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Genetic Control of Clubroot Disease at AAFC, Saskatoon

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Canada

Clubroot Disease

HWANG et al. 2012 *MOLECULAR PLANT PATHOLOGY*

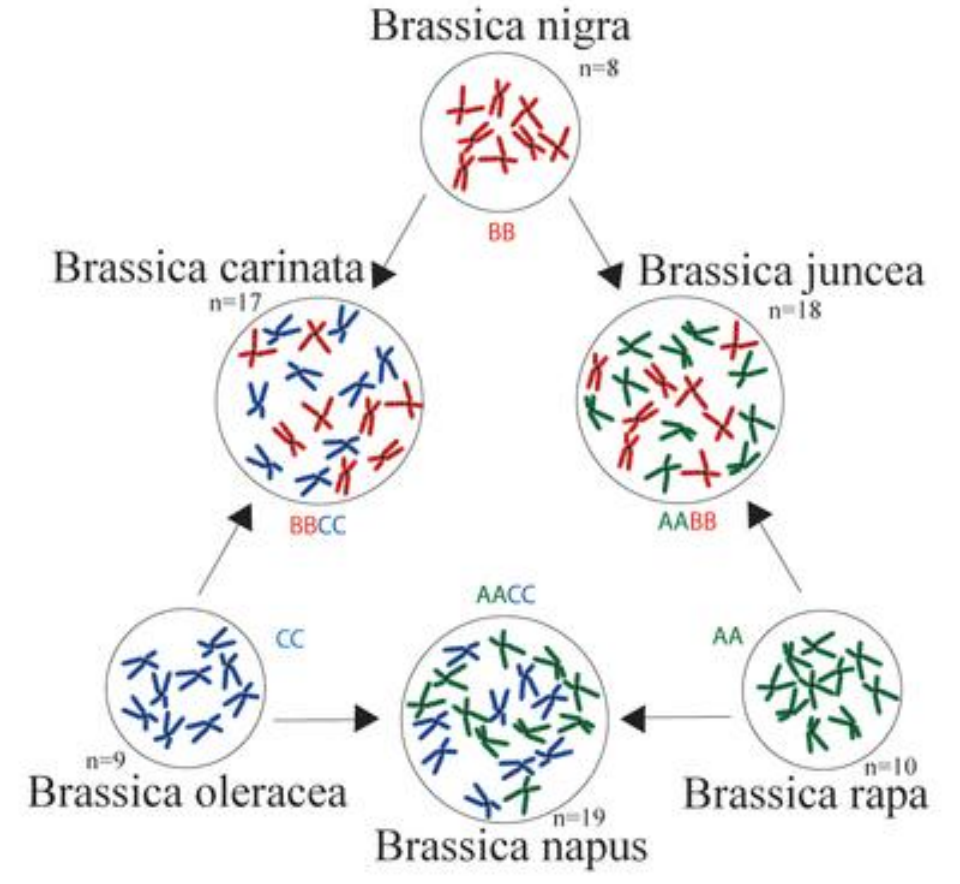
- Occurs in more than 60 countries and results in a 10%–15% reduction in yield on a global scale (Dixon, 2009)
- In Quebec, up to 91% yield loss in infected canola fields (Pageau *et al*., 2006)
- In Alberta, almost 100% yield loss in the most severely affected field (Strelkov *et al*., 2007).
- Significant decrease in seed oil content and an increase in chlorophyll content in the oil (Engqvist 1994; Pageau et al. 2006).

Development of resistant cultivars is considered an essential step to control this disease for all *Brassica* species.

