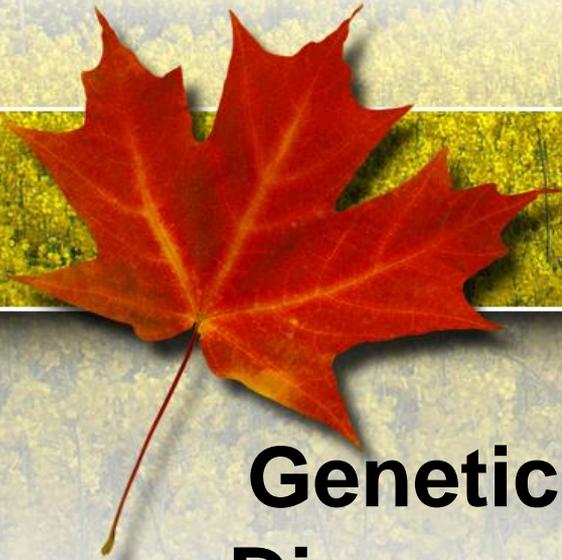




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Genetic Control of Clubroot Disease at AAFC, Saskatoon

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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).

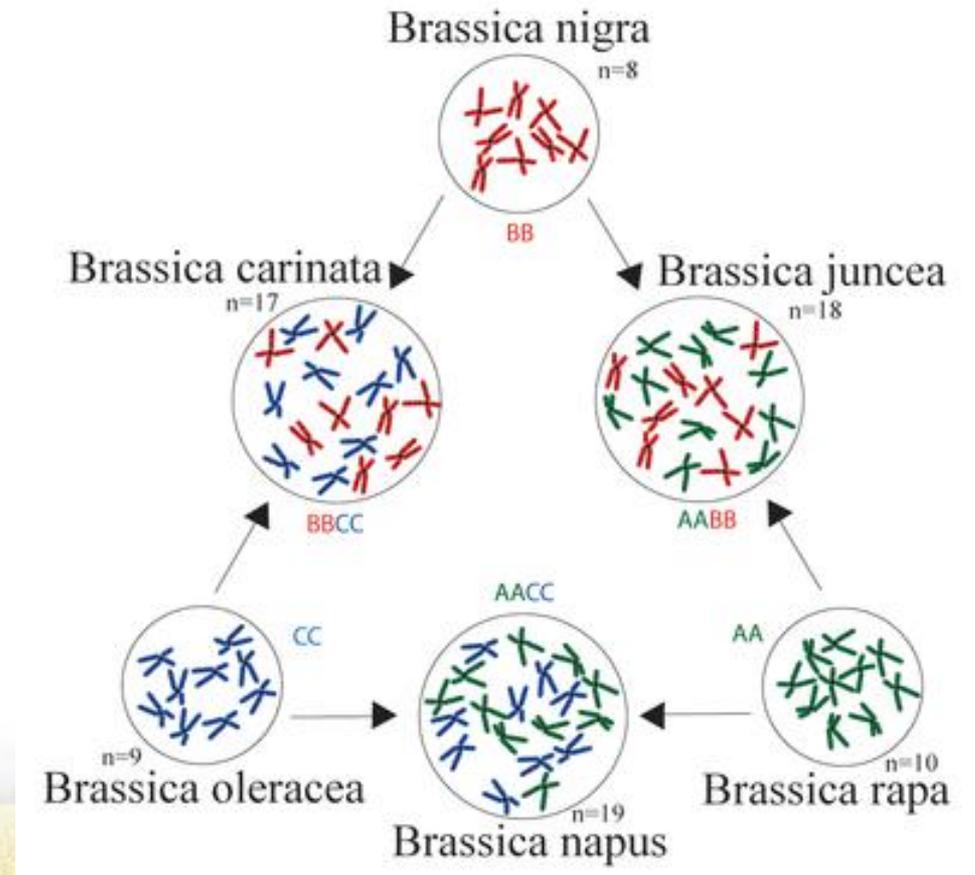
Development of resistant cultivars is considered an essential step to control this disease for all *Brassica* species.



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).

