



沈阳农业大学
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Clubroot Resistance in *Brassica rapa*: Genetics, Functional Genomics and Marker- Assisted Breeding



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Clubroot disease

- Clubroot disease is caused by *Plasmodiophora brassicae*, which specifically infect the crucifer plants
 - The infected plant has clubbed root and wilt in plant.
 - most dangerous disease in Brassica crops worldwide
 - decrease of yield about 10-15% in the world (Dioxin, 2009)



Clubroot disease in China

- It was firstly reported in 1955
- In recent years, it became the most serious problem in the cultivation area of Brassica crops, mainly Chinese cabbage, rapeseeds, zha-tsai and others



Control of clubroot disease

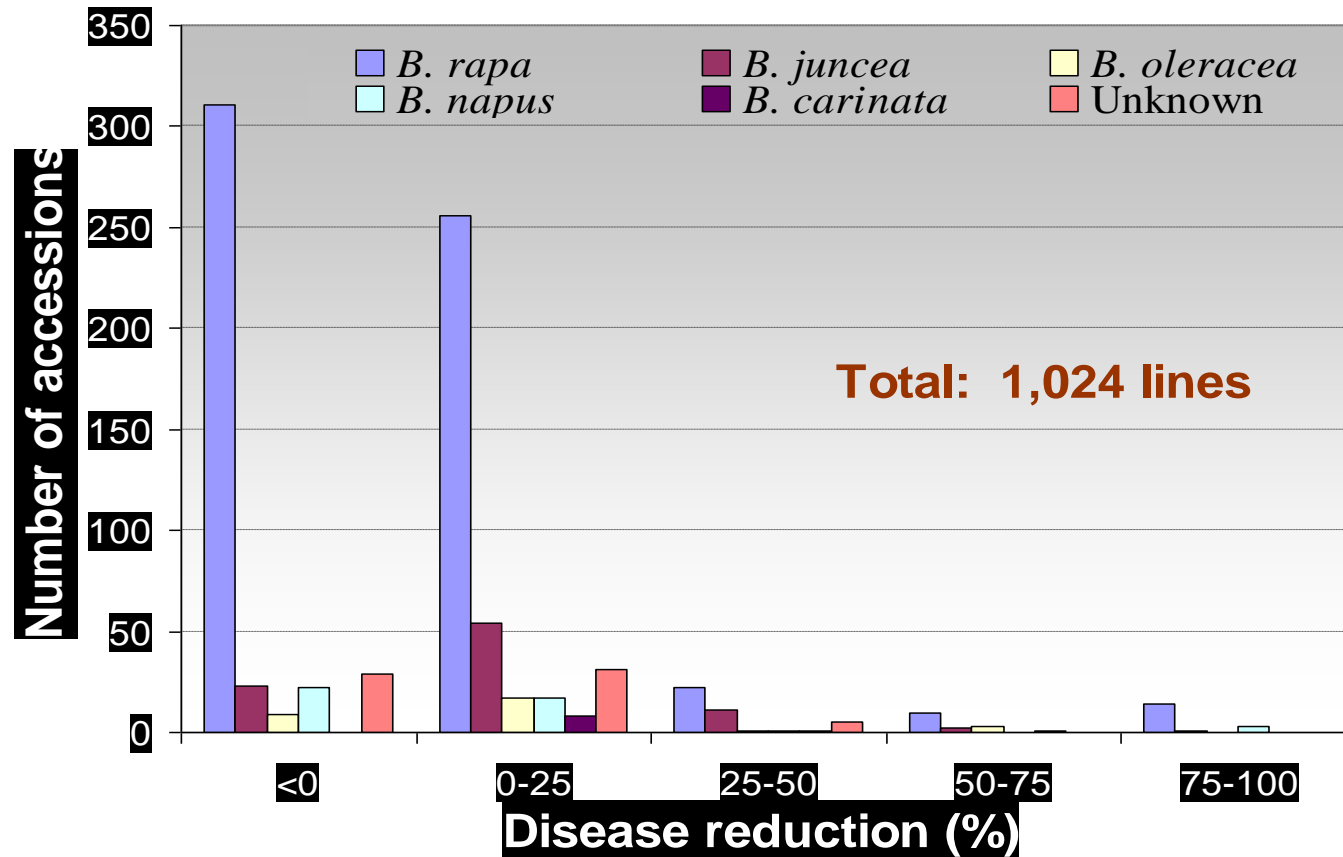
- **Chemical / microbial fungicide control**
 - **Fluazinam, Cyazofamid: more expensive for farmers**
 - **Reduce disease index, less effective**
- **Agronomical practice control**
 - **rotation: need more time, slightly diseased**
 - **application of lime: less effective**

Control of clubroot disease

- **Resistant breeding**
 - **most effective to control, and environmental friendly**
 - **identification of clubroot resistant (CR) genetic sources**
 - **characterize *CR* genes, including gene structure, inheritance and the model of gene action**
 - **the mechanism of resistance**
 - **CR breeding**

Genetics of clubroot resistance in *B. rapa*

■ CR genetic resources in Brassica crops

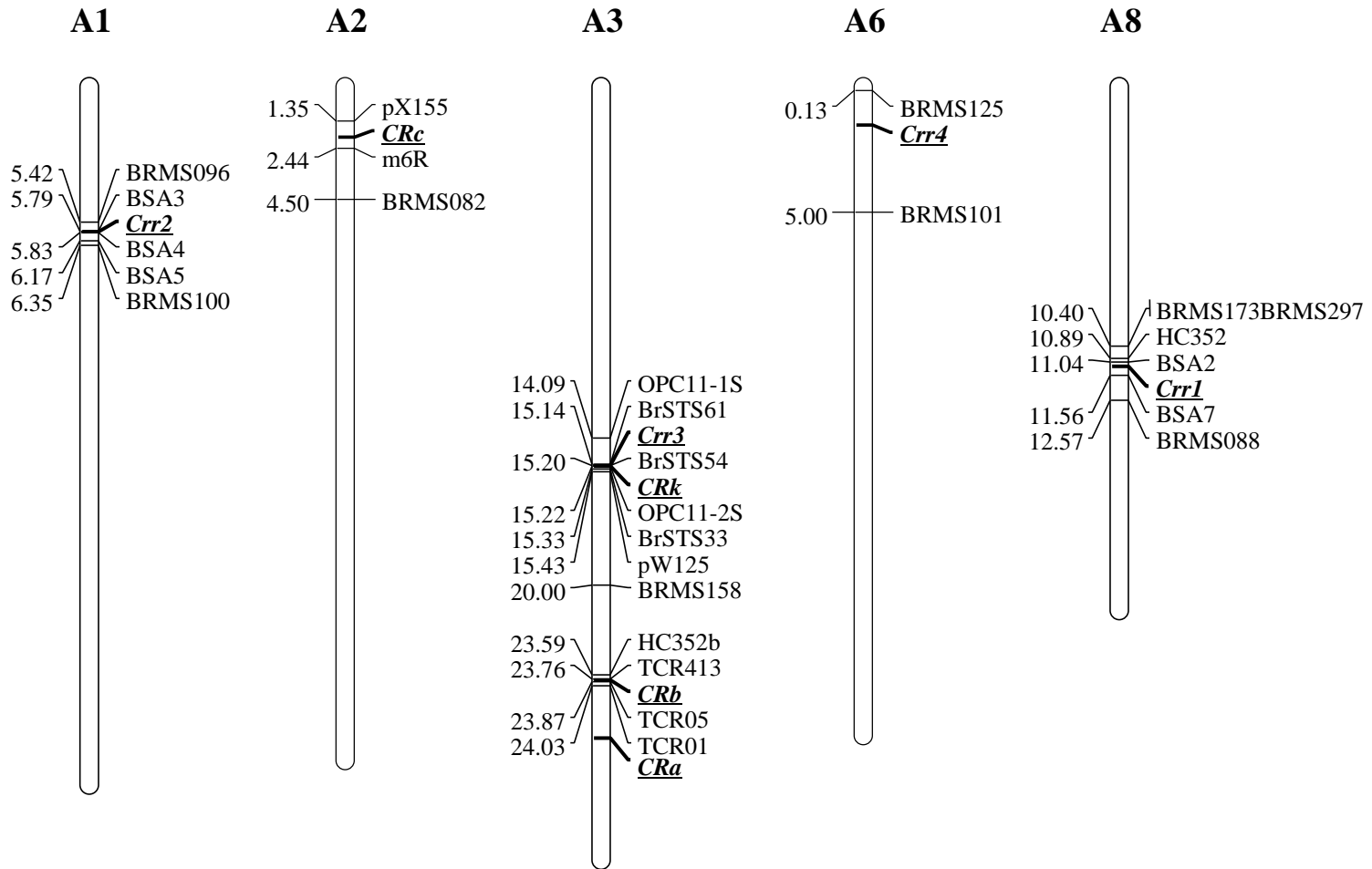


Peng et al.

