Agriculture and Agri-Food Canada Agriculture et Agroalimentaire Canada

Identification of clubroot-resistance genes and development of clubroot resistant canola germplasm

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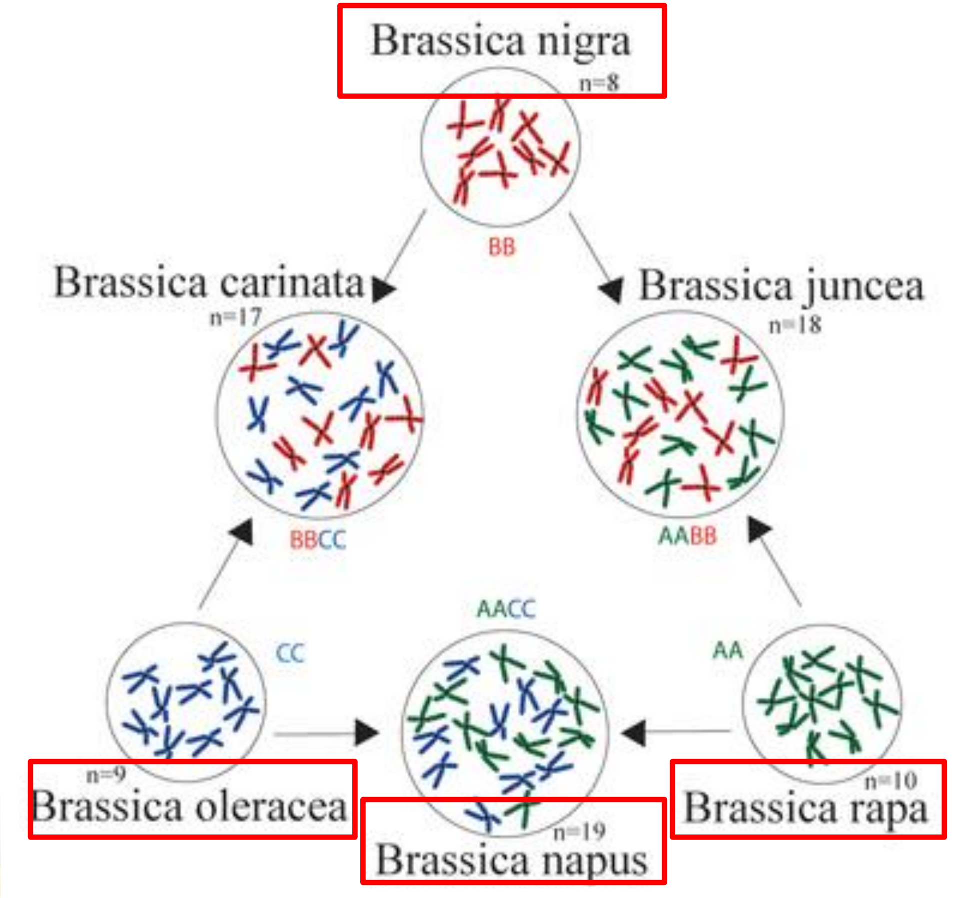
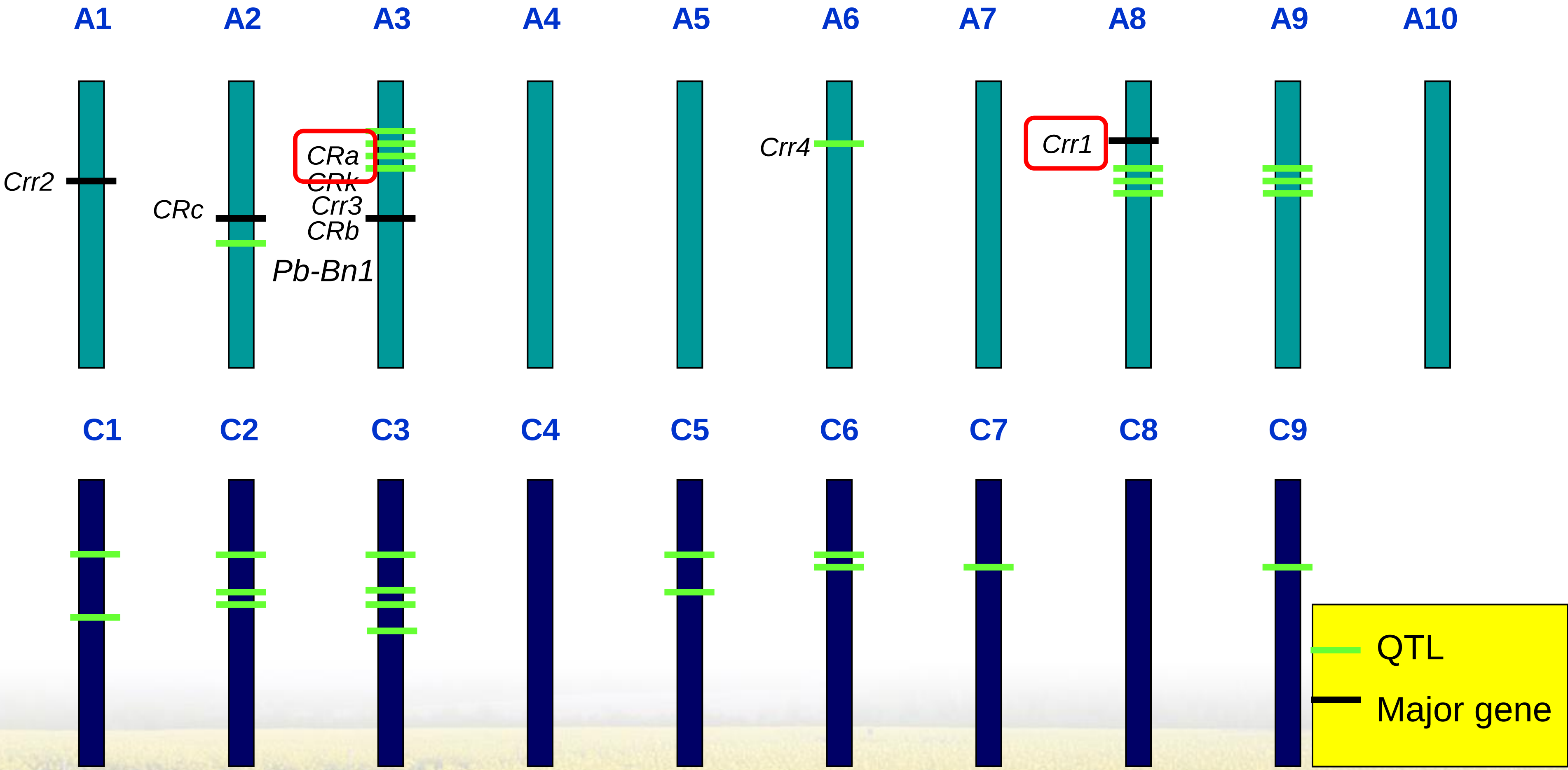
Canada