Sources of clubroot resistance in Brassica

Members of the plant genus Brassica - Triangle of U

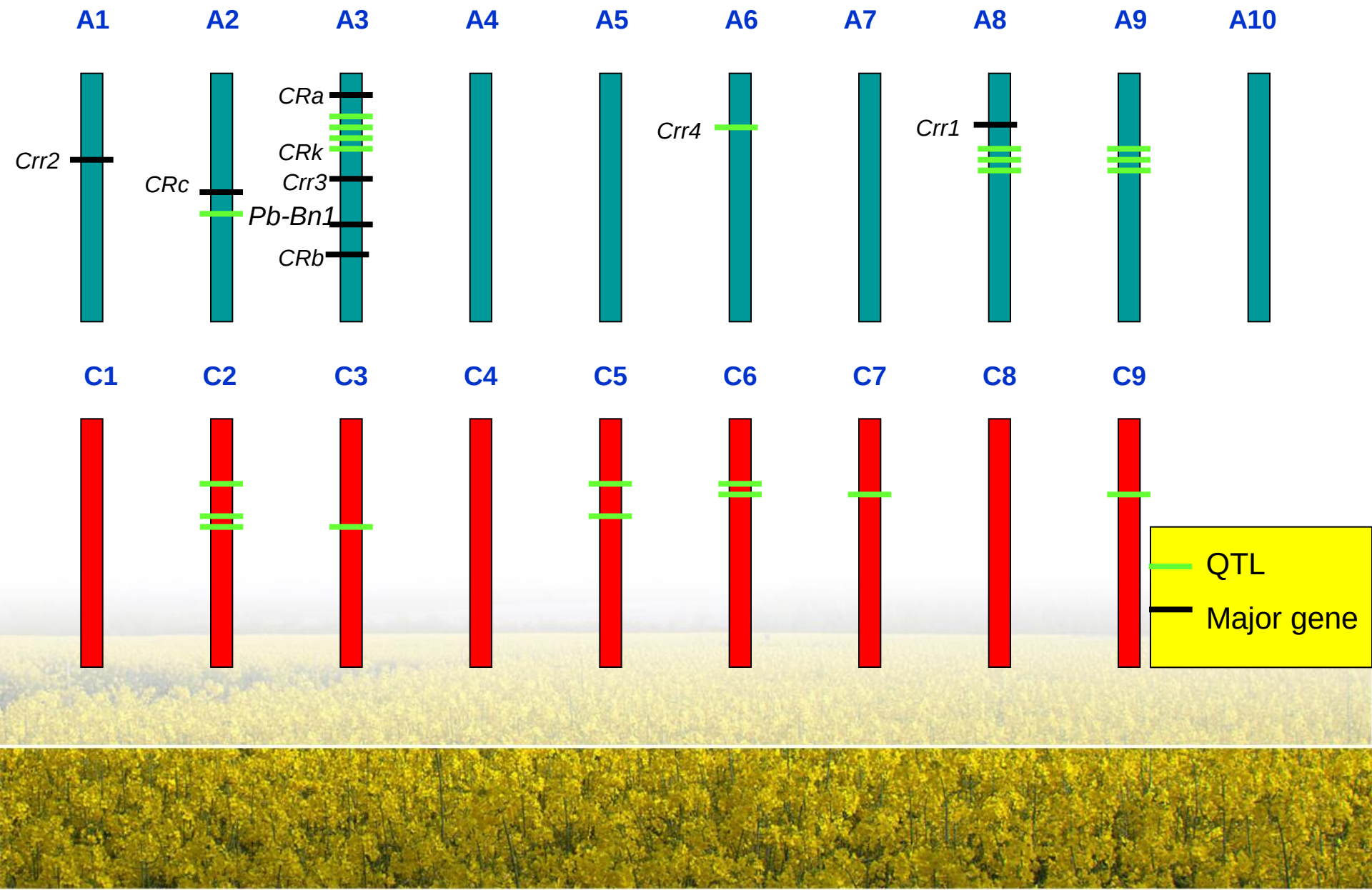
With the exception of *B. juncea* and *B. carinata*, genotypes with resistance to one or more pathotypes of *P. brassicae* can be found in all of the major brassica crops (Diederichsen *et al*., 2009).



Inheritance of Clubroot Resistance in Brassica species

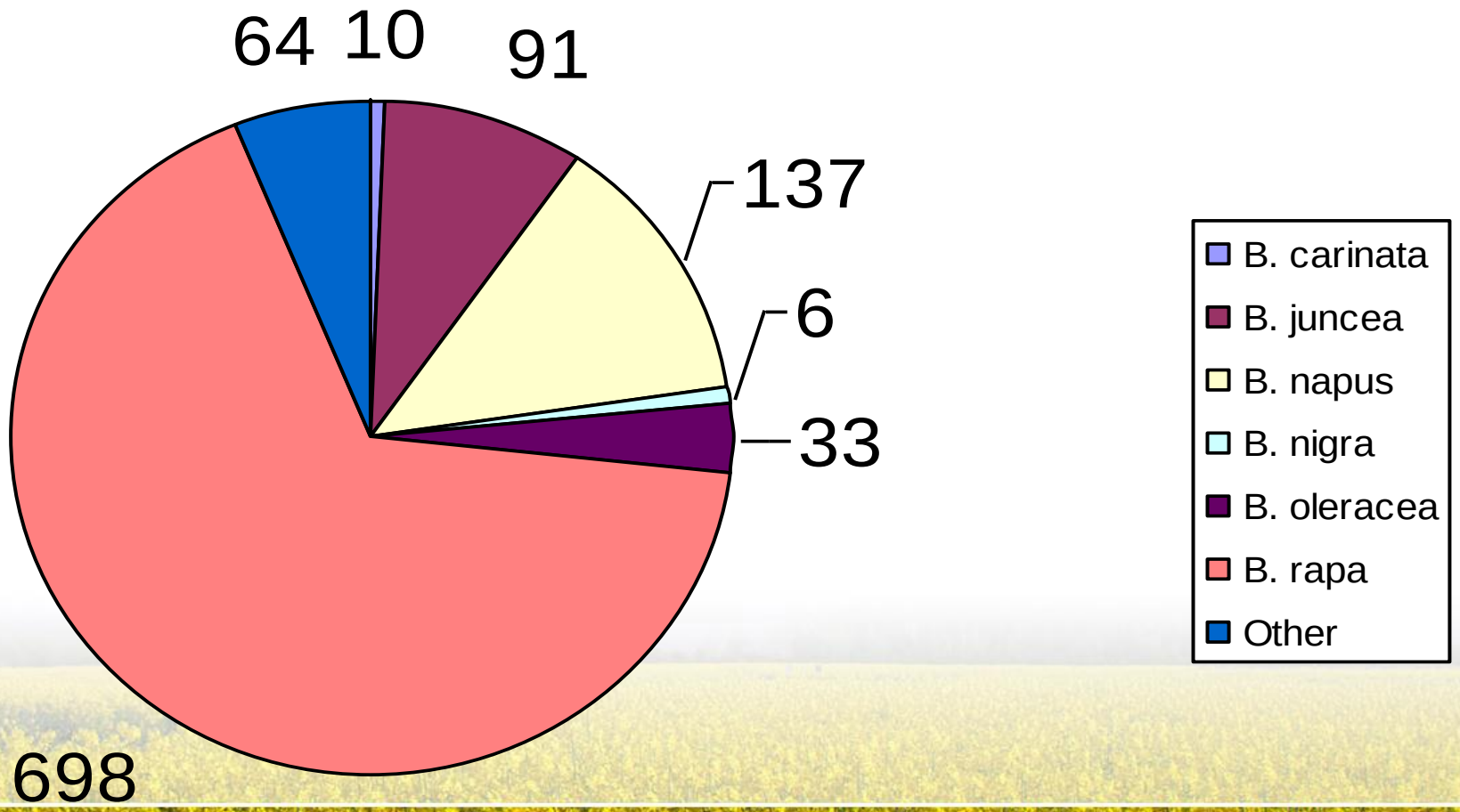
Species	Inheritance	Reference
B. rapa	Single or multiple dominant genes	Wit,1965; Strandberg et al,1967 Hirai et al 2004; Cho *et al*, 2008
	Crr1 and Crr2 complementary	Suwabe et al 2003
B. oleracea	Single or multiple dominant genes	Chiang et al 1983; Laurens and Thomas, 1993
	Multiple recessive genes	Walker and Larson 1951; Chiang and Crete 1970; Voorrips et al 1997; Carlsson et al 2004
	QTLs	Rocherieux et al 2004
B. nigra	NA	
B . napus	One major gene and two QTLs	Manzanares-Dauleux et al 2000
	QTLs	Werner et al 2008