The main CR resources in *B. rapa*

- Turnip (*B. rapa* ssp. *rapifera*)
 - ECD01 (B+C, *CRb*)
 - ECD02 (A+C, *CRa*)
 - ECD03 (A+B)
 - ECD04 (A+B+C)
 - Debra (*CRc*, *CRk*)
 - Siloga (*Crr1*, *Crr2*, *Crr4*)
 - Milan White (*Crr3*)

CR genes identified and mapped in *B. rapa*



CR* gene identified and mapped in *B. rapa

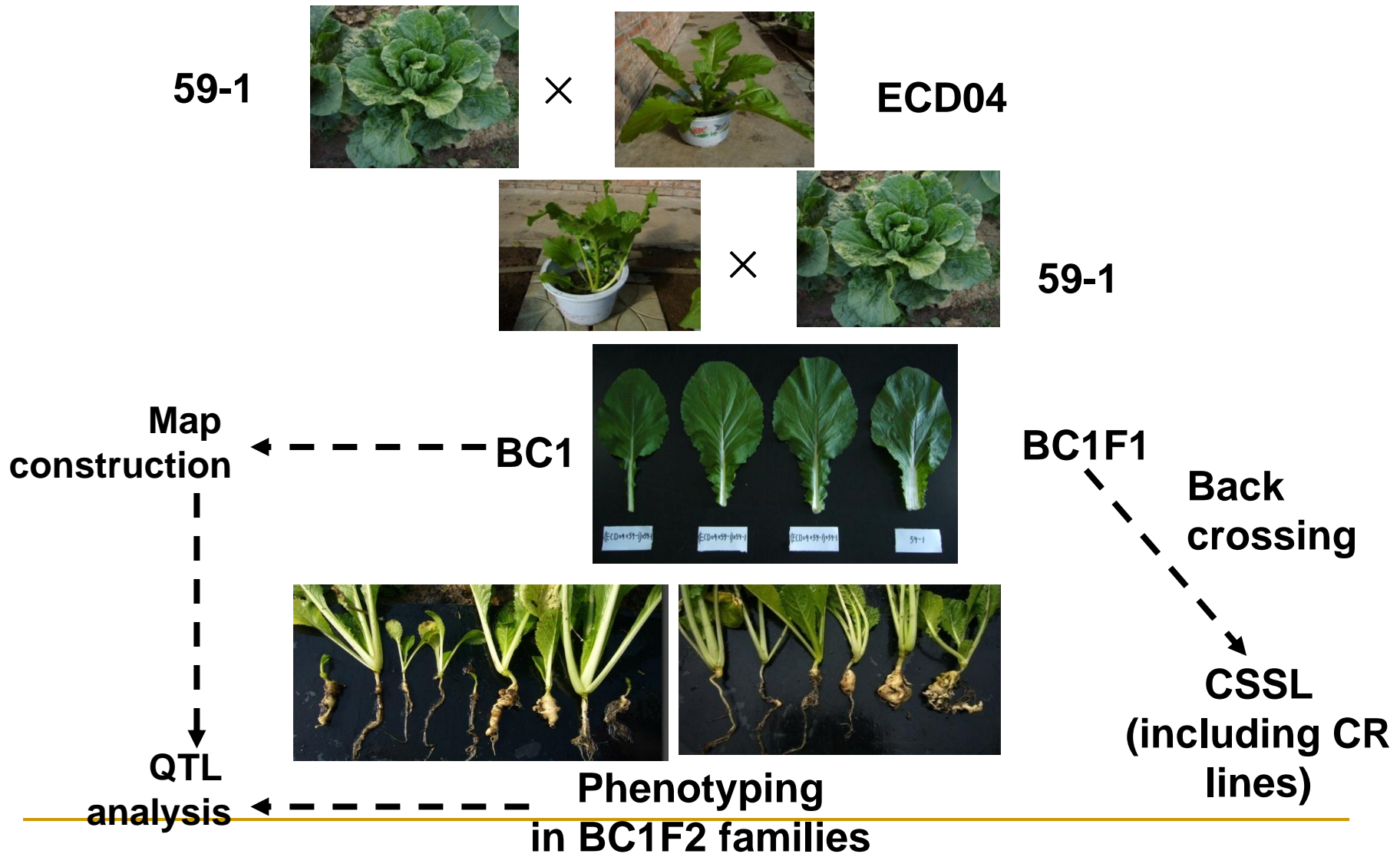
CR gene	CR origin	Chrom	Effect	Reference
<i>Crr1</i>	Siloga	A8	Major	Suwabe et al. (2006)
<i>Crr2</i>		A1	Modifier	
<i>Crr4</i>		A6	Minor	
<i>Crr3</i>	Milan White	A3	Major	Hirai et al. (2003)
<i>CRa</i>	ECD02	A3	Major	Matsumoto et al.(2005)
<i>CRb</i>	ECD01	A3	Major	Piao et al. (2004)
<i>CRc</i>	Debra	A3	Major	Sakamoto et al. (2008)
<i>CRk</i>	Debra	A2	Major	Sakamoto et al. (2008)

Pathotypes of *P. brassicae* in China

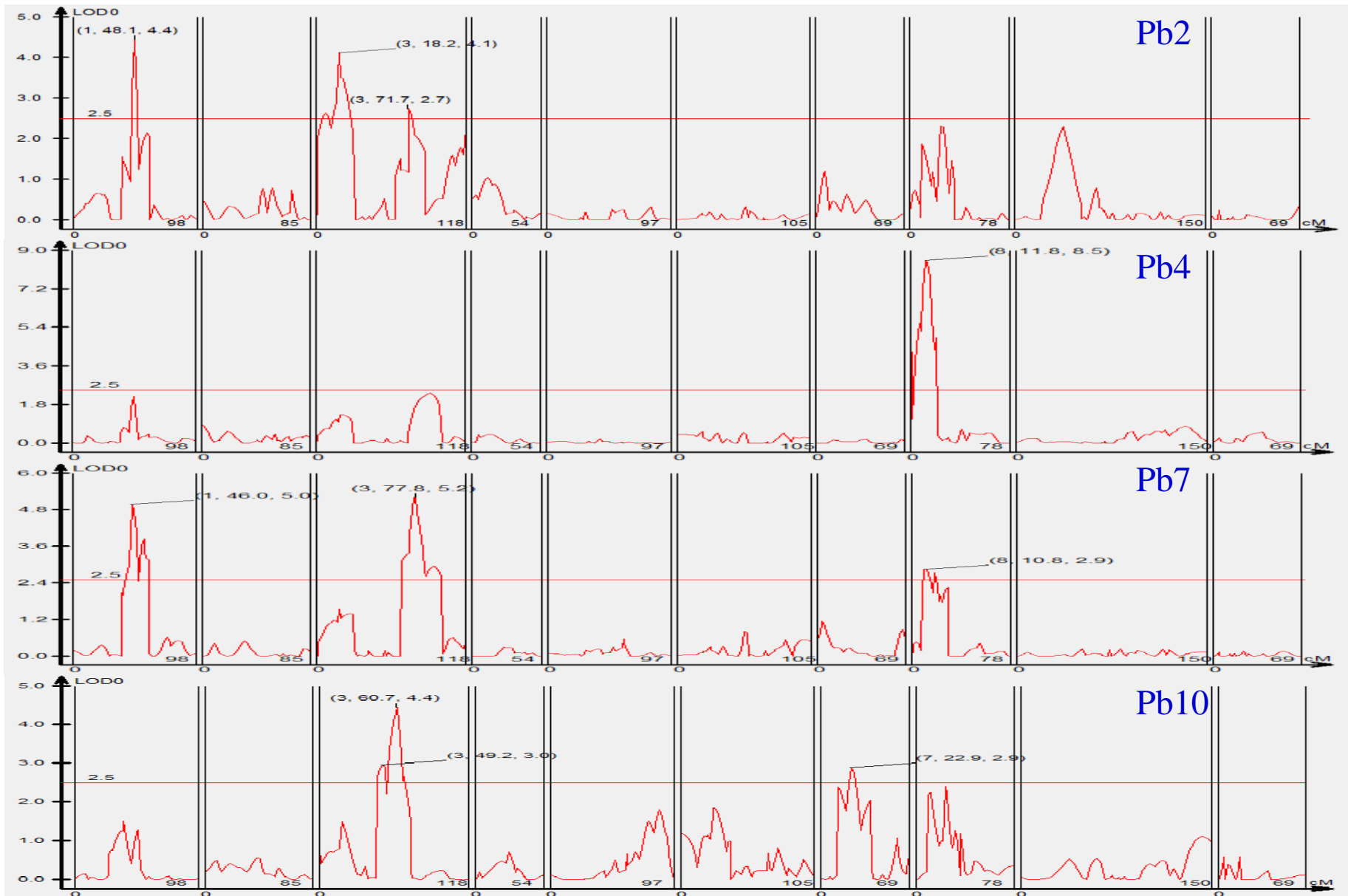
Province	Race	Province	Race
Yunan	1, 2, 4, 6, 7, 10, 11, 12, 13	Shanghai	2, 5, 7
Tibet	2, 4, 5, 7	Sichuan	1, 4, 5, 7, 10, 15
Guizhou	4	Liaoning	2, 4, 11, 14
Anhui	2, 4, 9, 13	Jilin	4
Shandong	2, 4, 7	Hubei	4
Hunan	1, 4, 9, 13		