Sources of resistance identified in Brassica species



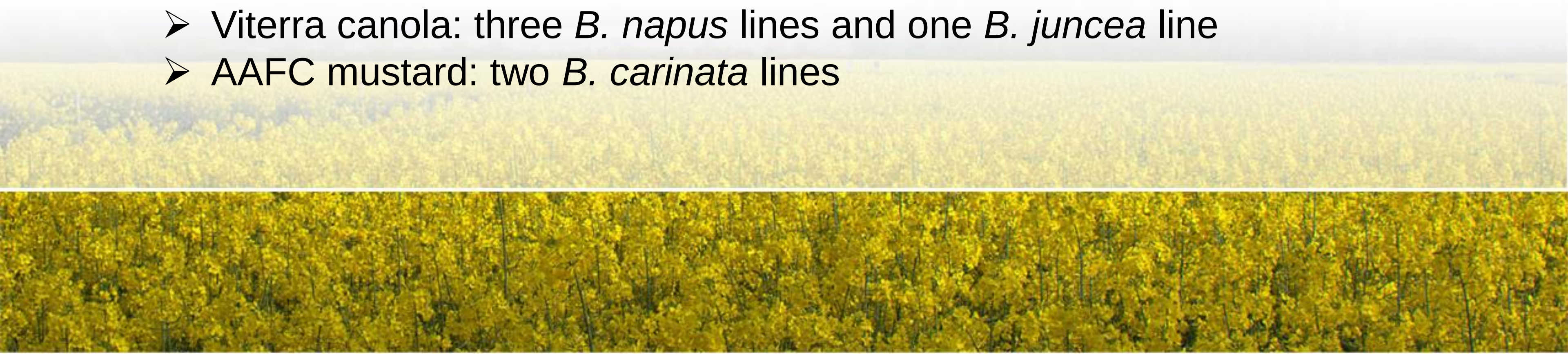
With the exception of *B. juncea* and *B. carinata*, genotypes with resistance to one or more pathotypes of *P. brassicae* can be found in all major brassica crops.