Map of clubroot resistance genes



Screening 1039 Brassica germplasms for resistance to clubroot at AAFC - Saskatoon

- *Plasmodiophora brassicae* pathotype 3



Resistant Brassica germplasm identified at AAFC - Saskatoon

Species	Number of lines	Number of R lines	R%
B. carinata	10	0	0.0
B. juncea	91	0	0.0
B. napus	137	3	2.2
B. nigra	6	2	33.3
B. oleracea	33	3	9.1
B. rapa	698	24	3.4
Other	64	0	0
Total	1039	32	

Development of segregating populations

B. rapa ACDC **X** *B. rapa* lines resistant to clubroot

self-compatible

self-incompatible

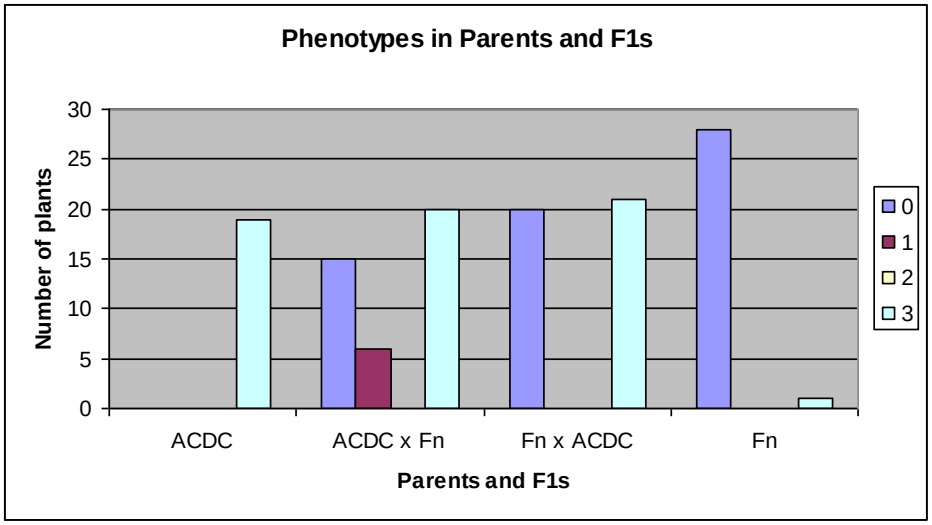
B. rapa ACDC **X** F1 F1 Phenotyping F1

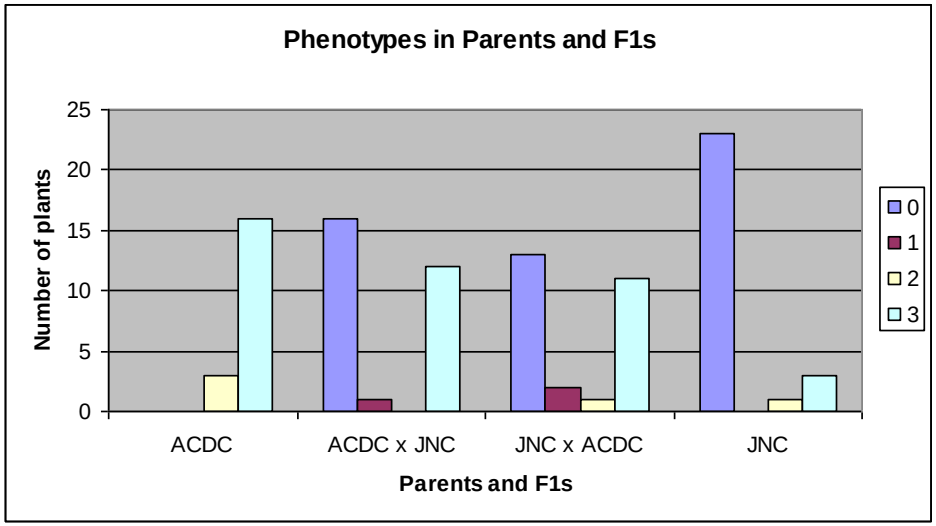
Non-segregation

segregated

BC1 → **Mapping**

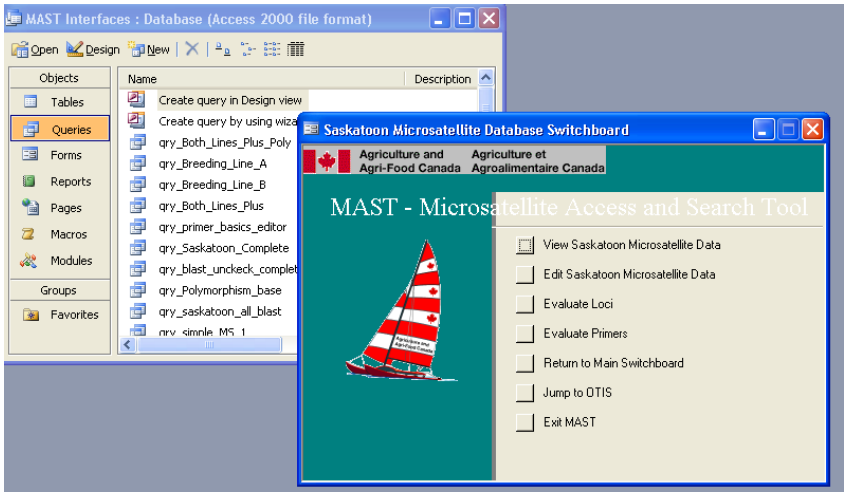
Inheritance of clubroot resistance in *B. rapa*





Cross	CR/CS plants	Expected ratio	X^2	P
ACDC x Fn	199/166	1:1	2.98	0.084
ACDC x JNC	640/639	1:1	0.00	0.978