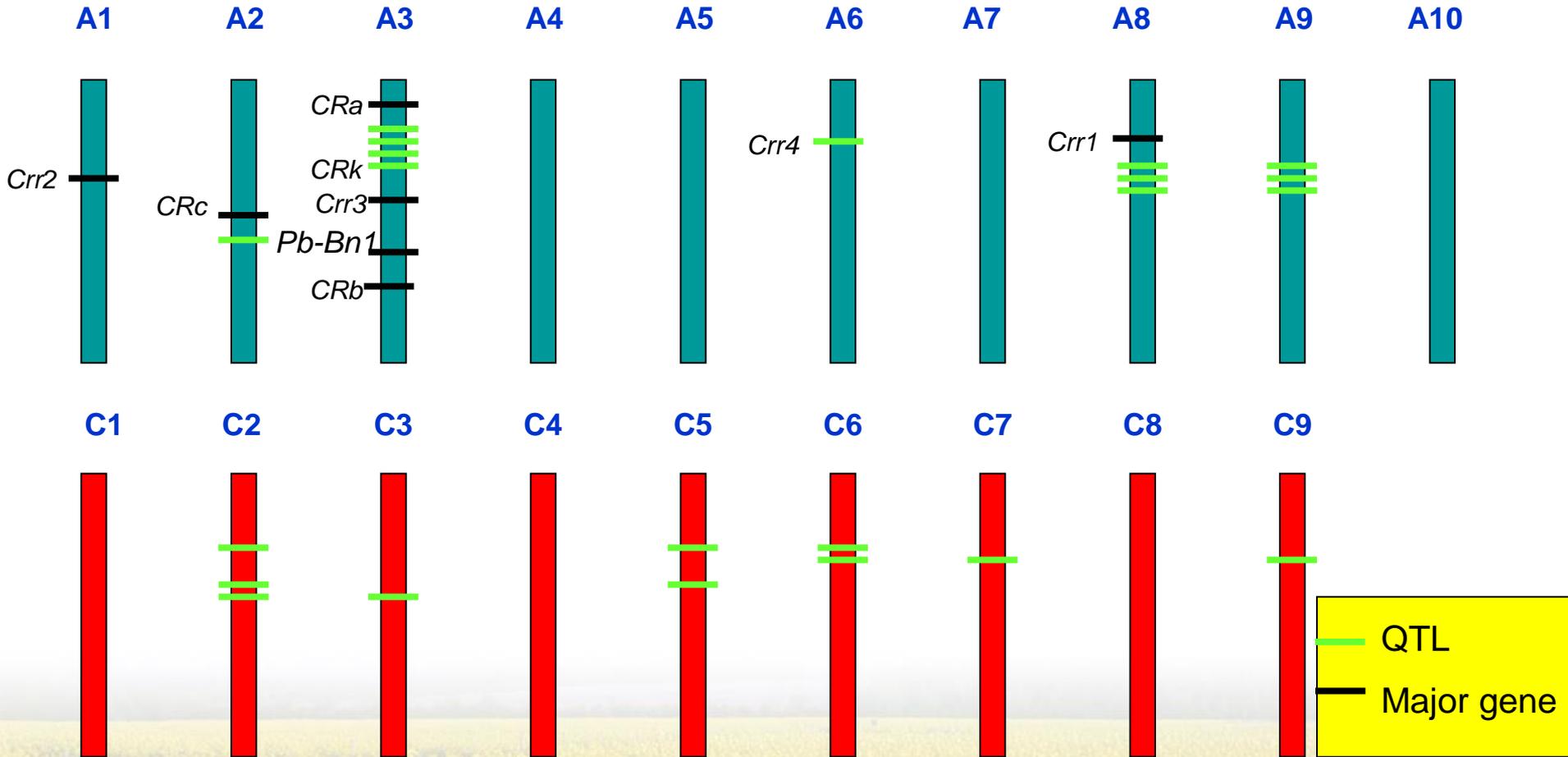


Inheritance of Clubroot Resistance in Brassica species

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

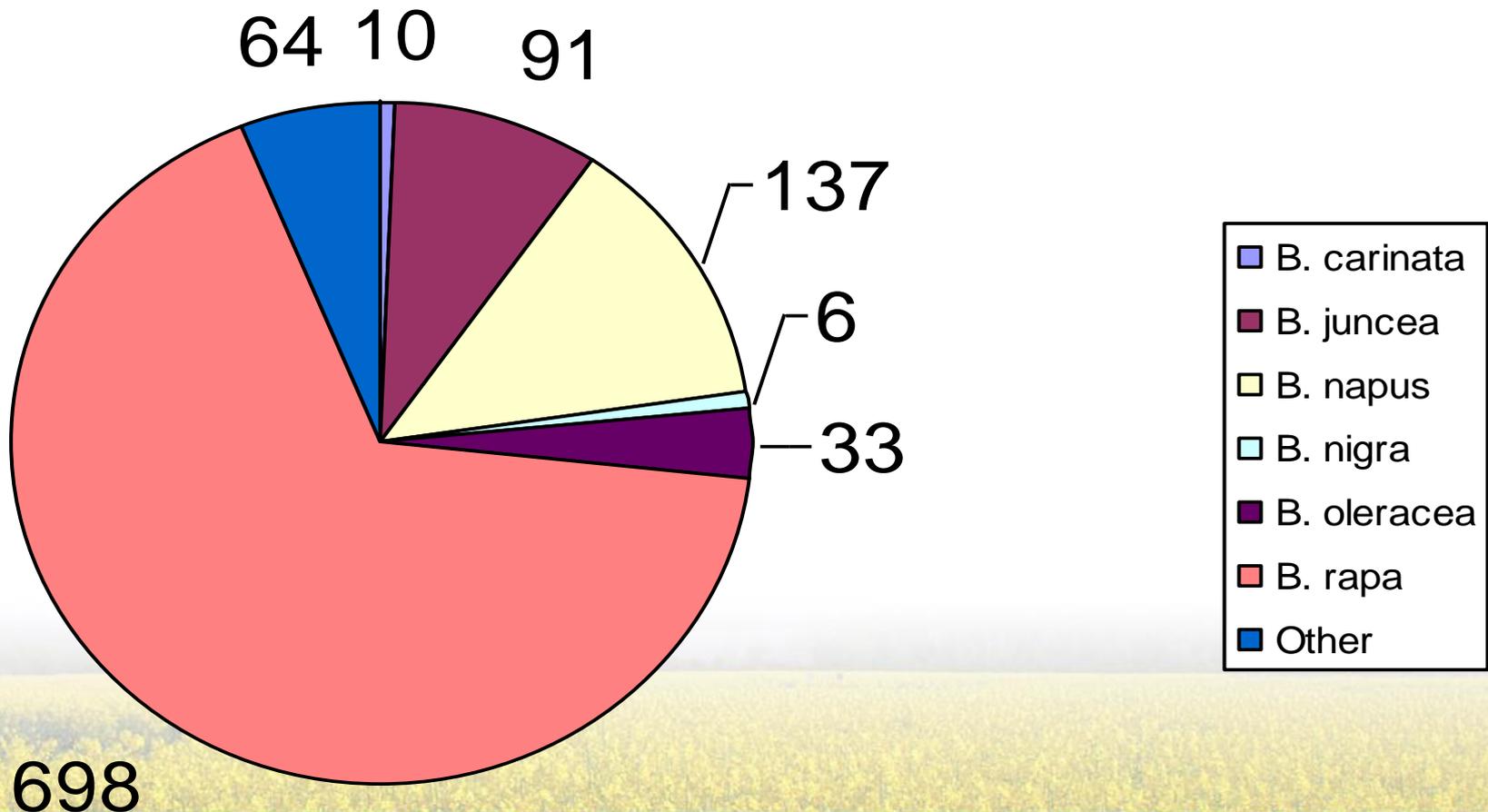


Map of clubroot resistance genes



