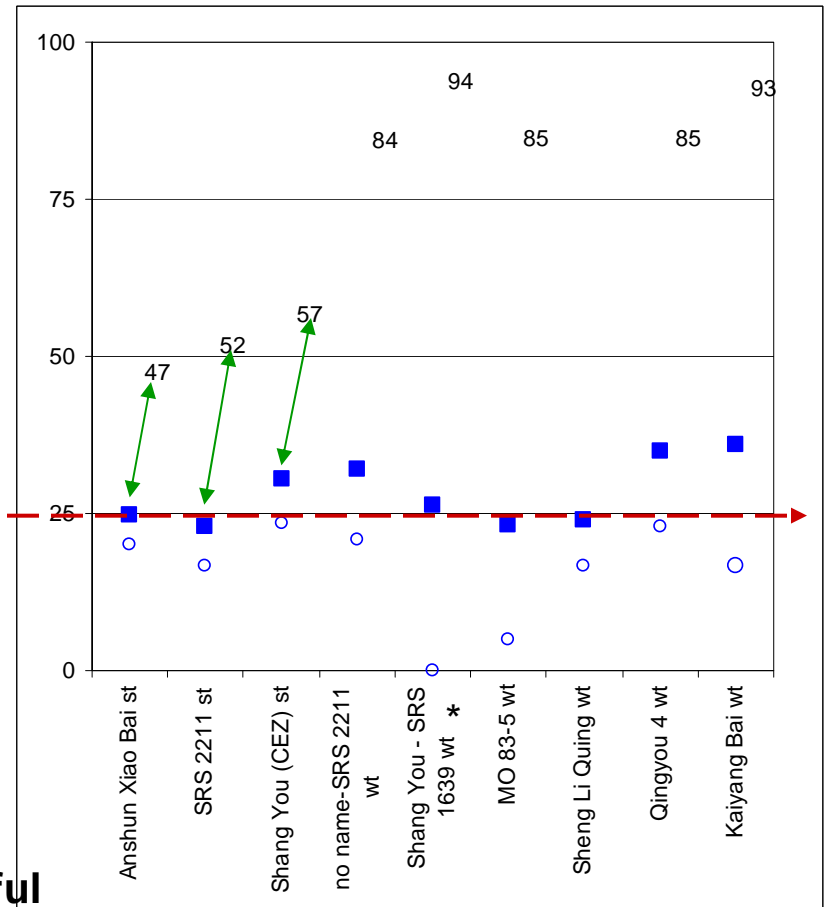


## Results from screening of Asian lines

### 15 lines from Pakistan



### 9 lines from China



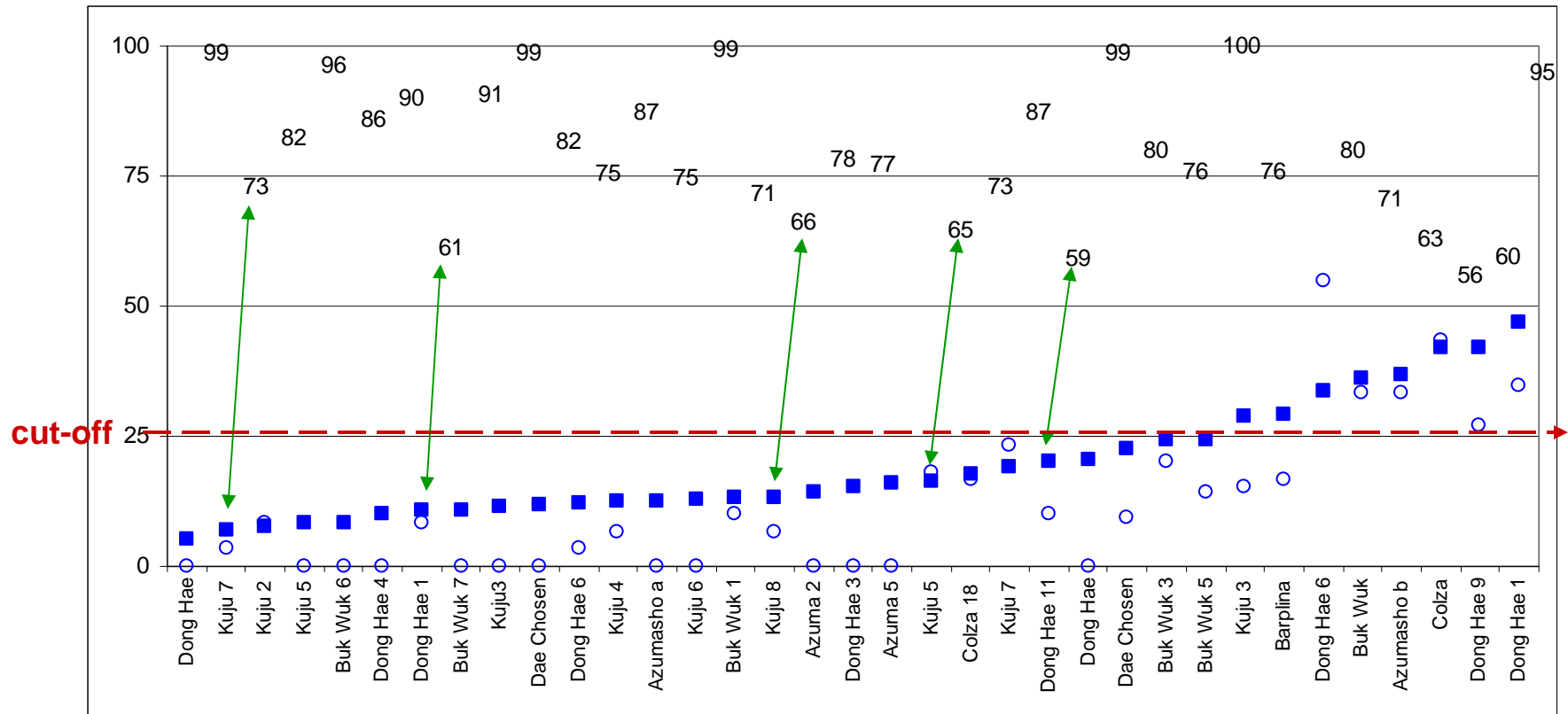
**4 lines from Pakistan and 3 from China with useful level of sclerotinia resistance and early flowering**