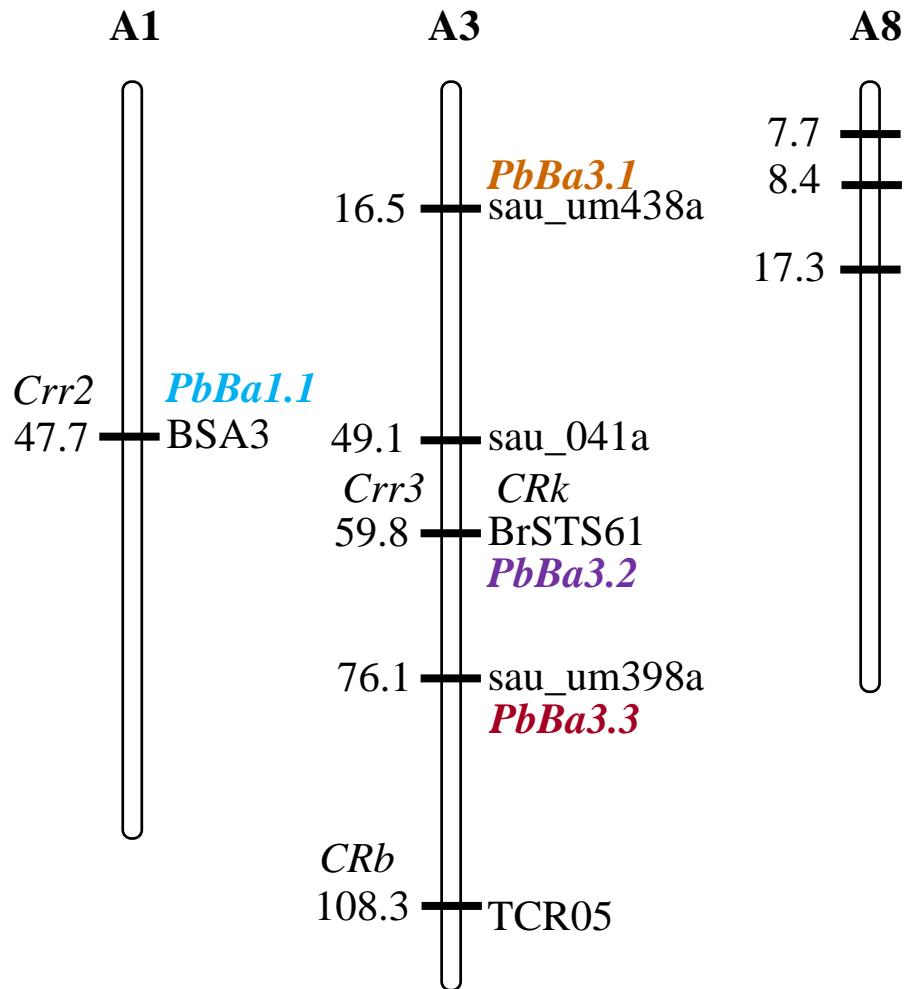
- Race 4 distributed in different area are the same pathotype?
- A new clubroot differentiation set is needed with CR plants which carry known CR gene.

QTL mapping for clubroot resistance



Isolate-specific resistance of CR

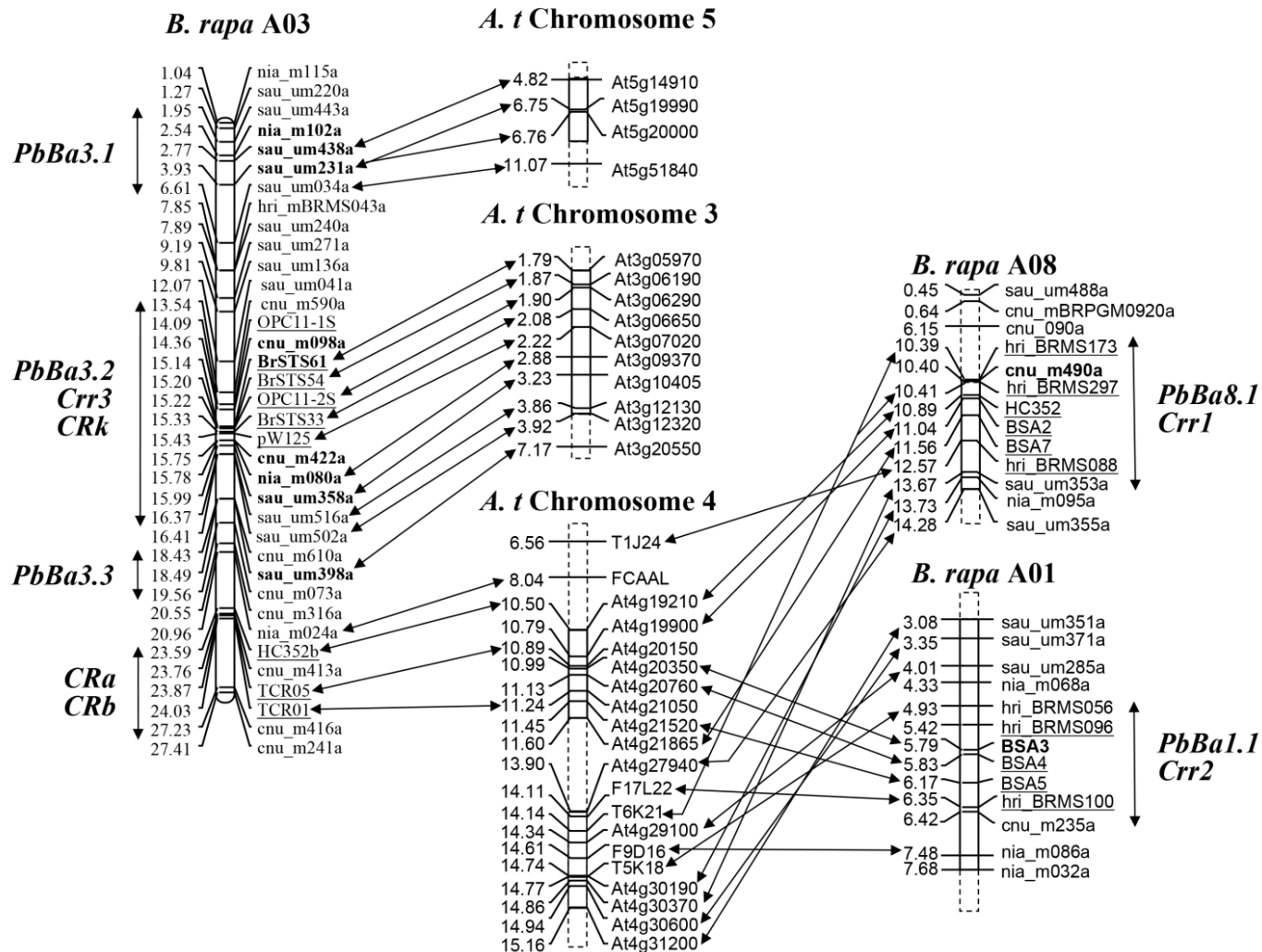




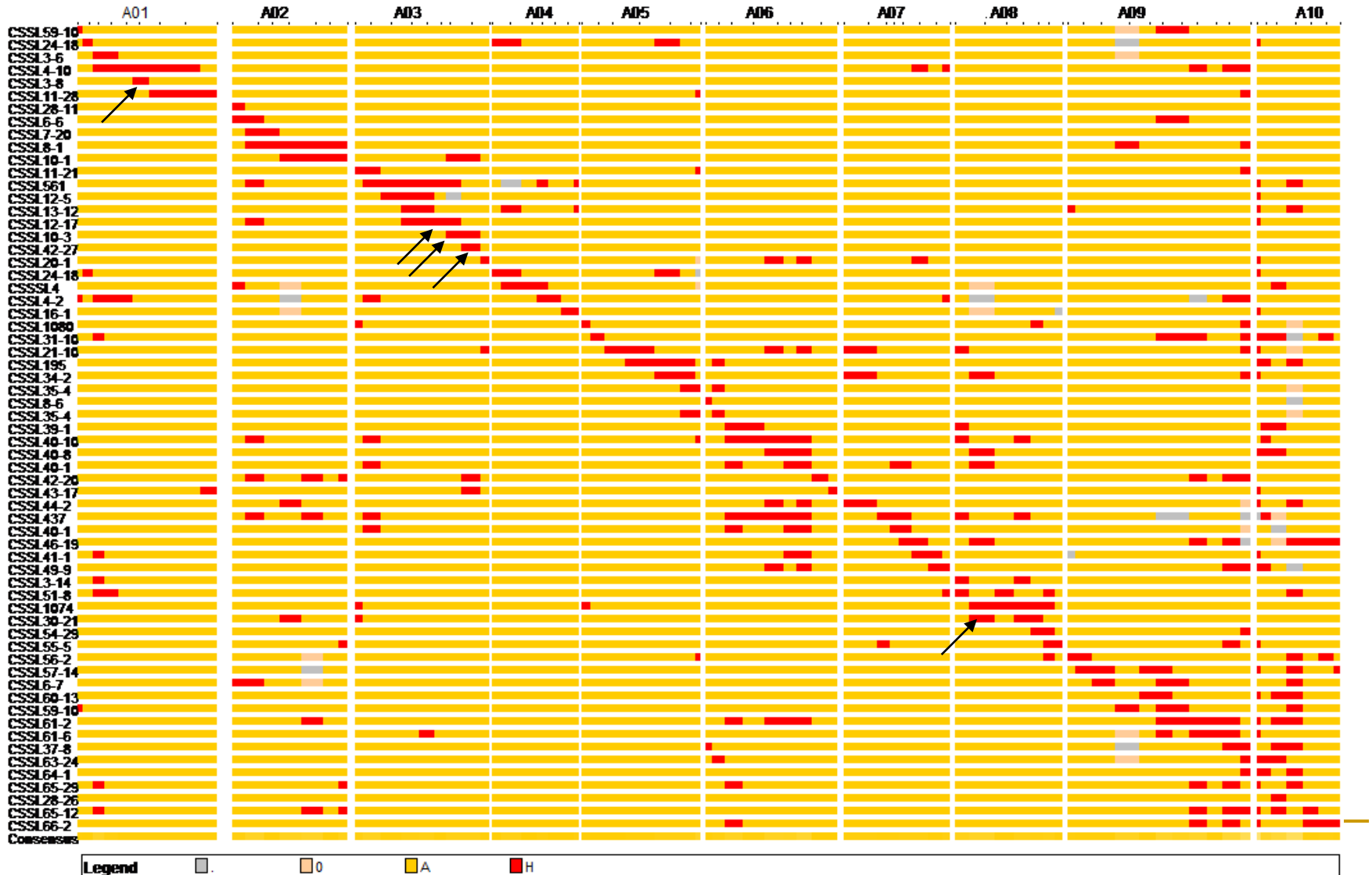
- **Five CR QTLs were identified.**
- **Two were novel.**
- **Three were located in the respective position of *Crr1*, *Crr2*, and *Crr3* (*CRk*)**