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

• *Plasmodiophora brassicae* pathotype 3



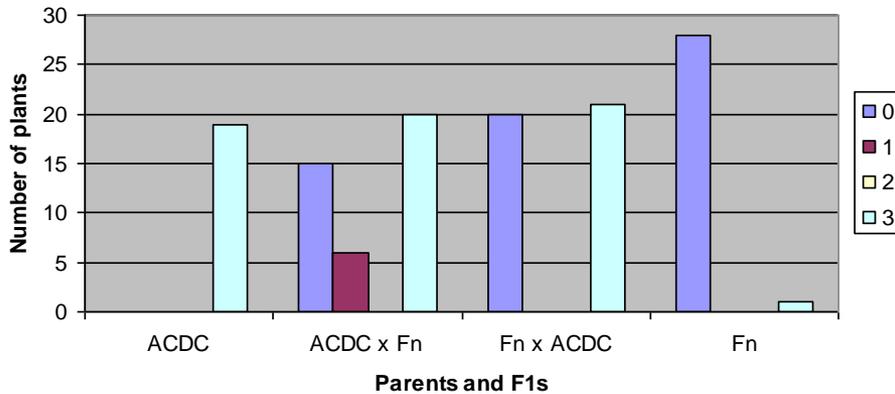
Resistant Brassica germplasm identified at AAFC - Saskatoon