Note that some names have been abbreviated



## Results from screening of Asian lines

35 lines from South Korea (~ 70 lines in the PGRC collection)

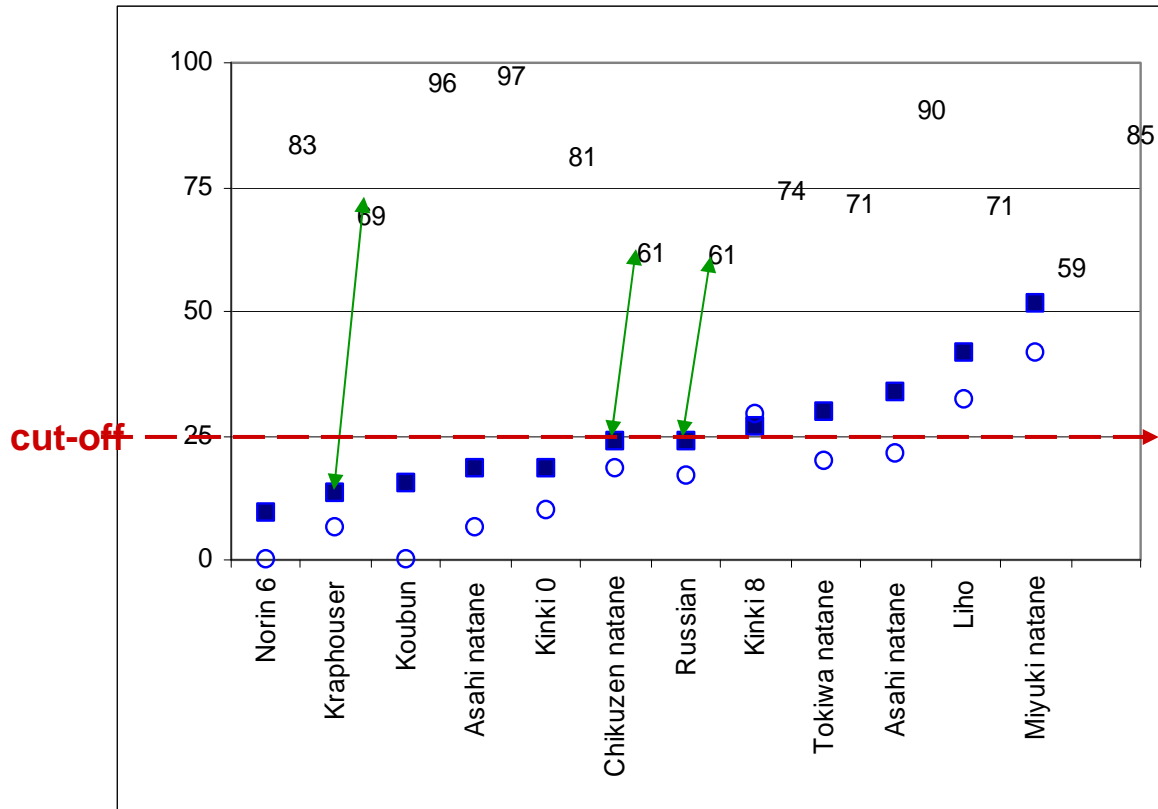


5 lines from South Korea with useful level of sclerotinia resistance and early flowering