Mapping of CR genes



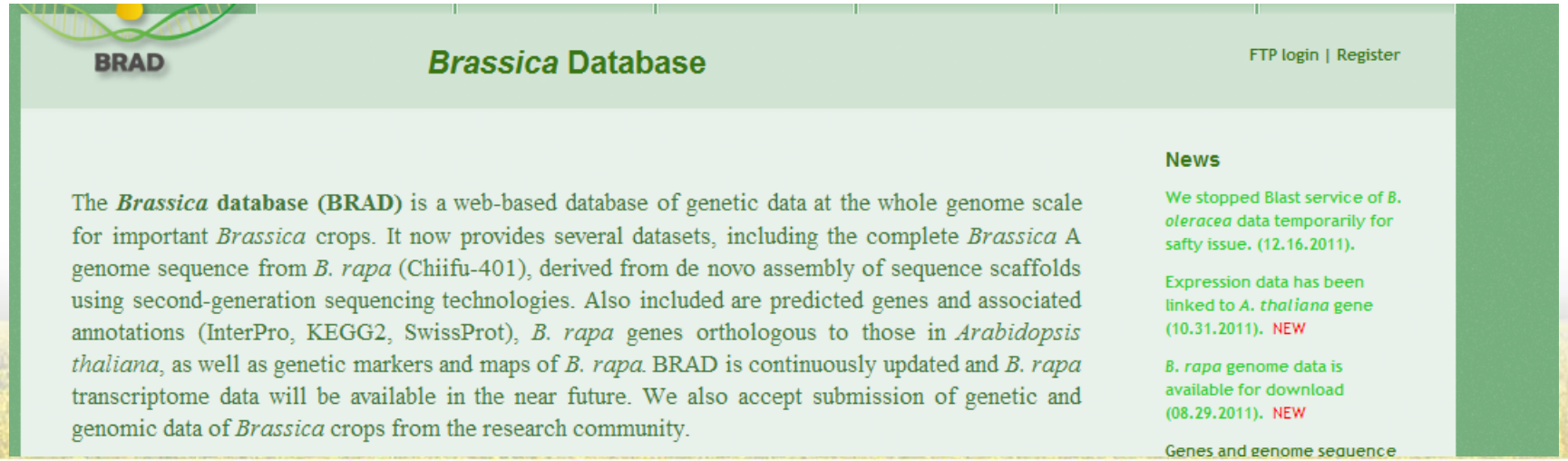
AAFC microsatellite markers

B. napus > 4000

B. juncea: 2085

B. rapa sequencing information at

http://brassicadb.org/brad/



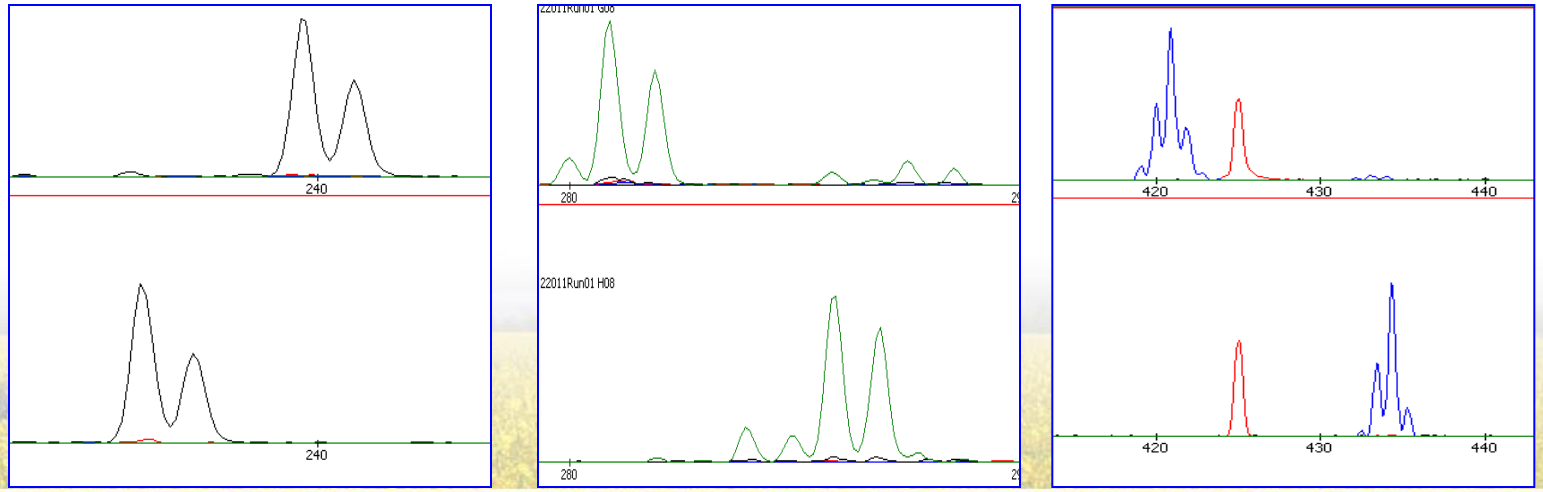
Mapping of CR gene in *B. rapa*

Populations
- ACDC x JNC
- ACDC x FN

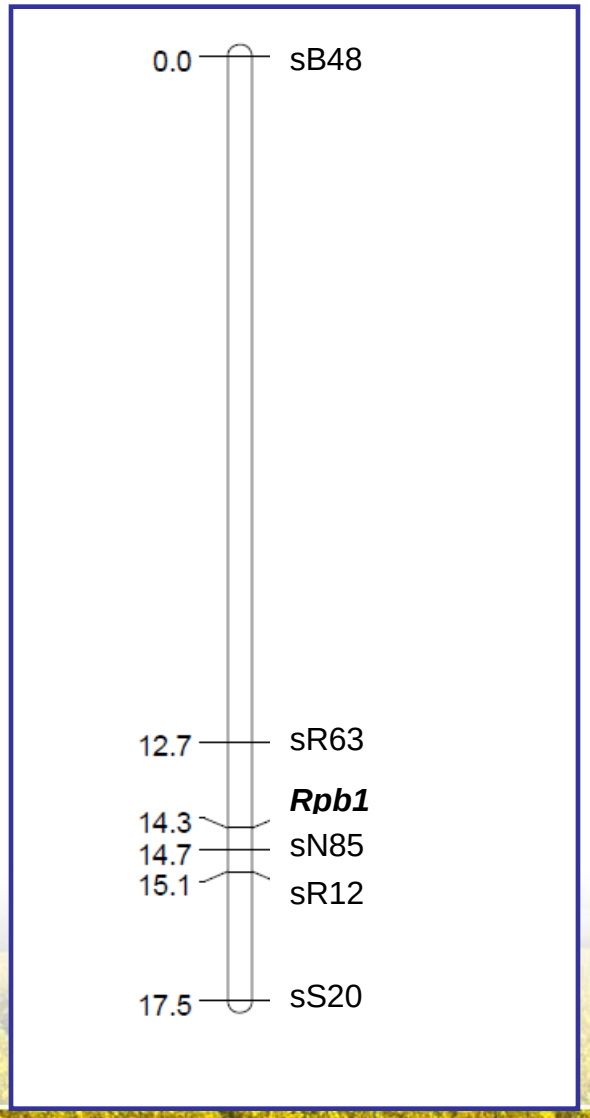
Microsatellite marker
- 500 robust markers
- evenly distributed in A genome (A1-A10)