Chen et al. 2013

Origin of the CR genes

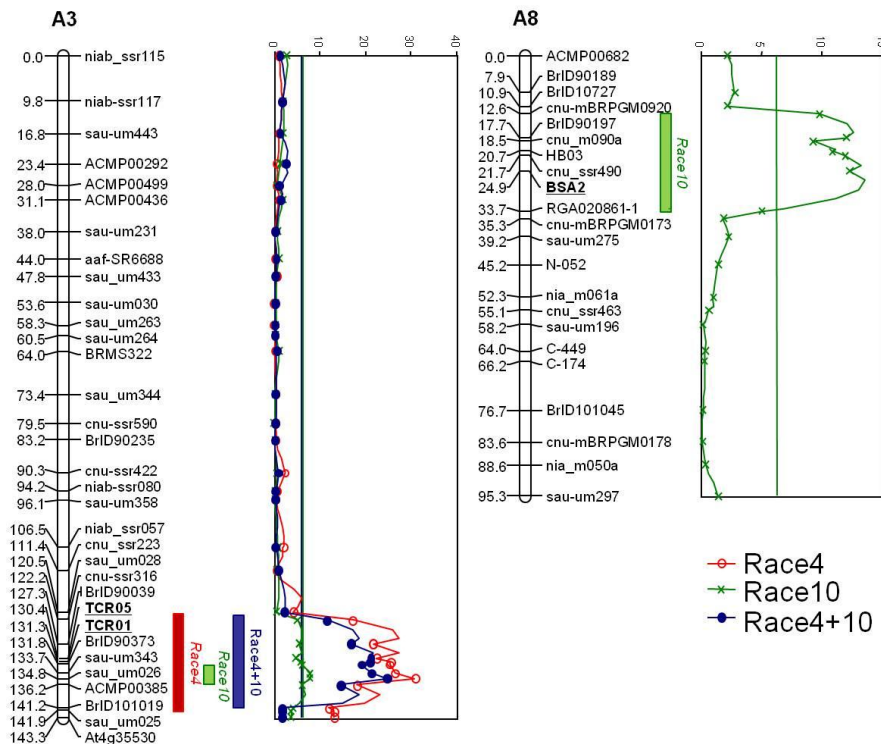


CSSL constructed by integration of chromosome fragment of ECD04 into Chinese cabbage



QTL mapping of clubroot resistance

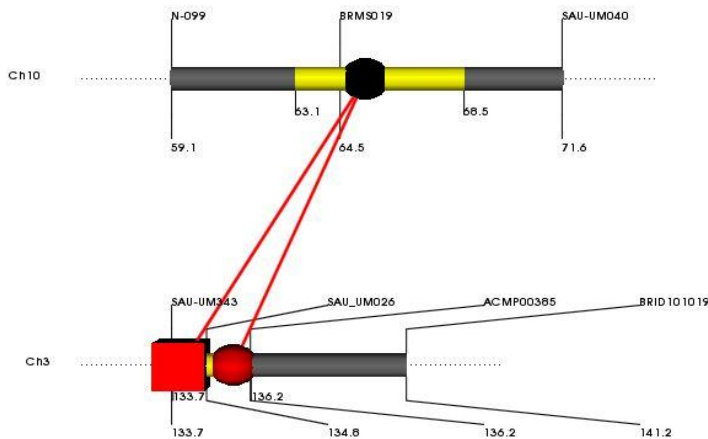
- In siliga, clubroot resistance is isolate-specific



- *Crr1* region possible also with the aids of *CRb* (*CRA*) against race 10
- *CRb* (*CRA*) to race 4
- *CRb* (*CRA*) to the mixture of race 4 and race 10
- Are there any unknown interaction between different pathotypes?

Analysis of epistatic interaction

- Clubroot resistance is also controlled by epistatic interaction between
 - CR QTL and non-CR QTL



Maker for QTL-a	Maker for QTL-b	Epistasis	Epistasis-value	H^2 (%)	P value
sau_um026 (A3)	BRMS019 (A10)	AA	-0.2739	0.0797	0.0006

Isolate-specific resistance of *CR* genes

CR gene	Race
<i>Crr1, Crr3, CRa, PbBa1.1 + PbBa3.1</i>	2
<i>Crr1+Crr2+Crr4, PbBa8.1</i>	4
<i>CRc</i>	K04
<i>CRk</i>	2, K04
<i>CRb</i>	2, 4, 8
<i>PbBa1.1 + PbBa3.3</i>	7
<i>PbBa3.2</i>	10

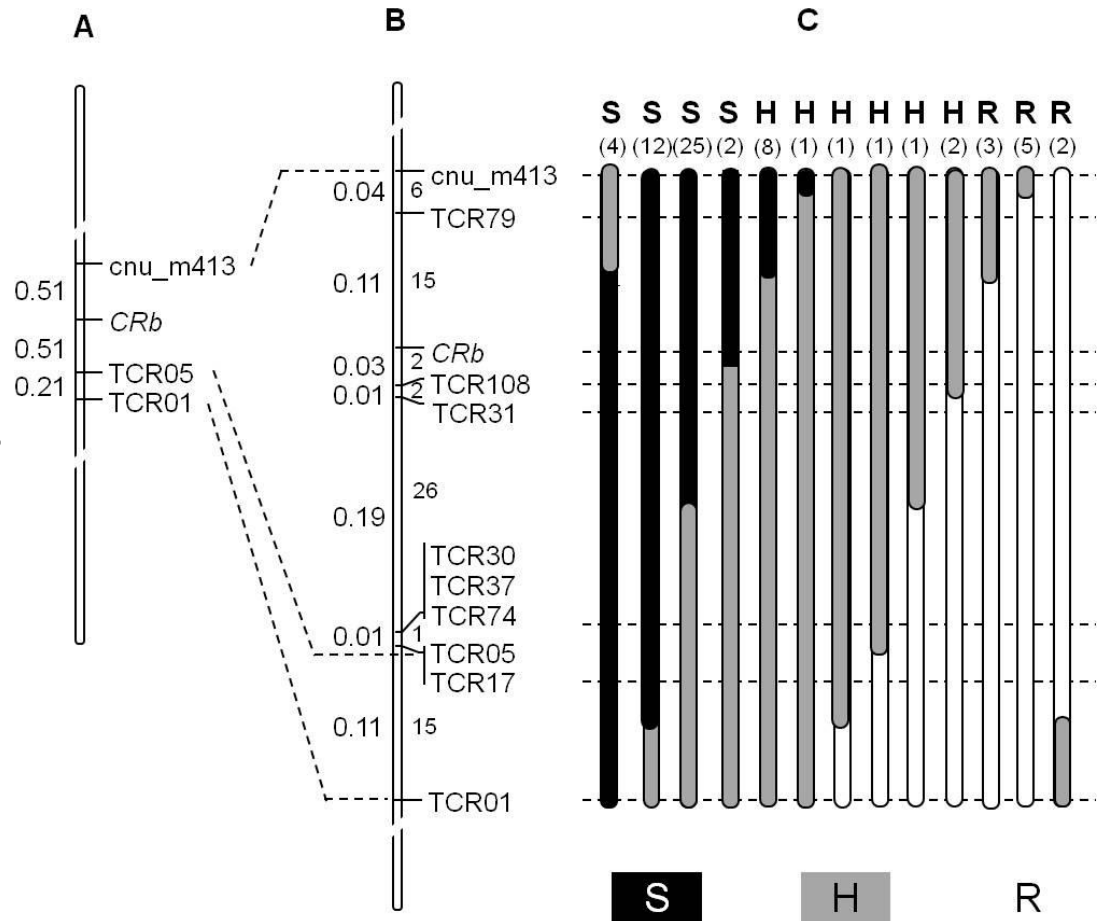
Are these *CR* genes resistance to other pathotype?

Inheritance of *CR* genes in *B. rapa*

- Single gene or QTL, depend on the CR resources
 - Single gene: *Crr3*, *CRa*, *CRb*, *CRCc*, *CRk*
 - QTL (Turnip) :
 - *Crr1*, *Crr2*, *Crr4*, *CRb* (*CRa*) (Siloga)
 - *PbBa1.1*, *PbBa3.1*, *PbBa3.2*, *PbBa3.3* and *PbBa8.1* (ECD04)
- Dominant or incomplete dominant
 - Dominant: *Crr3*, *CRa*, *CRb*, *CRCc*, *CRk*
 - Incomplete dominant: *Crr1* and *Crr2*