# Results from screening of Asian lines

## 12 lines from Japan



**3 lines from Japan with useful level of sclerotinia resistance and early flowering**



## Screening under semi-field conditions to confirm indoor disease scores



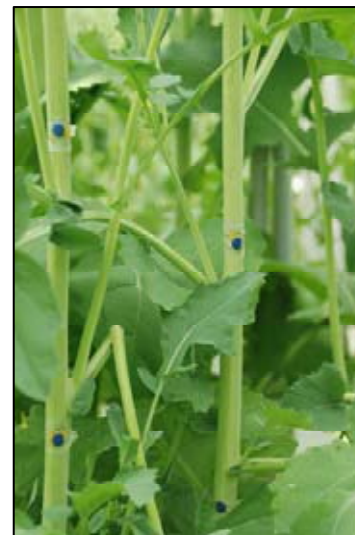
Walls and roof are retractable and plants grow in field soil at ambient temperature and light.



U-shaped hoops support plants in rows



Plants at full flower are inoculated and a over-head misting system ensures humidity



Two nodes per plant are inoculated



Lesions on a susceptible line

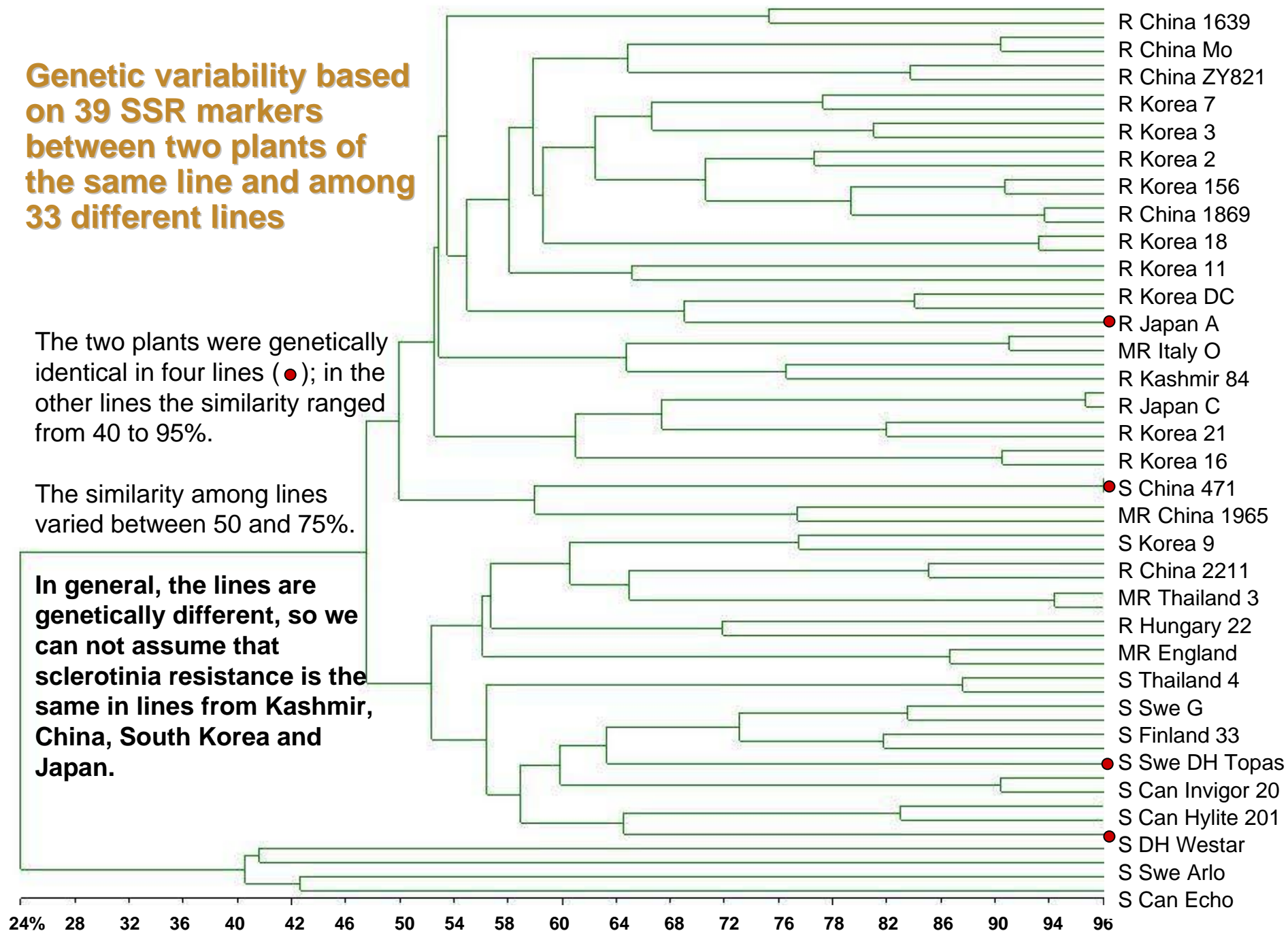


## Genetic variability based on 39 SSR markers between two plants of the same line and among 33 different lines

The two plants were genetically identical in four lines (●); in the other lines the similarity ranged from 40 to 95%.

The similarity among lines varied between 50 and 75%.

**In general, the lines are genetically different, so we can not assume that sclerotinia resistance is the same in lines from Kashmir, China, South Korea and Japan.**



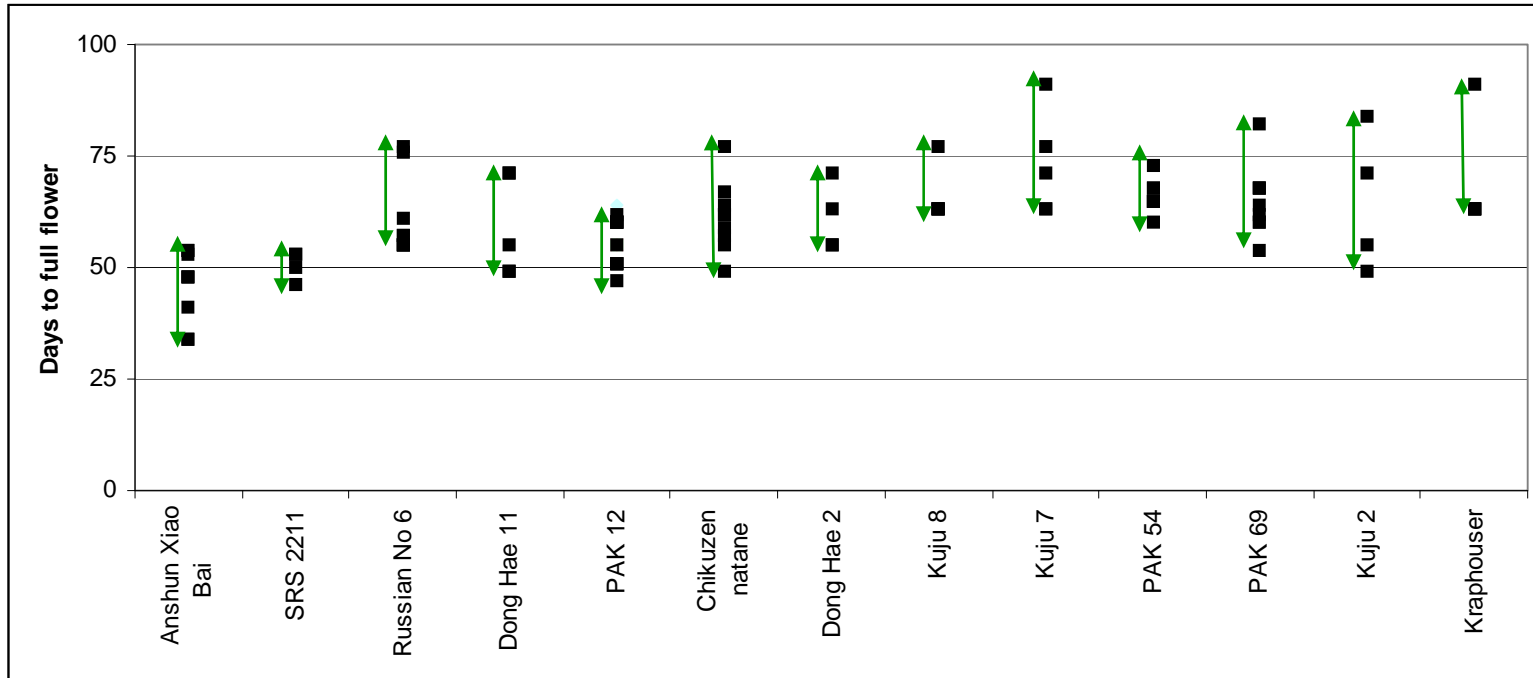
## Variability in sclerotinia scores among plants of the same line