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	

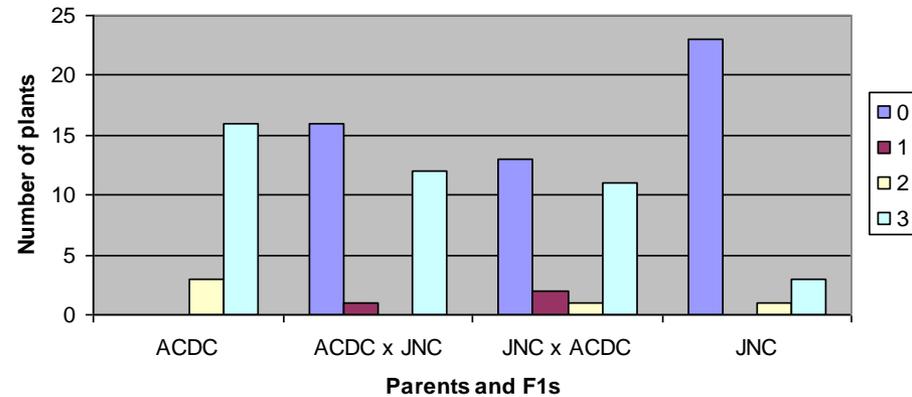


Inheritance of clubroot resistance in *B. rapa*

Phenotypes in Parents and F1s



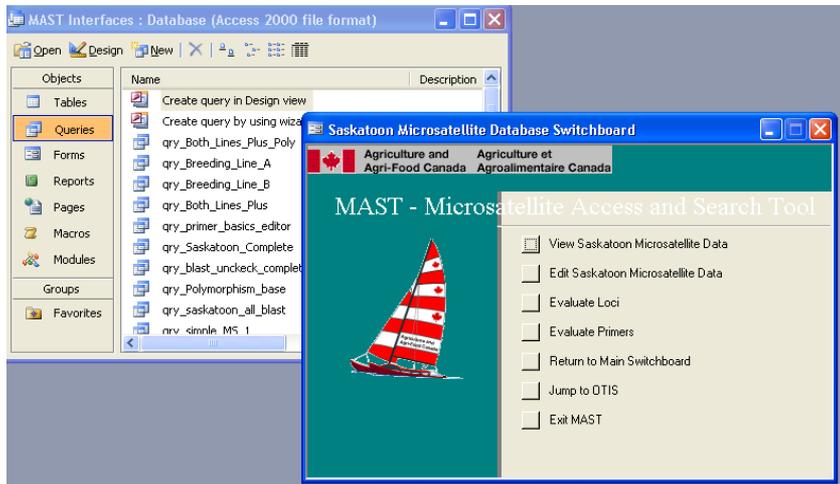
Phenotypes in Parents and F1s



Cross	CR/CS plants	Expected ratio	χ^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/>

The image is a screenshot of the BRAD Brassica Database website. At the top left is the BRAD logo, which includes a stylized DNA double helix. The main header is 'Brassica Database' in a green serif font. To the right of the header are links for 'FTP login' and 'Register'. Below the header is a large text block describing the database. To the right of this text is a 'News' section with several entries. The background of the website is a light green color with a subtle image of a field of yellow flowers.

BRAD **Brassica Database** [FTP login](#) | [Register](#)

The **Brassica database (BRAD)** is a web-based database of genetic data at the whole genome scale for important *Brassica* crops. It now provides several datasets, including the complete *Brassica A* genome sequence from *B. rapa* (Chiifu-401), derived from de novo assembly of sequence scaffolds using second-generation sequencing technologies. Also included are predicted genes and associated annotations (InterPro, KEGG2, SwissProt), *B. rapa* genes orthologous to those in *Arabidopsis thaliana*, as well as genetic markers and maps of *B. rapa*. BRAD is continuously updated and *B. rapa* transcriptome data will be available in the near future. We also accept submission of genetic and genomic data of *Brassica* crops from the research community.

News

We stopped Blast service of *B. oleracea* data temporarily for safety issue. (12.16.2011).