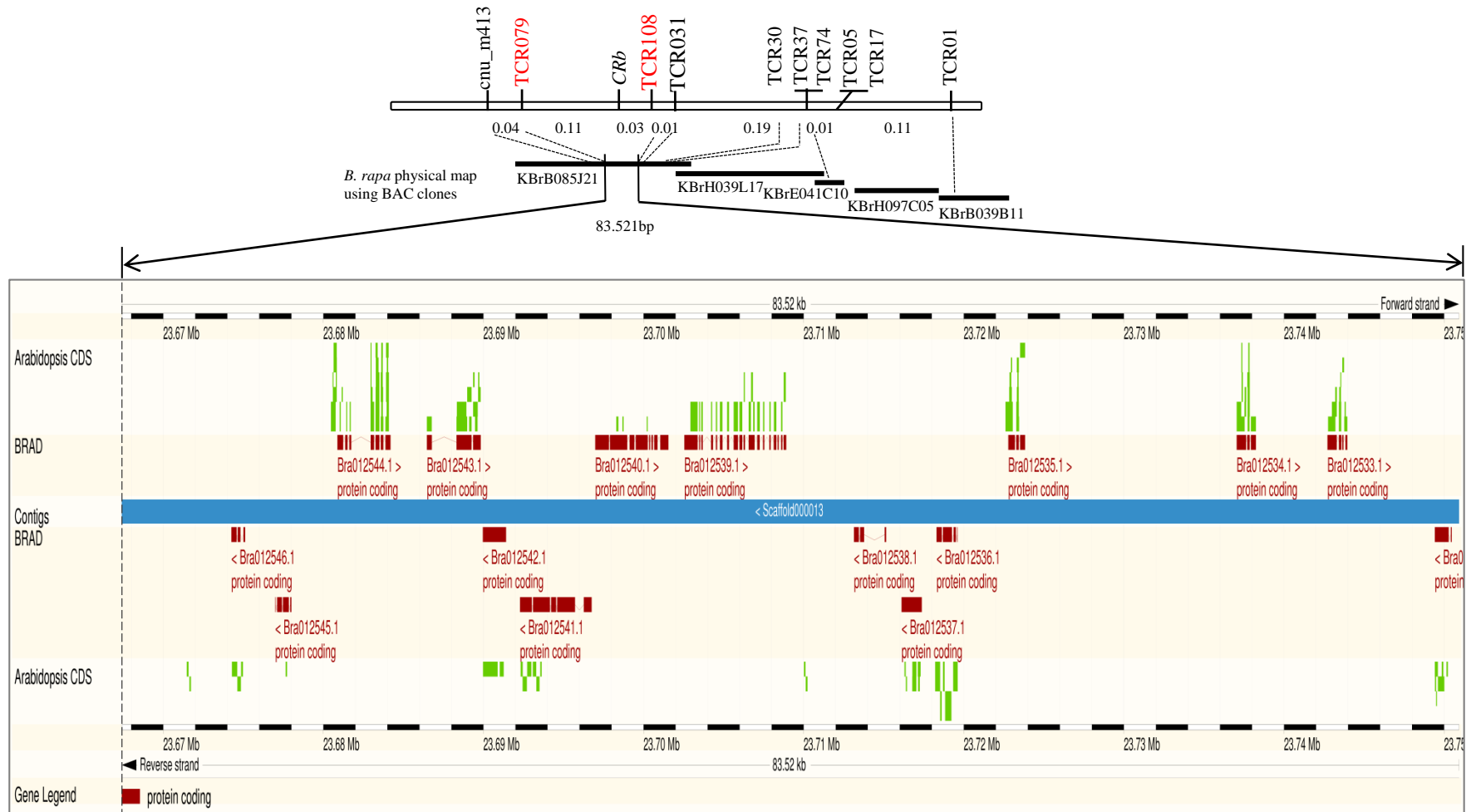
Fine mapping of the *CRb* gene

- A total of 2,896 and 1,486 (susceptible plants selected from 5,800) F2 individuals were used for fine mapping of *CRb*.
- 67 recombinants were found

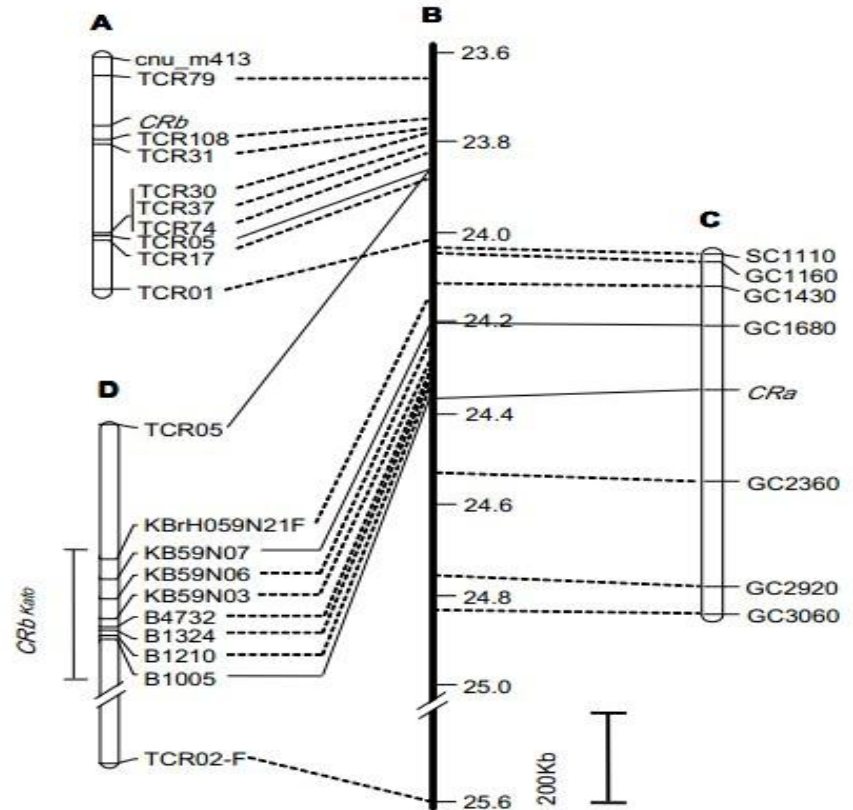
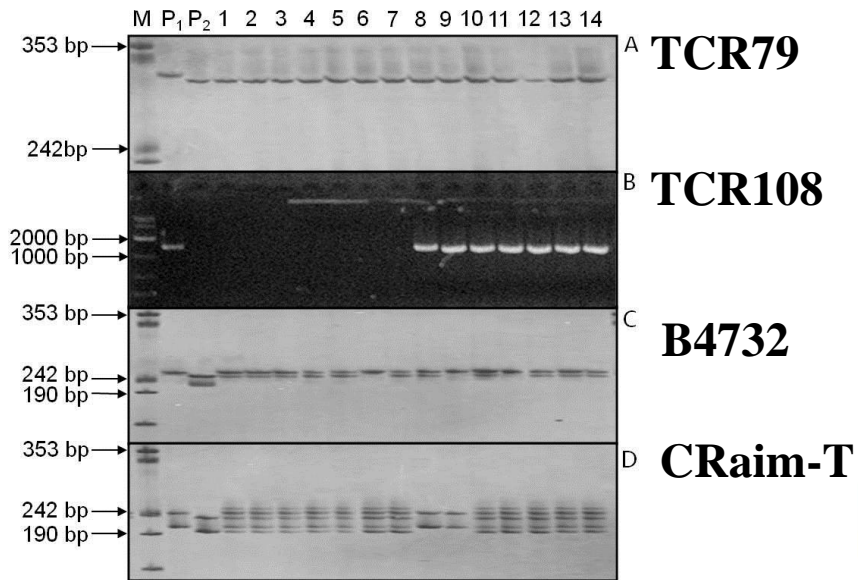