| COUNTRY | Line name | Plant number | Days to flower | After 7 days |          | After 14 days |          | After 21 days |          |
|---------|-----------|--------------|----------------|--------------|----------|---------------|----------|---------------|----------|
|         |           |              |                | Lesion 1     | Lesion 2 | Lesion 1      | Lesion 2 | Lesion 1      | Lesion 2 |
| Kashmir | 12        | 1            | 51             | 27           | 20       | 54            | 45       | 75            | 90       |
|         |           | 2            | 51             | 5            | 7        | 9             | 9        | 9             | 10       |
|         |           | 3            | 51             | 26           | 7        | 38            | 8        | 39            | 13       |
|         |           | 4            | 51             | 8            | 7        | 10            | 7        | 10            | 7        |
|         |           | 5            | 64             | 13           | 10       | 13            | 19       | 13            | 22       |
| Kashmir | 54        | 1            | 75             | 17           | 22       | 18            | 40       | 20            | 49       |
|         |           | 2            | 82             | 3            | 39       | 4             | 56       | 5             | 70       |
|         |           | 3            | 75             | 8            | 5        | 10            | 9        | 10            | 10       |
|         |           | 4            | 75             | 6            | 3        | 7             | 4        | 9             | 4        |
|         |           | 5            | 75             | 7            | 6        | 8             | 6        | 9             | 7        |
| Korea   | 6         | 1            | 50             | 46           | 87       | 127           | 98       | 130           | 125      |
|         |           | 2            | 61             | 24           | 36       | 96            | 103      | 185           | 130      |
|         |           | 3            | 61             | 4            | 15       | 6             | 17       | 8             | 22       |
|         |           | 4            | 61             | 17           | 24       | 18            | 72       | 22            | 104      |
|         |           | 5            | 50             | 57           | 55       | 130           | 98       | 130           | 110      |
| Korea   | 27        | 1            | 50             | 16           | 7        | 45            | 15       | 79            | 15       |
|         |           | 2            | 50             | 55           | 43       | 110           | 98       | 160           | 150      |
|         |           | 3            | 71             | 5            | 7        | 9             | 16       | 9             | 16       |
|         |           | 4            | 50             | 11           | 7        | 12            | 8        | 13            | 9        |
|         |           | 5            | 61             | 9            | 6        | 9             | 11       | 15            | 27       |
| Korea   | 29        | 1            | 75             | 4            | 13       | 9             | 16       | 11            | 17       |
|         |           | 2            | 57             | 24           | 11       | 34            | 24       | 39            | 26       |
|         |           | 3            | 61             | 11           | 9        | 17            | 9        | 19            | 14       |
|         |           | 4            | 75             | 22           | 4        | 52            | 12       | 91            | 52       |
|         |           | 5            | 67             | 19           | 22       | 22            | 33       | 22            | 36       |
| Japan   | 22        | 1            | 81             | 10           | 3        | 14            | 12       | 16            | 12       |
|         |           | 2            | 61             | 11           | 3        | 11            | 7        | 11            | 19       |
|         |           | 3            | 71             | 8            | 12       | 13            | 20       | 13            | 20       |
|         |           | 4            | 71             | 9            | 10       | 12            | 11       | 21            | 11       |
|         |           | 5            | 81             | 12           | 19       | 17            | 19       | 17            | 19       |

Examples of stem lesion measurements (mm) showing that line 6 and 27 from Korea consists of susceptible (white) and resistant (green) plants. The table also shows early (yellow) and late (blue) flowering plants.



## Variability in days to flower among plants of the same line



**Plants of the same line flower within 10 days of one another other lines within 30 days; many lines are therefore heterogeneous.**

**Before the new lines from Asia can be utilized for sclerotinia resistance it is necessary to select single plants form early flowering lines, collect seed and confirm progenies from the same line do not segregate for sclerotinia resistance, time to flowering and are genetically similar.**