- Approx. 40% of the markers are polymorphic in the populations

JNC

ACDC



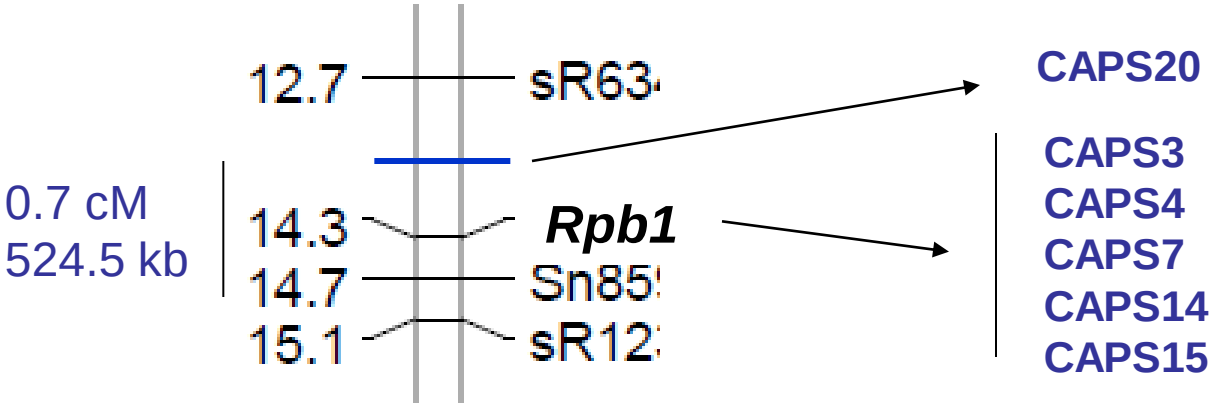
Mapping of CR gene in *B. rapa*



- Two R lines (FN and JNC)
- A CR gene *Rpb1* was mapped on *B. rapa* linkage group A3
- Microsatellite markers linked to the genes:

  an interval of 2 cM
- As *B. rapa* genome sequence was released on August 29, 2011, further fine mapping and marker development have been carried out.