Zhang et al. in press

Candidates of the *CRb* genes



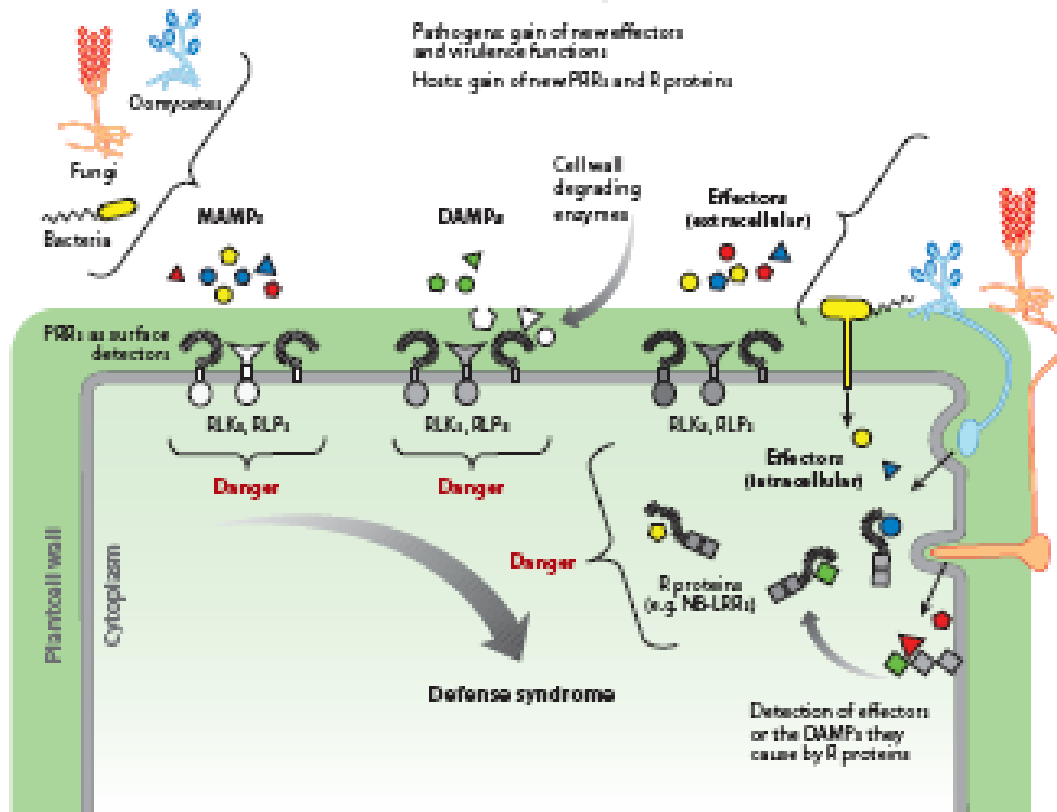
CRa is not identical to *CRb*



Conclusion

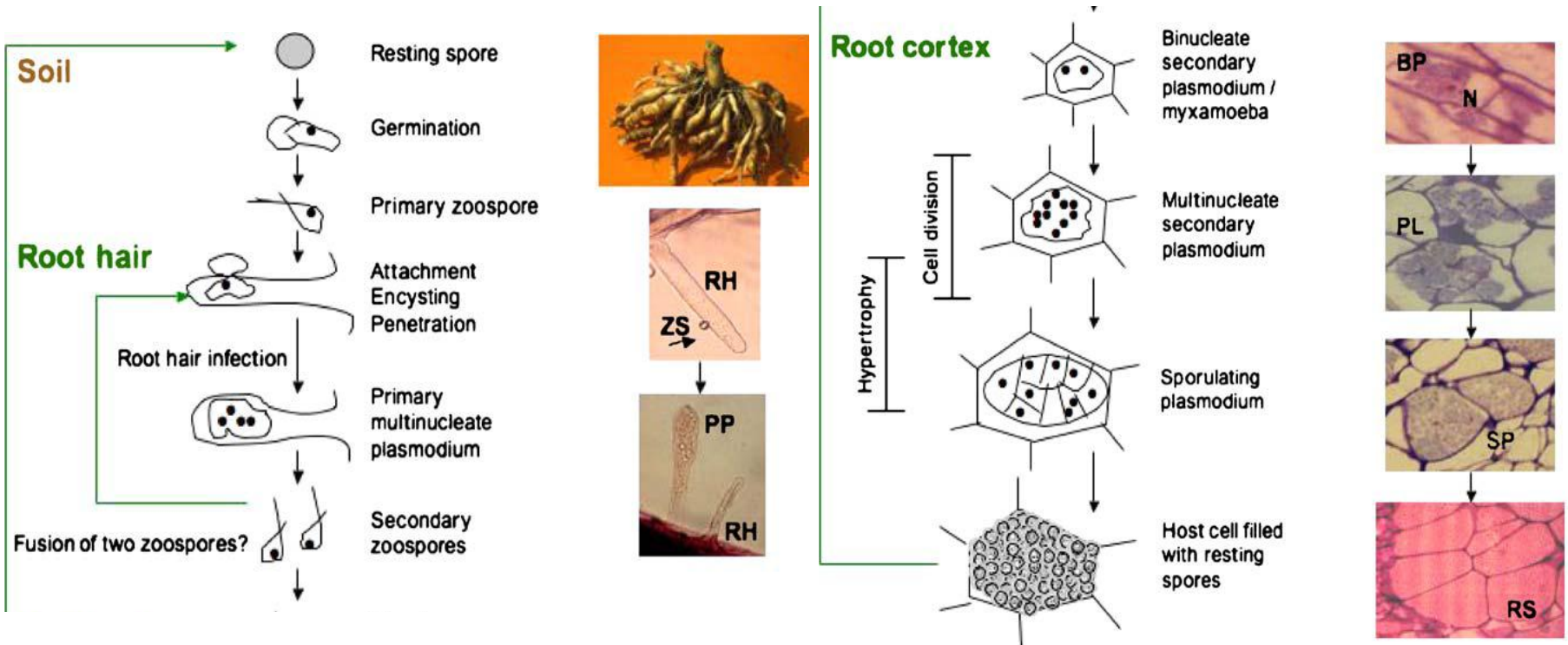
1. Clubroot resistance is controlled either by single gene or polygenes depend on the CR germplasm.
 2. Ten *CR* genes were identified in *B. rapa*.
 3. *CR* genes are isolate-specific.
 4. Epistatic effects is also present in clubroot resistance.
 5. *CR* genes might be originated by at least 3 common ancestors.
 6. *CRa* and *CRb* are not the same gene.
-

Functional Genomics of Clubroot Resistance in *B. rapa*



- PAMPs: pathogen-associated molecular patterns
- PRRs: pattern recognition receptors
- PTI: PAMP-triggered immunity
- ETI: Pathogen effectors-triggered immunity

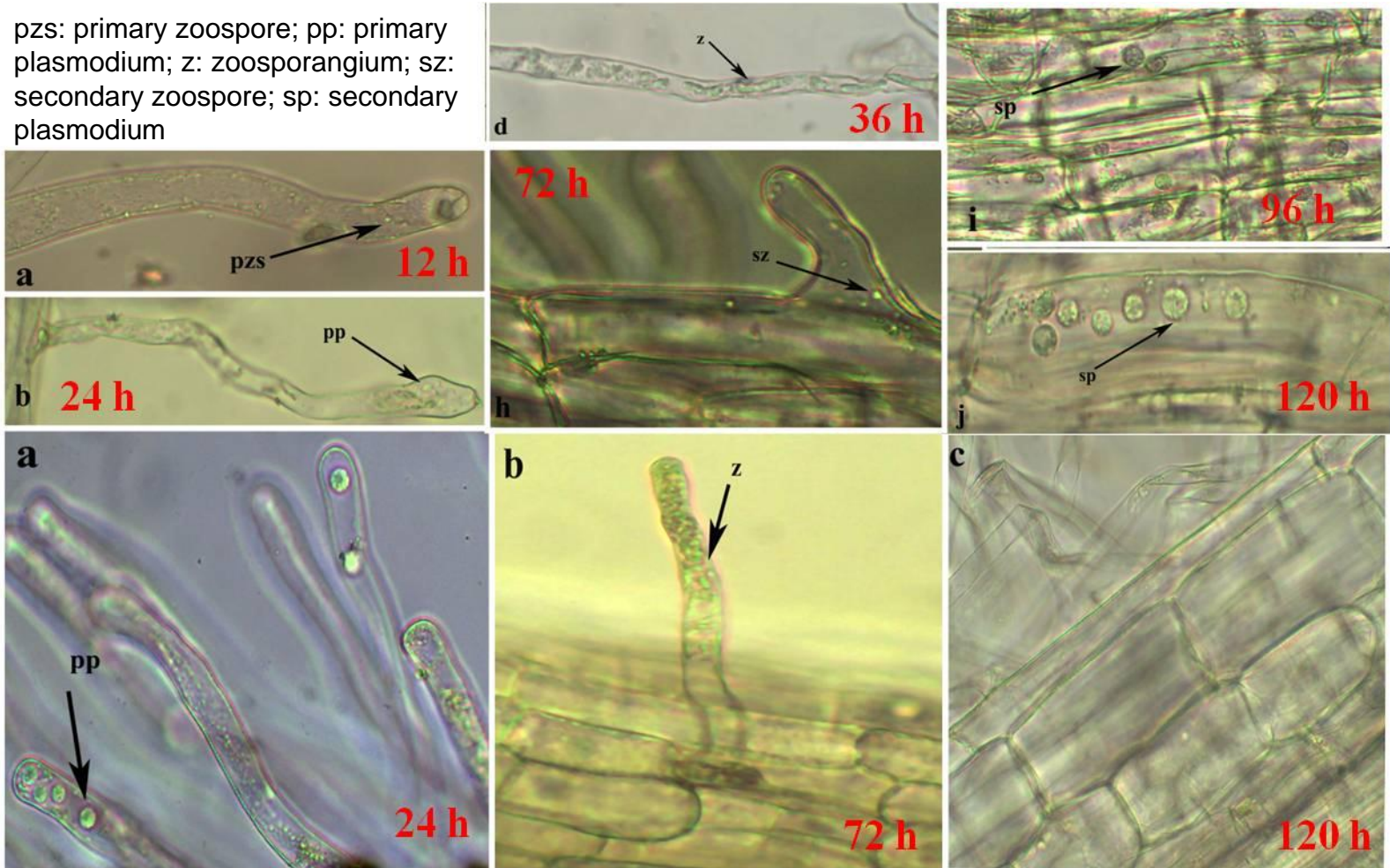
Life cycle of *P. brassicae*



Primary infection of *P. brassicae* into CR line

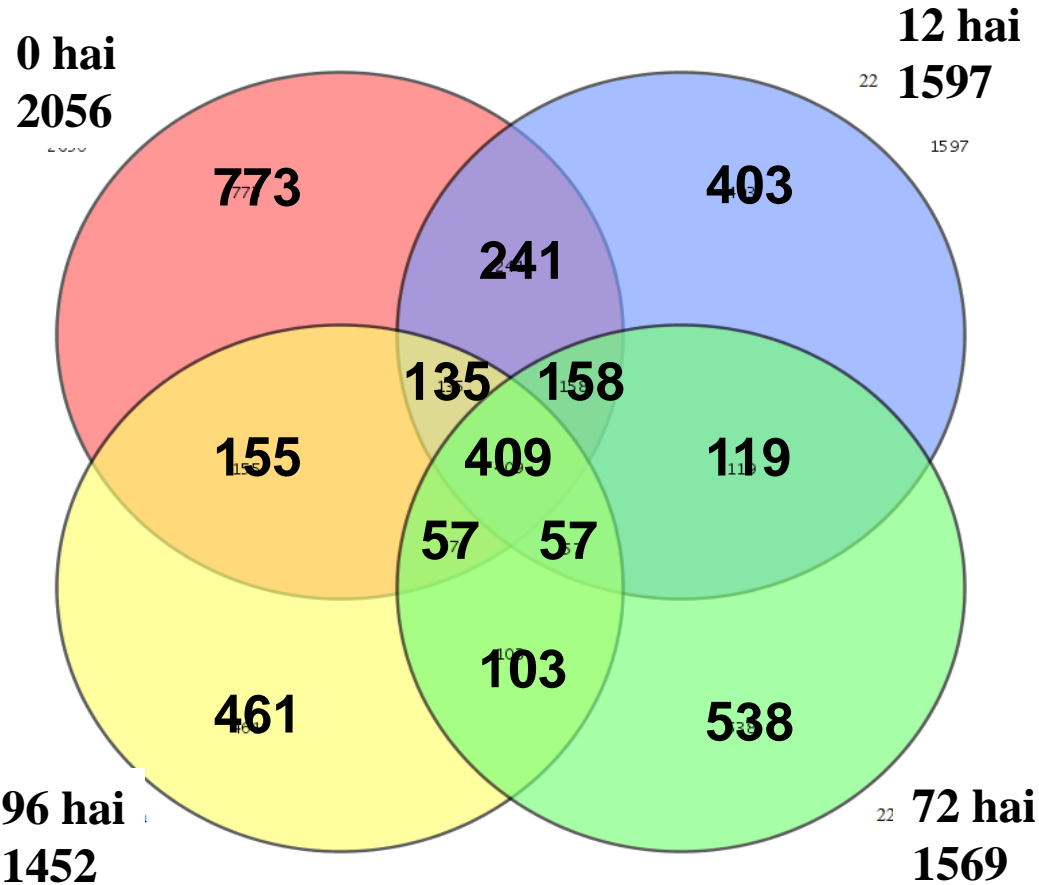
pzs: primary zoospore; pp: primary plasmodium; z: zoosporangium; sz: secondary zoospore; sp: secondary plasmodium