## Projects at AAFC based on the Chinese variety Zhongyou 821

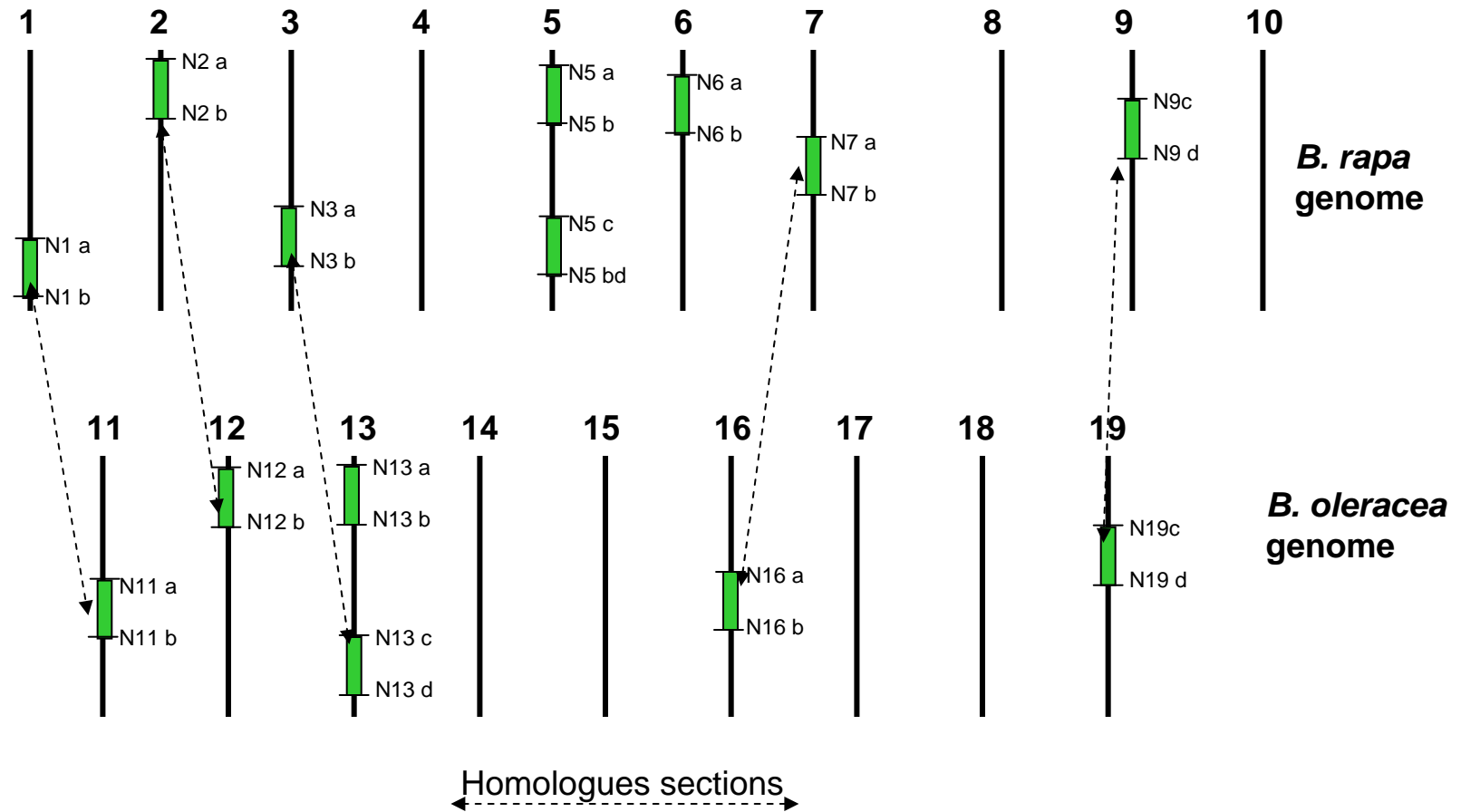


**Project A:**  
Molecular markers linked to  
resistance in Zhongyou 821

**Project B:**  
Defense genes expressed only  
in Zhongyou 821



# Project A: Molecular markers linked to resistance in Zhongyou 821



19 linkage groups of canola showing the locations of genes contributing to sclerotinia resistance which are also known as quantitative trait loci (QTL)