Fine mapping of *Rpb1*



- Two segregating populations:

  > 1500 individuals each

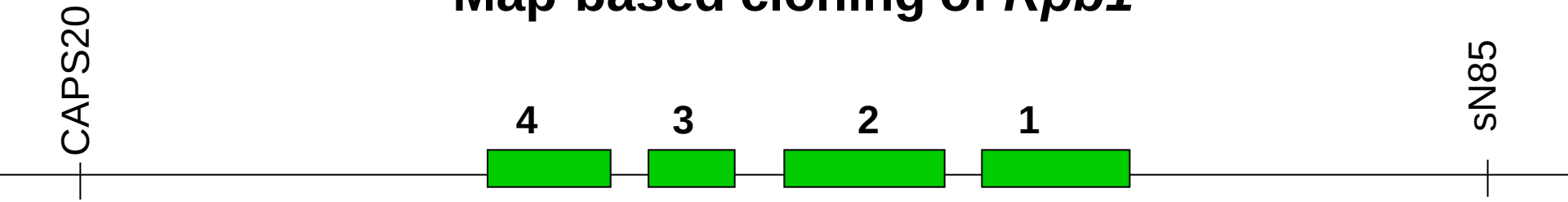
- CAPS markers tightly linked to the genes:

  Flanking markers: 0.7 cM

  5 markers co-segregated with the R gene

- Putative disease resistance proteins (TIR-NBS-LRR class) in the region

Map-based cloning of *Rpb1*



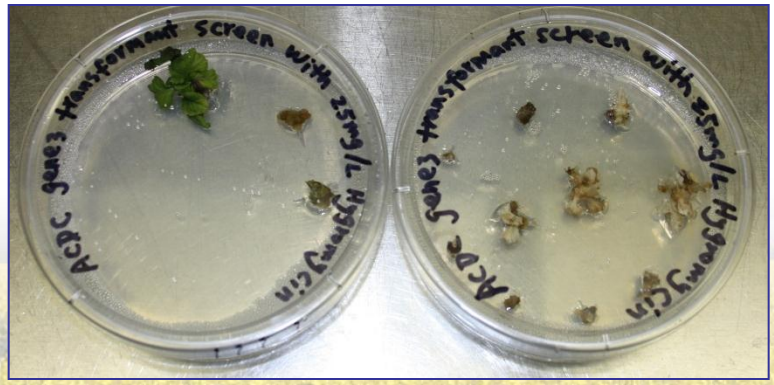
In the interval of CAPS20 and sN89:

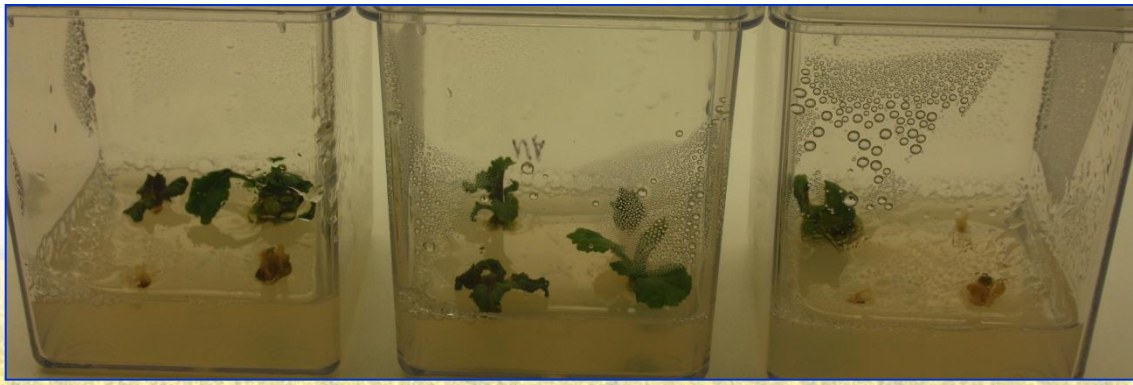
Total 73 genes

Four genes are TIR-NBS-LRR class

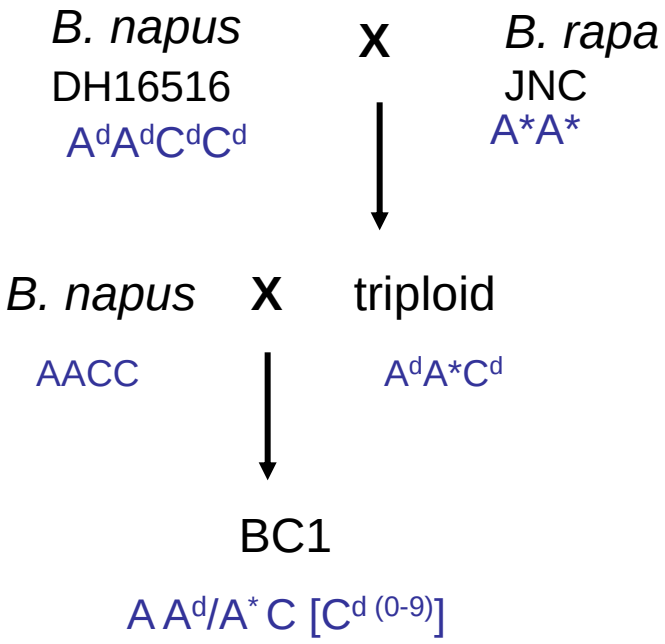
Isolated and sequenced three genes (gene2 to 4)