CS NIL

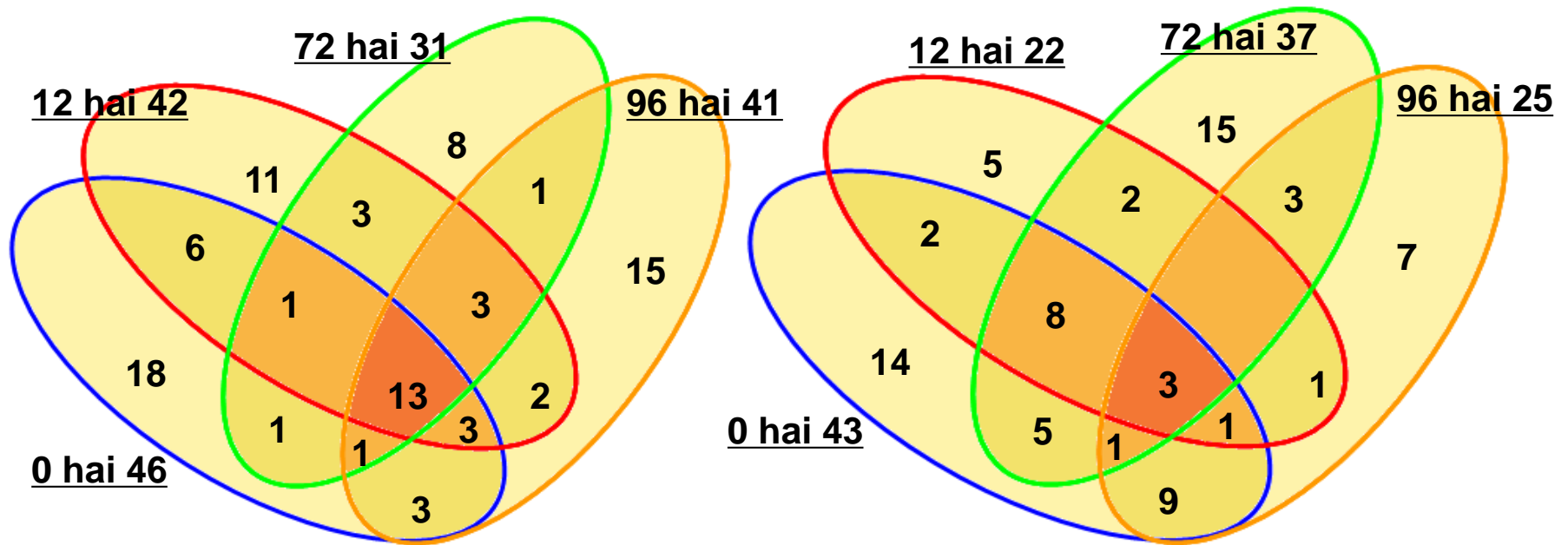


Statistics of illumina 100 reads and comparison to the *B. rapa* reference genome

Samples	Times (hai)	Total reads	Total nucleotides	Mapped reads %	
				Uniq	Multiple
CR NIL	0	22,595,546	4,563,380,909	76.0	2.3
	12	23,182,353	4,681,760,927	76.3	2.4
	72	19,372,251	3,912,648,648	75.7	2.3
	96	23,484,527	4,743,122,682	76.2	2.3
CS NIL	0	21,745,045	4,391,765,197	75.4	2.2
	12	22,598,749	4,563,816,668	75.9	2.3
	76	20,736,266	4,187,963,943	74.4	2.3
	96	26,470,268	5,345,954,462	75.8	2.3
Total		180,185,005	36.4 Gb		

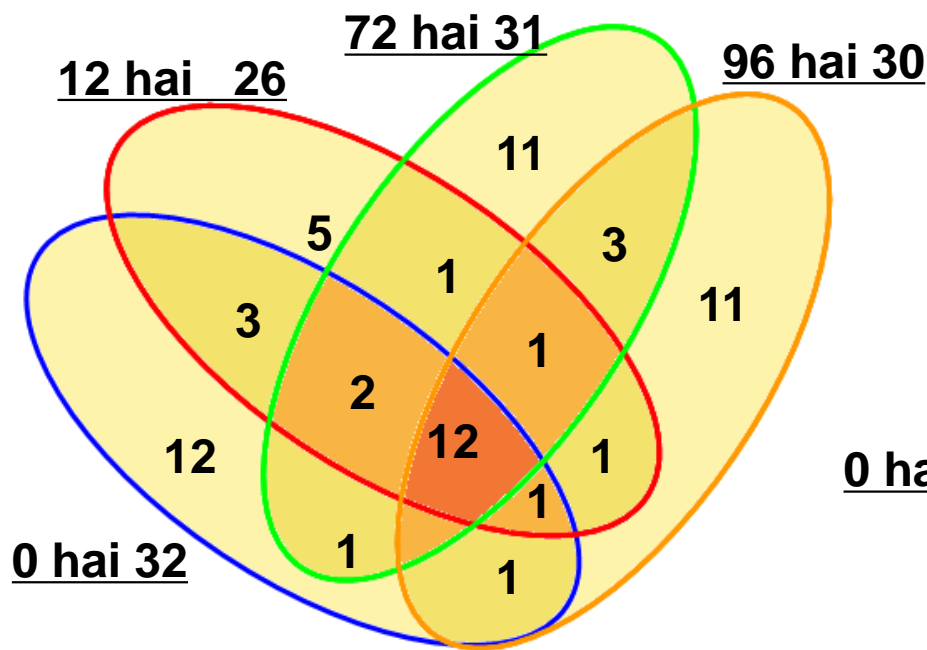


Number of transcripts in the CR NIL that were differentially expressed (FDR<0.01, Fold change>2.0 or <-2.0) compared to CS NIL.

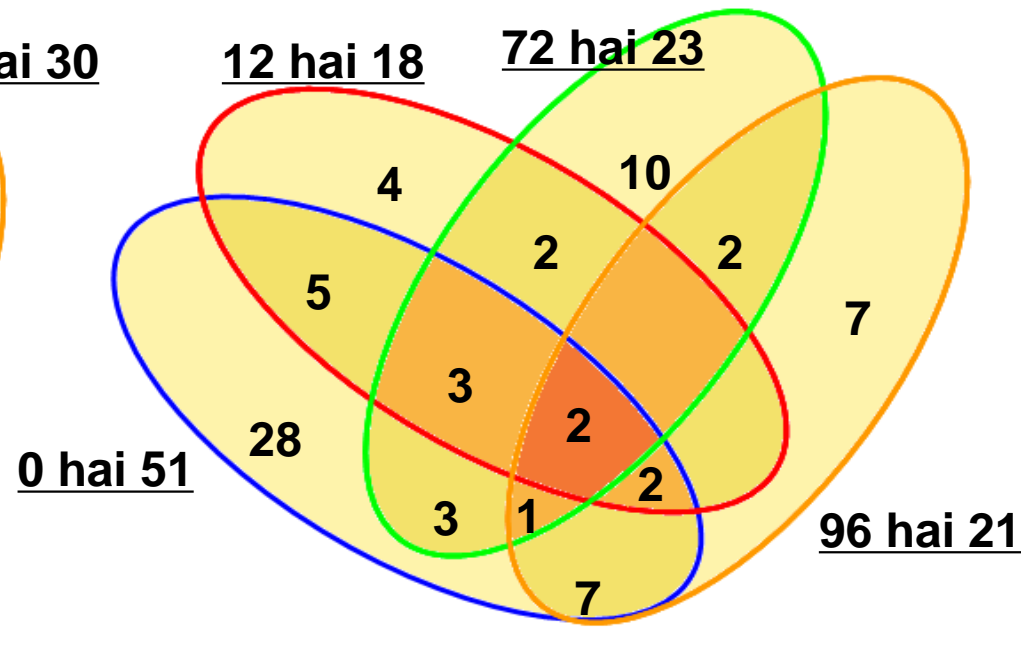


89 genes were down-regulated 76 genes were up-regulated

- A total of 165 unique genes related to ‘defense response’ were differentially expressed in CR NIL at various times after inoculation.
- Of these, 89 were up-regulated and 76 were down-regulated at 0, 12, 72, and 96 hai in CR NIL.
- Of these, only 16 genes were differentially regulated at each time point.