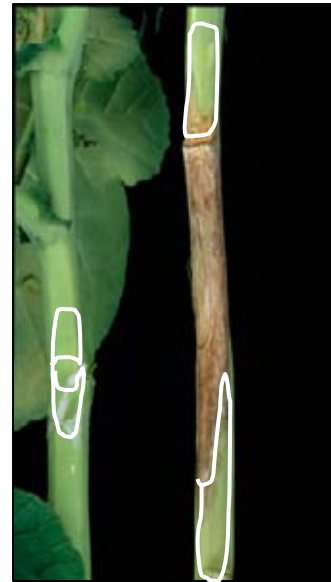


## Project B: Gene subtractions to identify defense genes unique to Zhongyou 821



**Resistant Zhongyou 821:**  
DNA collected from both uninoculated and inoculated stem tissue.

**Subtraction (microarray):**  
Genes expressed in inoculated tissue minus “house keeping” genes.



**Susceptible Westar:**  
DNA collected from one uninoculated stem and one inoculated with sclerotinia.

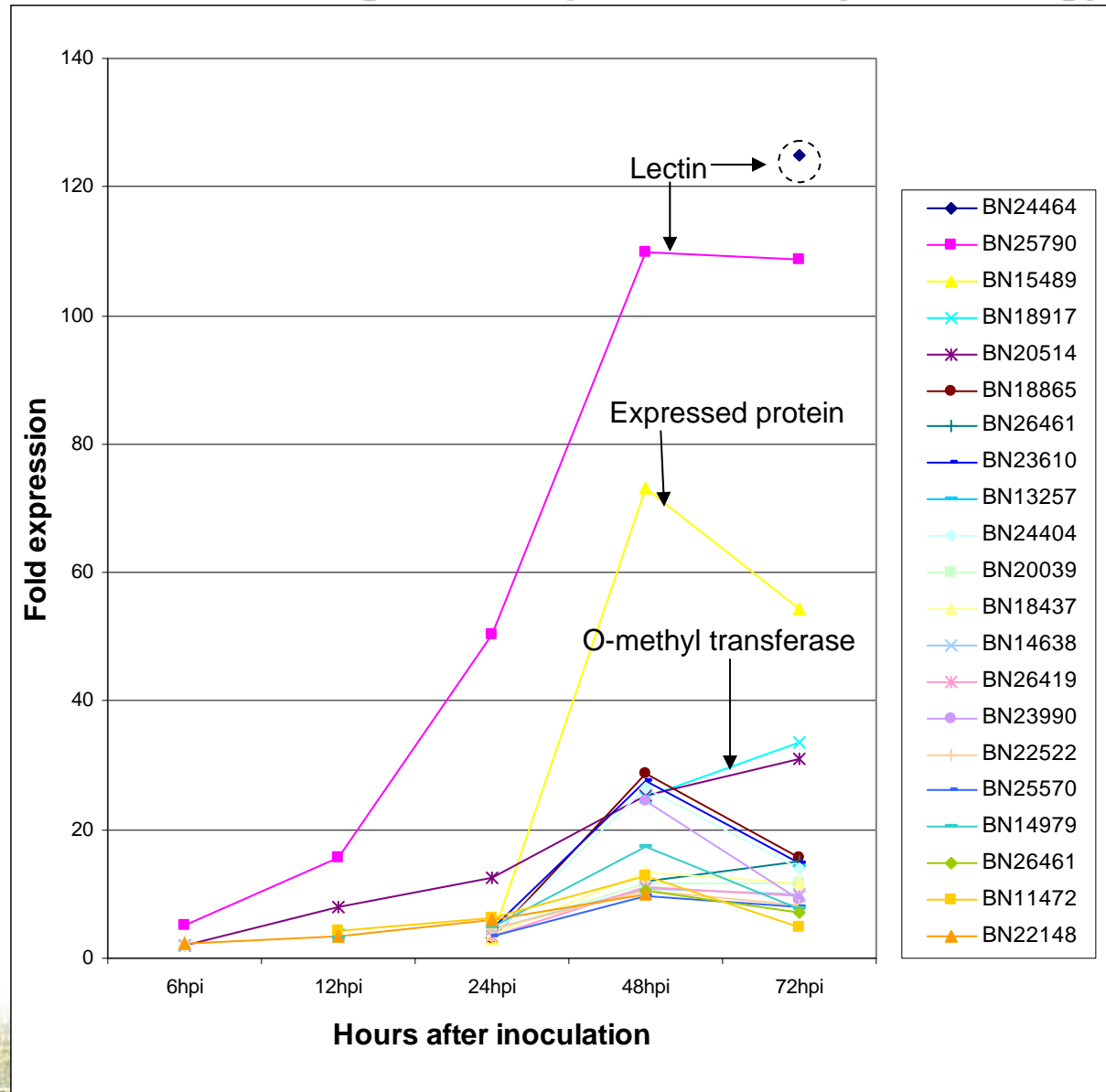
**Subtraction (microarray):**  
Genes expressed in inoculated tissue minus “house keeping” genes.