Expression data has been linked to *A. thaliana* gene (10.31.2011). **NEW**

B. rapa genome data is available for download (08.29.2011). **NEW**

[Genes and genome sequence](#)

Mapping of CR gene in *B. rapa*

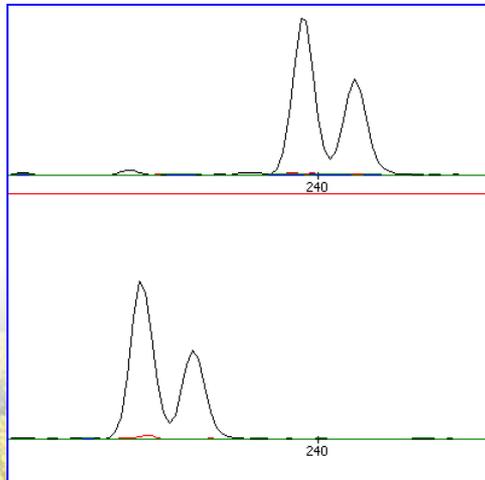
Populations

- ACDC x JNC
- ACDC x FN

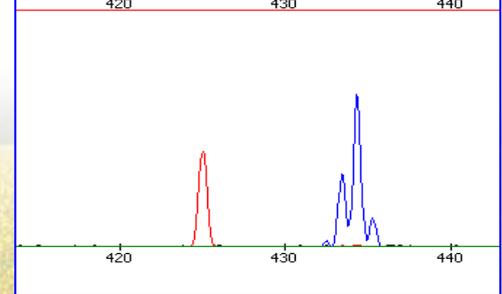
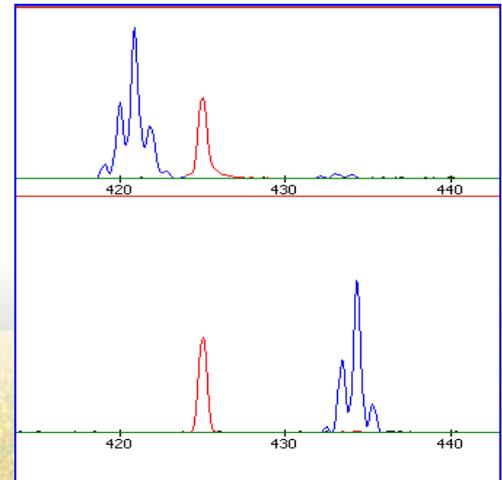
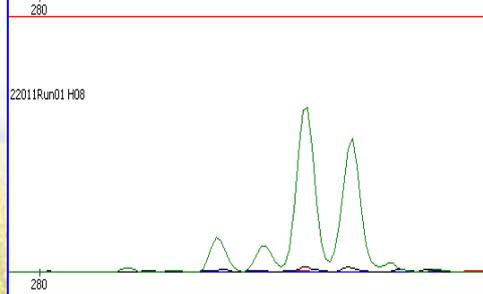
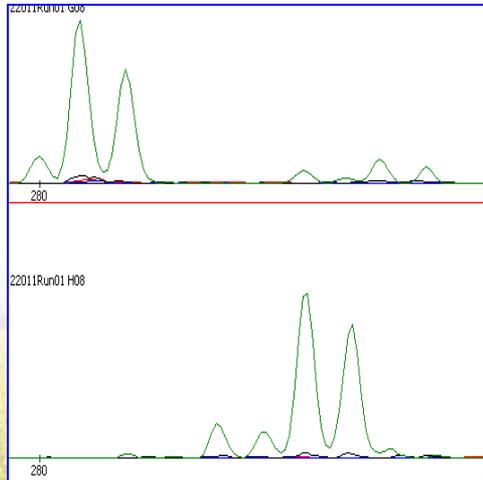
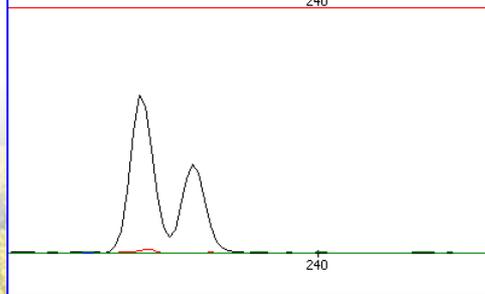
Microsatellite marker