Obtained transformants from gene3 in *B. rapa* and *B. napus*



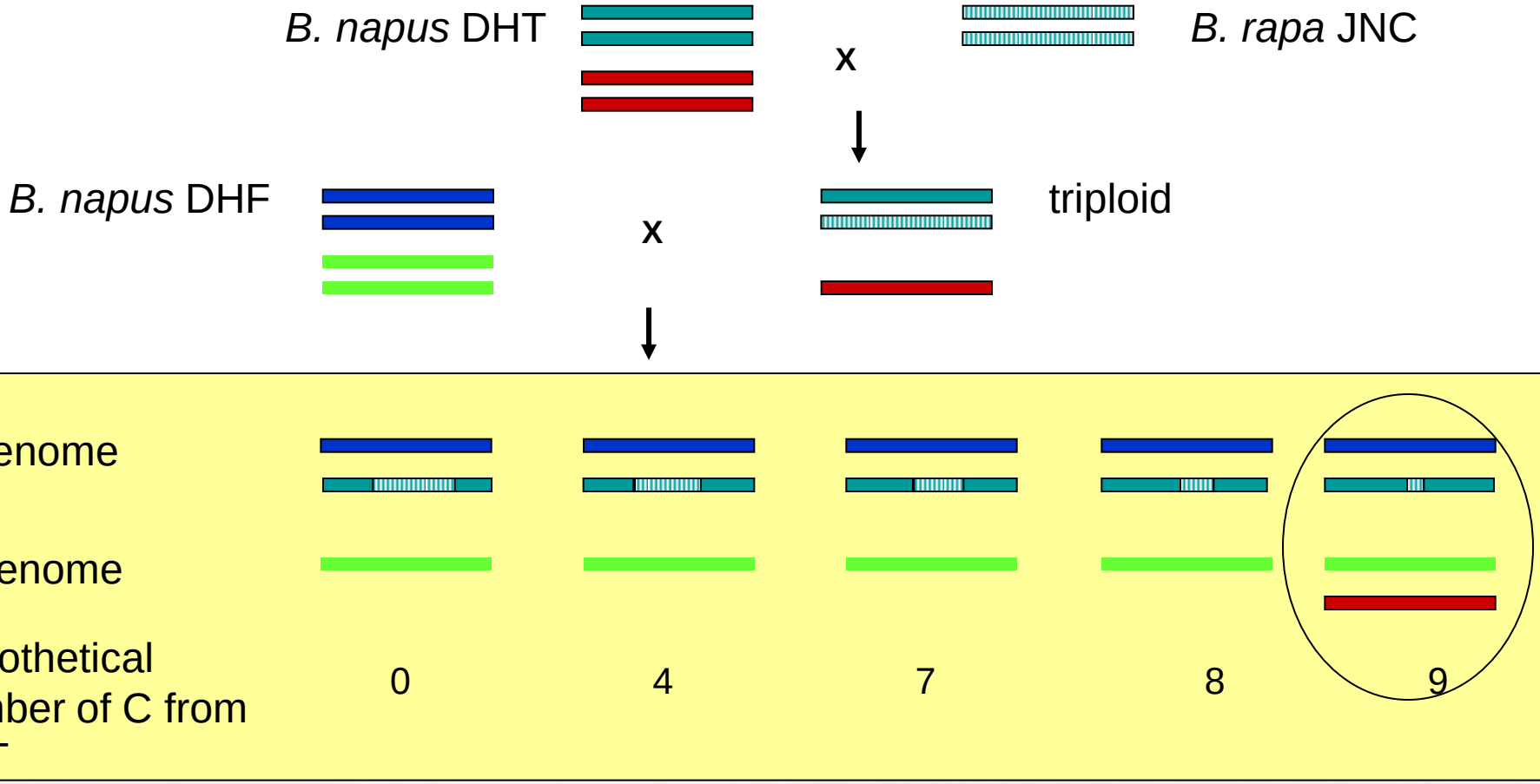


Introgression of CR genes into *B. napus*



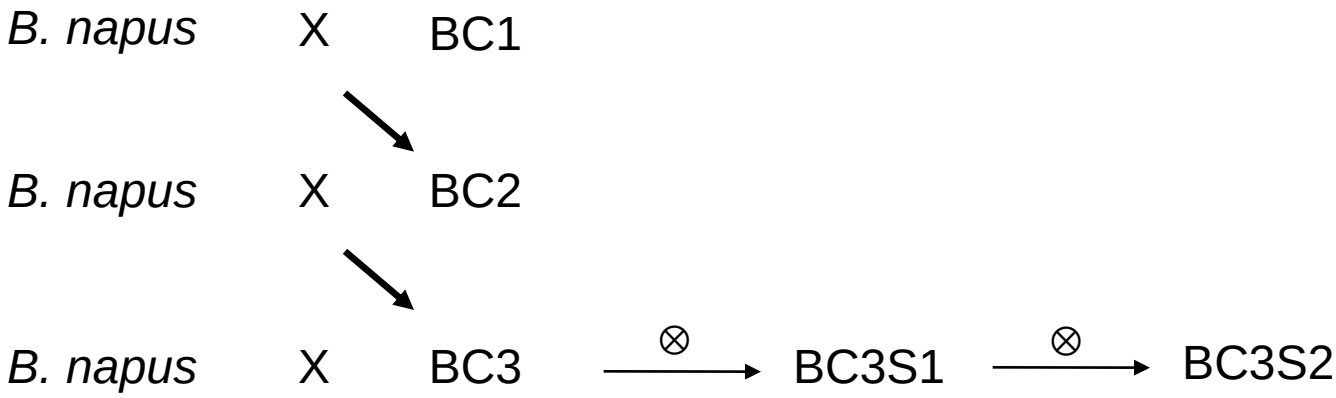
- Transfer resistance genes from JNC and FN into *B. napus*
- Eliminate unnecessary genetic background from donor sources
- Recover C-genome chromosomes