This excise was carried out at 6, 12, 24, 48 and 72 hours after inoculation

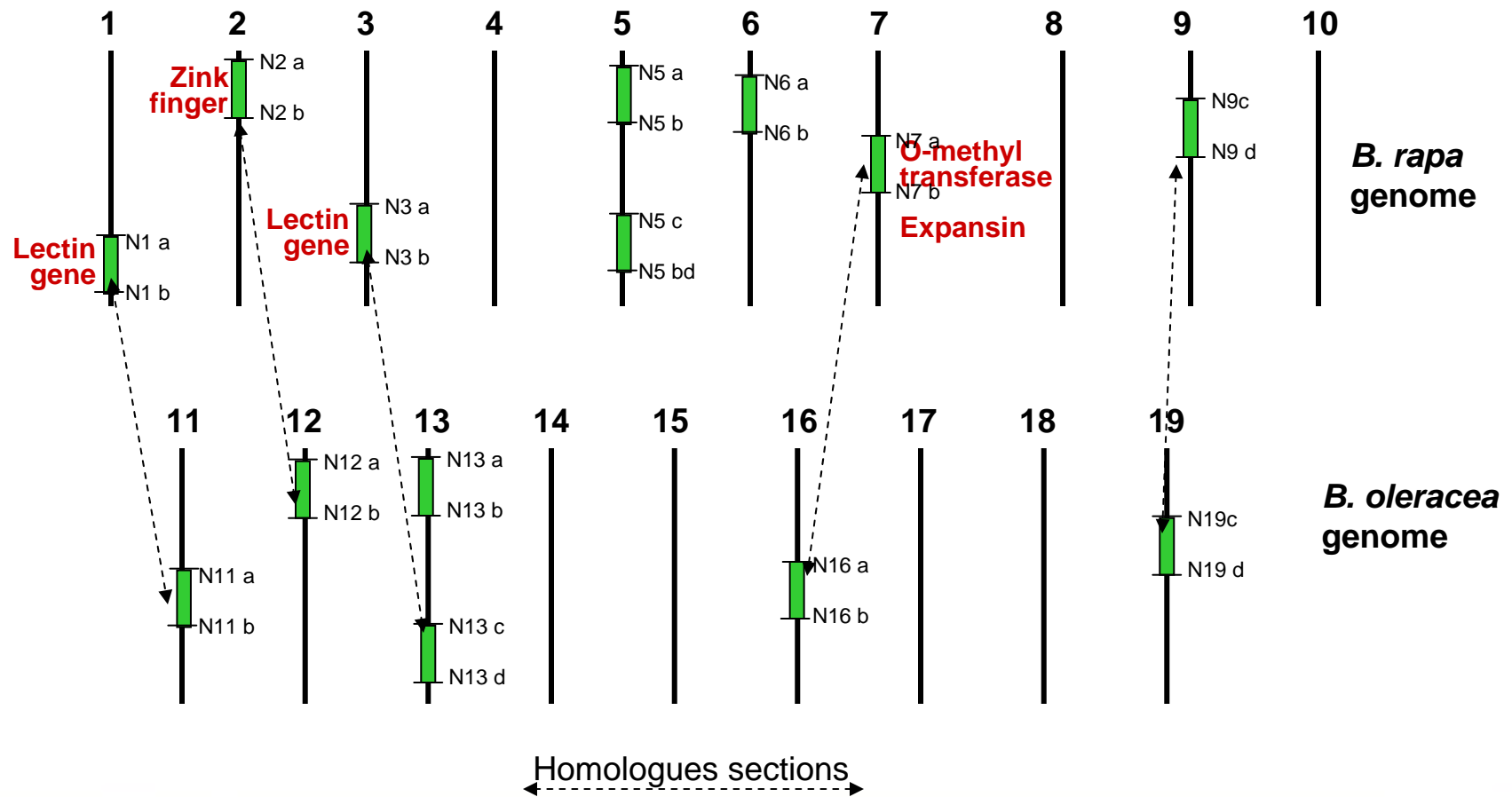
**Subtraction of genes in the data base:**  
Genes expressed in the Zhongyou 821 line minus those also expressed in the Westar resulting in genes expressed only in Zhongyou 821 as a result of inoculation with sclerotinia mycelium.



## Project B: Defense genes expressed only in Zhongyou 821



## Co-location of quantitative trait loci (QTL) for sclerotinia resistance and defense genes only expressed in Zhongyou 821



## **Steps in utilization of sclerotinia resistance in lines from Asia**

**Select single plants from the most sclerotinia resistant spring type lines and confirm sclerotinia resistance in progenies from these plants**

**Develop molecular markers linked to resistance to be utilized by plant breeders**

**Identify the most important defense genes which could be used for transformation**



# Acknowledgements

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## Co-location of markers and defense genes

Dr. Isobel Parkin

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