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

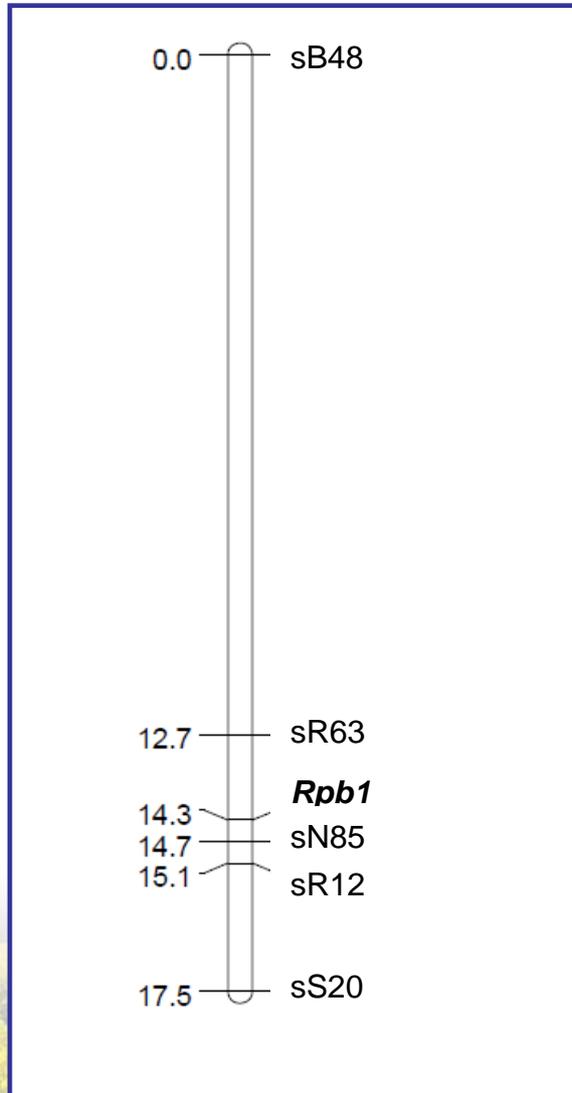
JNC



ACDC

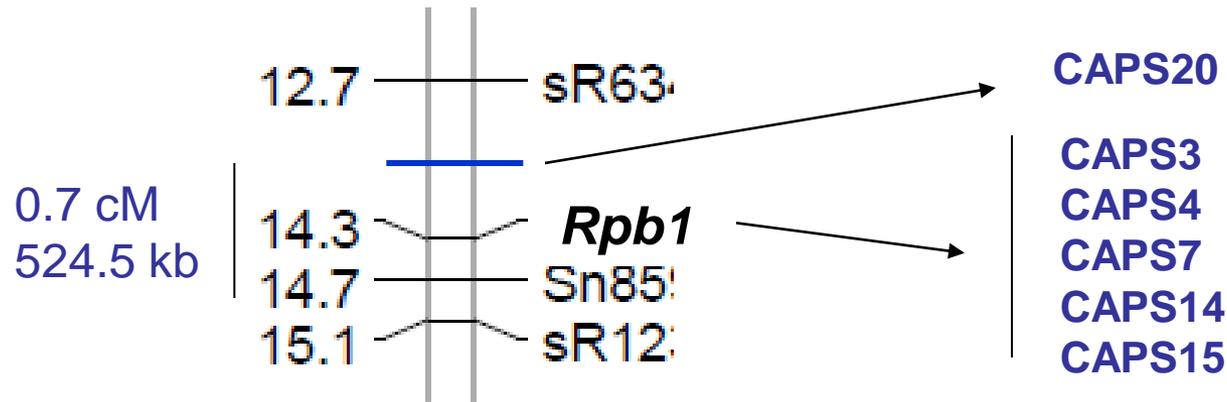


Mapping of CR gene in *B. rapa*



- 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

CAPS20

Map-based cloning of *Rpb1*

sN85



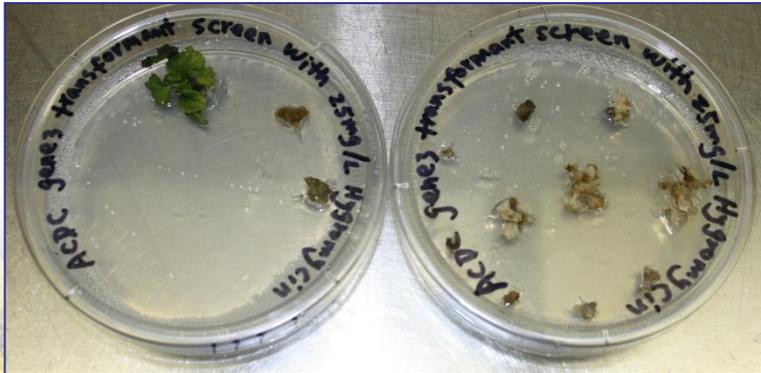
In the interval of CAPS20 and sN89:

Total 73 genes

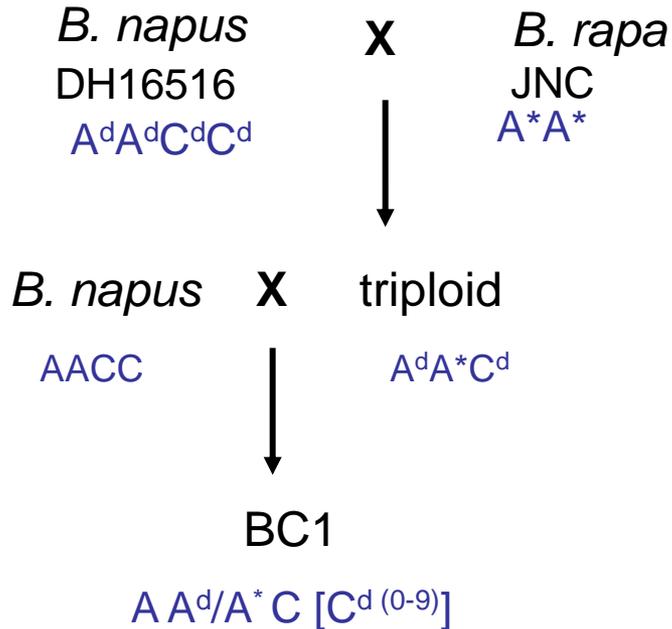
Four genes are TIR-NBS-LRR class

Isolated and sequenced three genes (gene2 to 4)

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



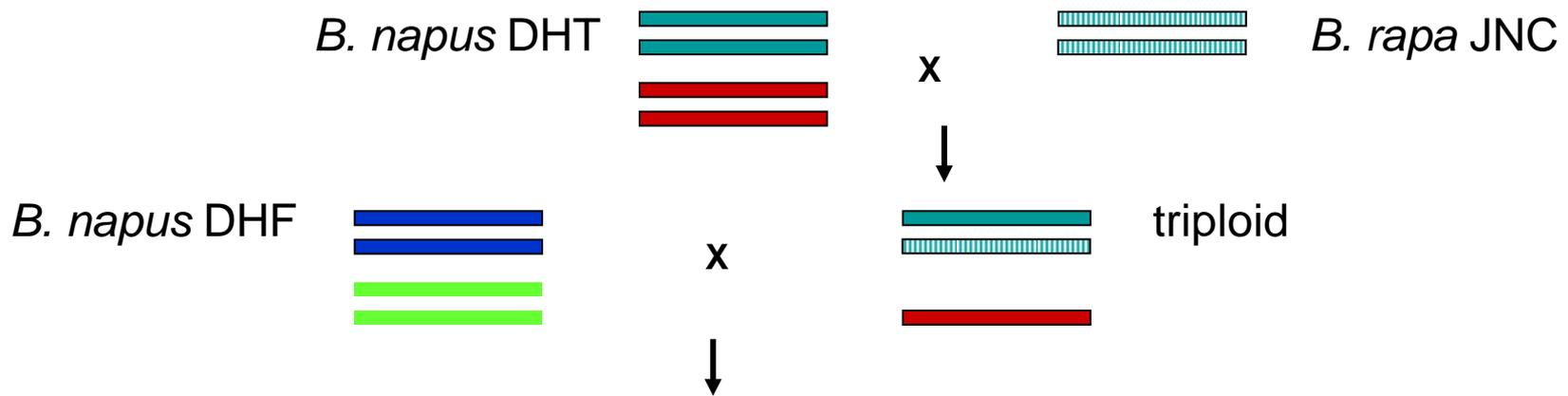
Introgression of CR genes into *B. napus*