Genome-wide marker assisted selection in BC1



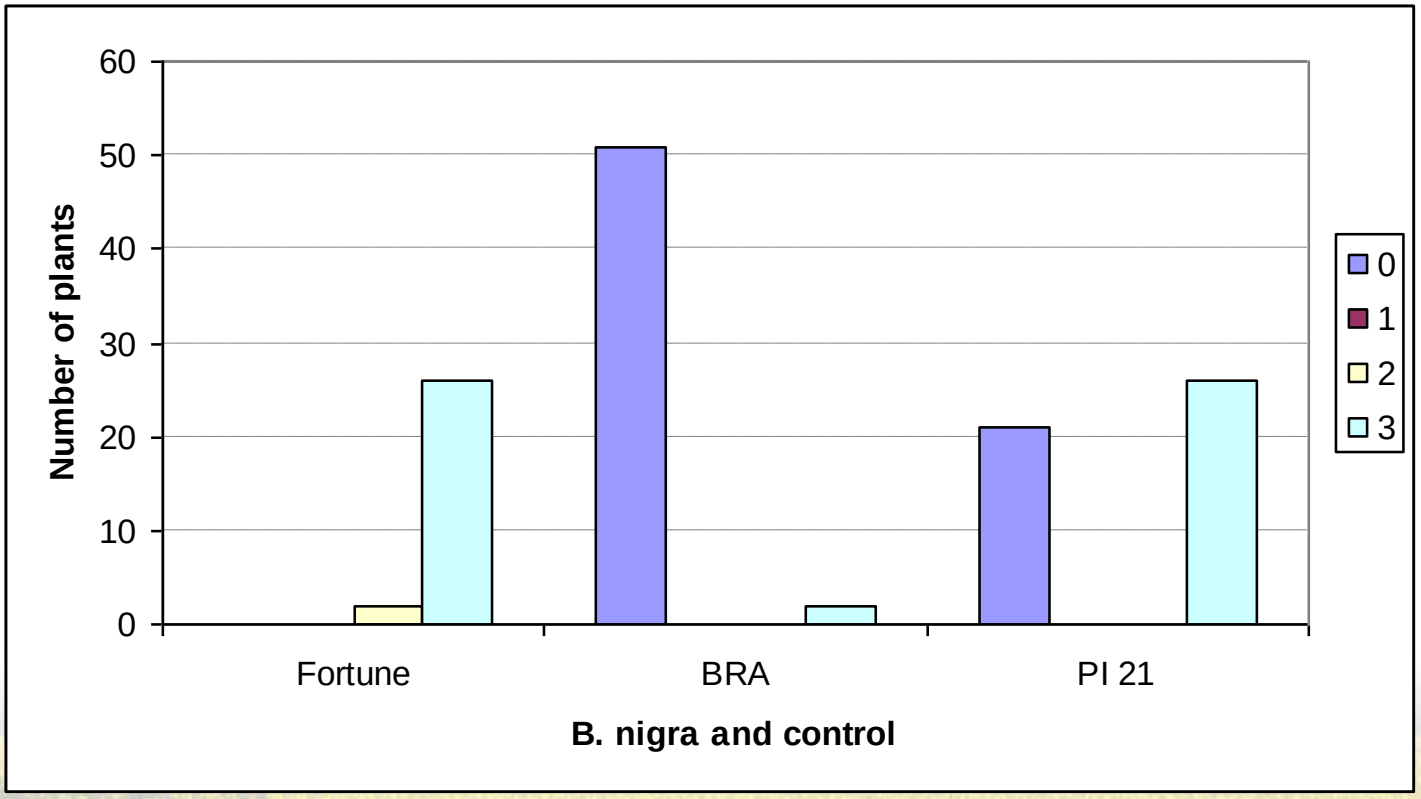
- Genome-wide SNP marker analysis using Illumina Golden Gate SNP assay

Backcross breeding and marker assisted selection



- BC1 plants will be tested for clubroot resistance
- Choose CR BC1 plants with limited Chinese cabbage background and more C-genome chromosomes based on genome-wide marker assisted selection
- Molecular marker assisted selection will be carried out in each generation
- Backcross breeding will be carried out for introgression of the CR gene into *B. rapa* canola and *B. juncea*

Two *B. nigra* lines (BRA and PI 21) highly resistant to *P. brassicae*



Inheritance of CR gene(s) in B genome

Cross	CR/CS plants	Expected ratio	X^2	P
1830 x BRA	11/14	1:1	0.36	0.549
1768 x BRA	17/8	1:1	3.24	0.072
1830 x PI 21	36/35	1:1	0.01	0.906
1768 x PI 21	13/12	1:1	0.04	0.841

Single dominant gene controls CR in both R lines

B. carinata DH lines1768 or 1830 x BRA or PI 21

Mapping of the CR gene(s) and introgression of resistance in to *B. carinata* and *B. juncea* are in progress

Further work

• Assess resistance spectrum for the CR material against different races of *P. brassicae*

• Map CR genes in CR lines from *B. napus, B. nigra, B. oleracea*, canola and turnip *B. rapa* lines

• Develop robust SNP markers for MAS

• Introgress CR genes into *B. napus*, *B. carinata*, *B. juncea* and *B. rapa* through backcross breeding

• Re-synthesize amphidiploid species (*B. napus*, *B. juncea* and *B. carinata*) highly resistant to clubroot using CR diploid species (*B. rapa*, *B. oleacea* and *B. nigra*).

• Molecular cloning and characterization of CR genes

Summary

• A large collection of Brassica germplasm was found to be highly resistant to clubroot disease.

• Microsatellite and CAPS markers tightly linked to *Rpb1* and genome-wide markers assisted selection are available for canola breeding.

• Cloning of *Rpb1*, identification of more CR genes and introgression of CR genes into canola are in progress.

• Further collaboration with industry partners and universities will be pursued.

• Additional funding and staffing is needed.

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Thank you!

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