65 genes down-regulation



76 genes up-regulation

- A total of 137 unique genes that related to immune response in GO terms were up- or down regulated in CR NIL at various times after inoculation.
- Of these, 76 were up-regulated and 65 were down-regulated at 0, 12, 72, and/or 96 hai in CR NIL.
- Of these, 14 genes were differentially regulated at each time point.

Conclusion

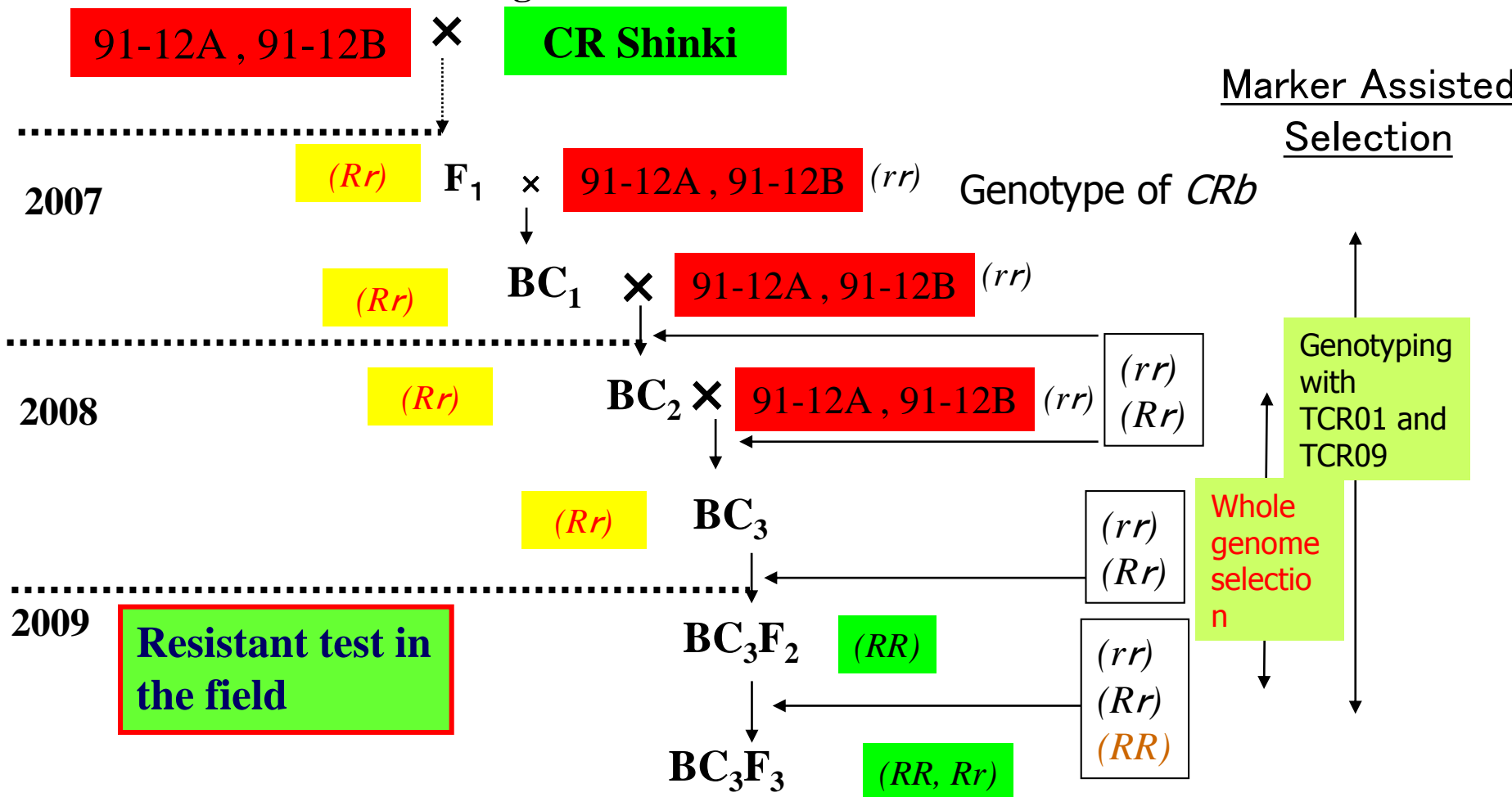
1. Among the putative Chinese cabbage defence response genes identified (GO:0006952), 165 exhibited significant differences in expression between the CR and CS NILs.
 2. Pathogen-associated molecular pattern (PAMP) receptors and the genes induced by these receptors were highly expressed at 0 hai in the CR NIL.
-

Breeding of Clubroot resistance in *B. rapa*

- Traditional breeding
- Marker-assisted breeding of single gene
- Pyramiding *CR* genes with linked markers

Marker-assisted breeding

An elite line of Chinese cabbage





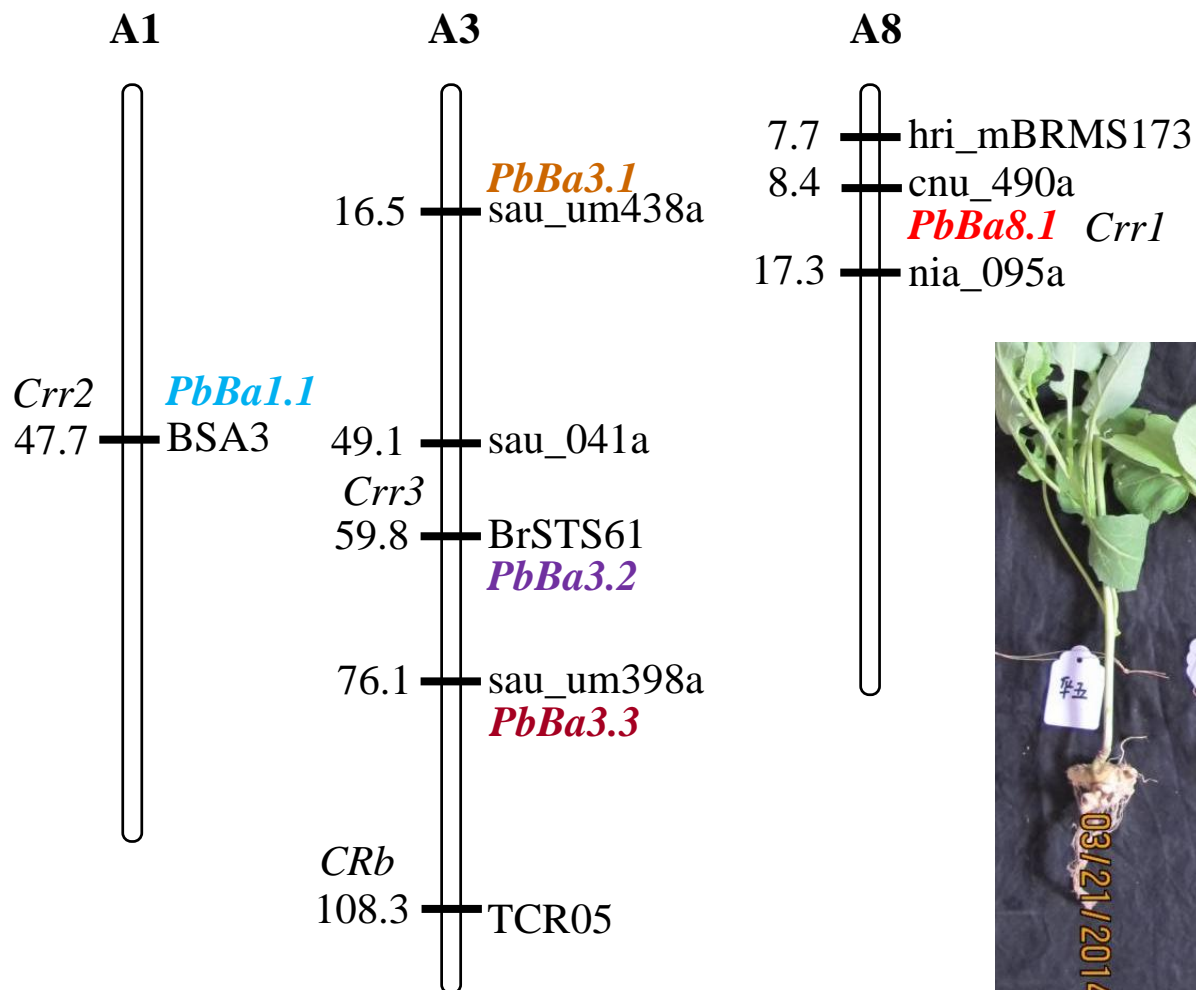
CR12

CR17



青梗菜 ‘CR 702’

Integration of *CR* genes into *B. napus* from ECD04



Some considerations in CR breeding

- ❖ Isolate-specific resistance of *CR* genes
 - ❖ Breeding and cultivation of CR cultivars resistance to specific pathotypes
- ❖ Action of *CR* genes
 - ❖ Dominant genes → CMS or SI → F1 hybrid between CR parent (*CRa* and *CRb*) and non-CR parent
 - ❖ Incomplete dominant → CMS or SI → F1 hybrid between two CR parents with same CR allele (*Crr1* and *Crr2*)

Some considerations in CR breeding

- ❖ **Gene interaction with additive effects**
 - ❖ **interaction between *CR* genes (*Crr1* and *Crr2*)**
 - ❖ **interaction between *CR* gene and non-*CR* loci**
- ❖ **Pyramiding *CR* genes from *CR* turnip or other *CR* resource for durable resistance**
 - ❖ **Transfer several genes simultaneously**
 - ❖ **Transfer *CR* gene to an elite line once a time, then combine them all**

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Thank you