- 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

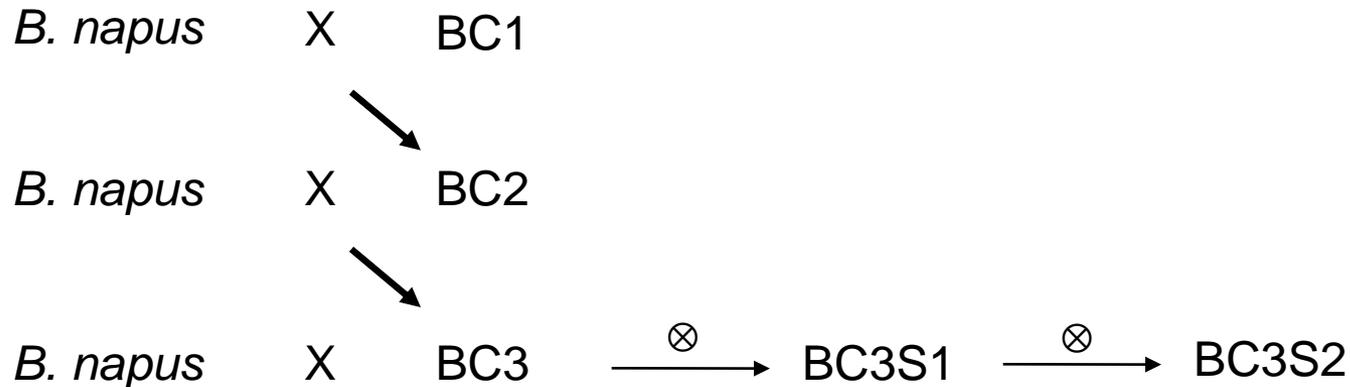


A genome					
C genome					
Hypothetical number of C from DHT	0	4	7	8	9

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

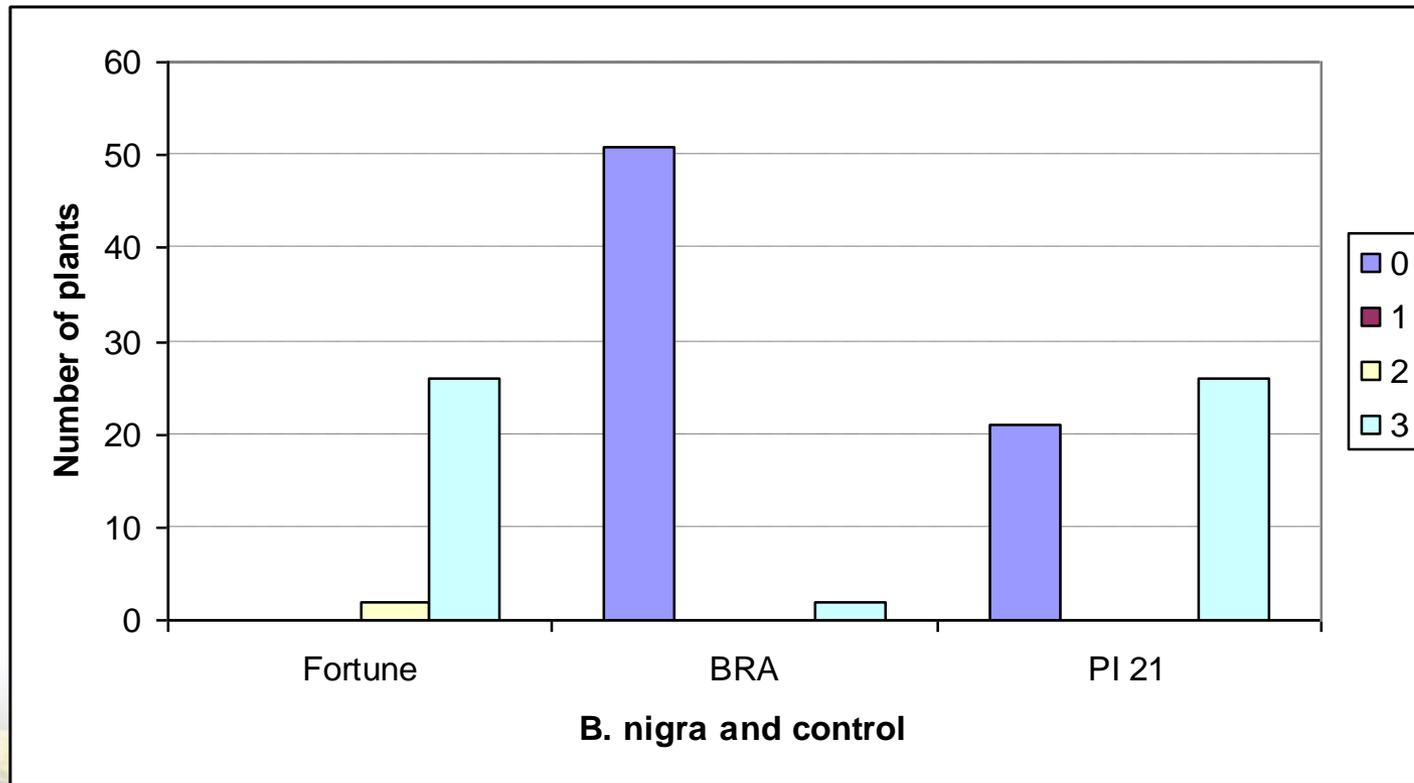


Backcross breeding and marker assisted selection



- 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 lines 1768 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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