Mapping of clubroot resistance genes



Developing CR canola and mustard at Saskatoon, AAFC

- Methods
  - molecular genetics
  - conventional breeding
- Unique CR materials identified in Saskatoon Research Centre
  - A total of 955 accessions in six cultivated species for resistance to pathotype 3 of *P. brassicae*
  - Selected CR lines tested with pathotypes 2, 5, 6 & 8
  - Eight accessions in diploid species highly resistant to all Canadian pathotypes
- Identification of CR genes through genetic mapping
- Development of molecular markers closely linked to CR genes
- Molecular cloning of CR genes
- Introgression of CR genes into canola and mustard
  - AAFC canola: two *B. napus* lines and one *B. rapa* line
  - Viterra canola: three *B. napus* lines and one *B. juncea* line
  - AAFC mustard: two *B. carinata* lines



Sources of clubroot resistance used at Saskatoon, AAFC

B. rapa ssp. *pekinensis*
Chinese cabbage

B. rapa ssp. *chinensis*
Bok choy

B. rapa ssp. *rapifera*
Turnip

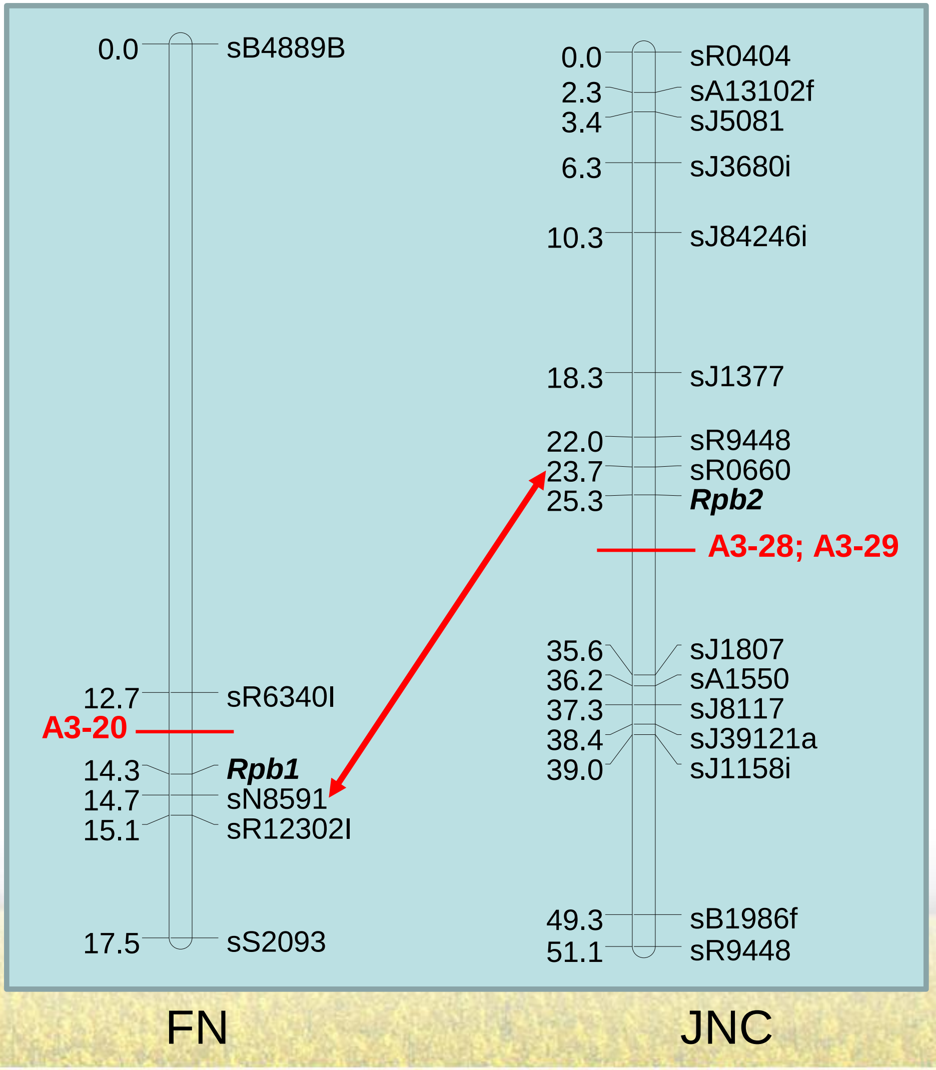
B. oleracea ssp. *botrytis*
Cauliflower

B. oleracea ssp. *capitata*
Cabbage

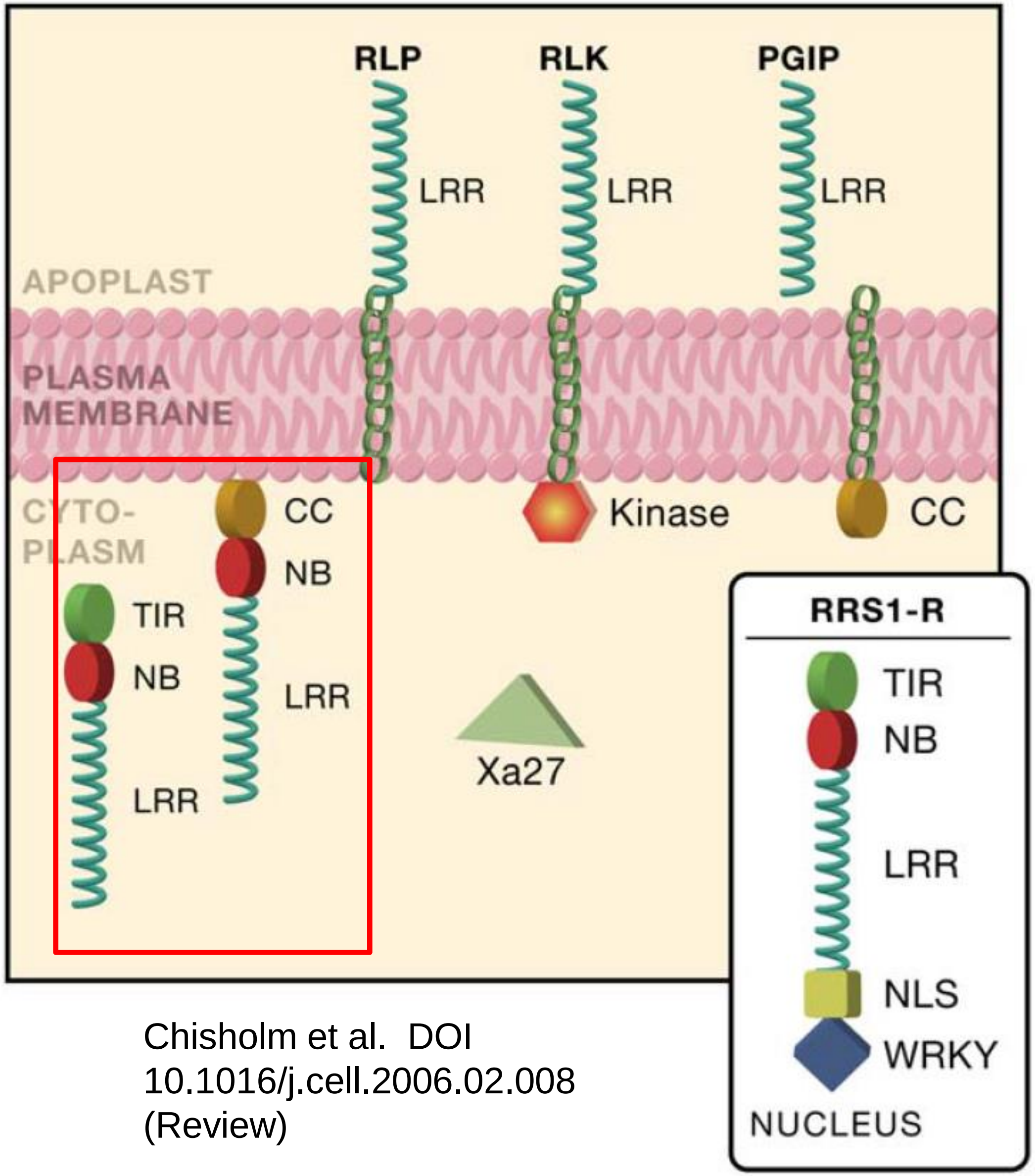
B. nigra
Black mustard

Mapping of two CR genes in *B. rapa* into A3

- Two vegetable cultivars
  - *B. rapa* ssp. *chinensis*, cv FN
  - *B. rapa* ssp. *pekinensis*, cv JNC
- AAFC microsatellite markers
  - A-genome of *B. napus*
- CAPS markers developed for fine mapping
  - *B. rapa* sequencing information at http://brassicadb.org/brad/
- Molecular markers available for MAS



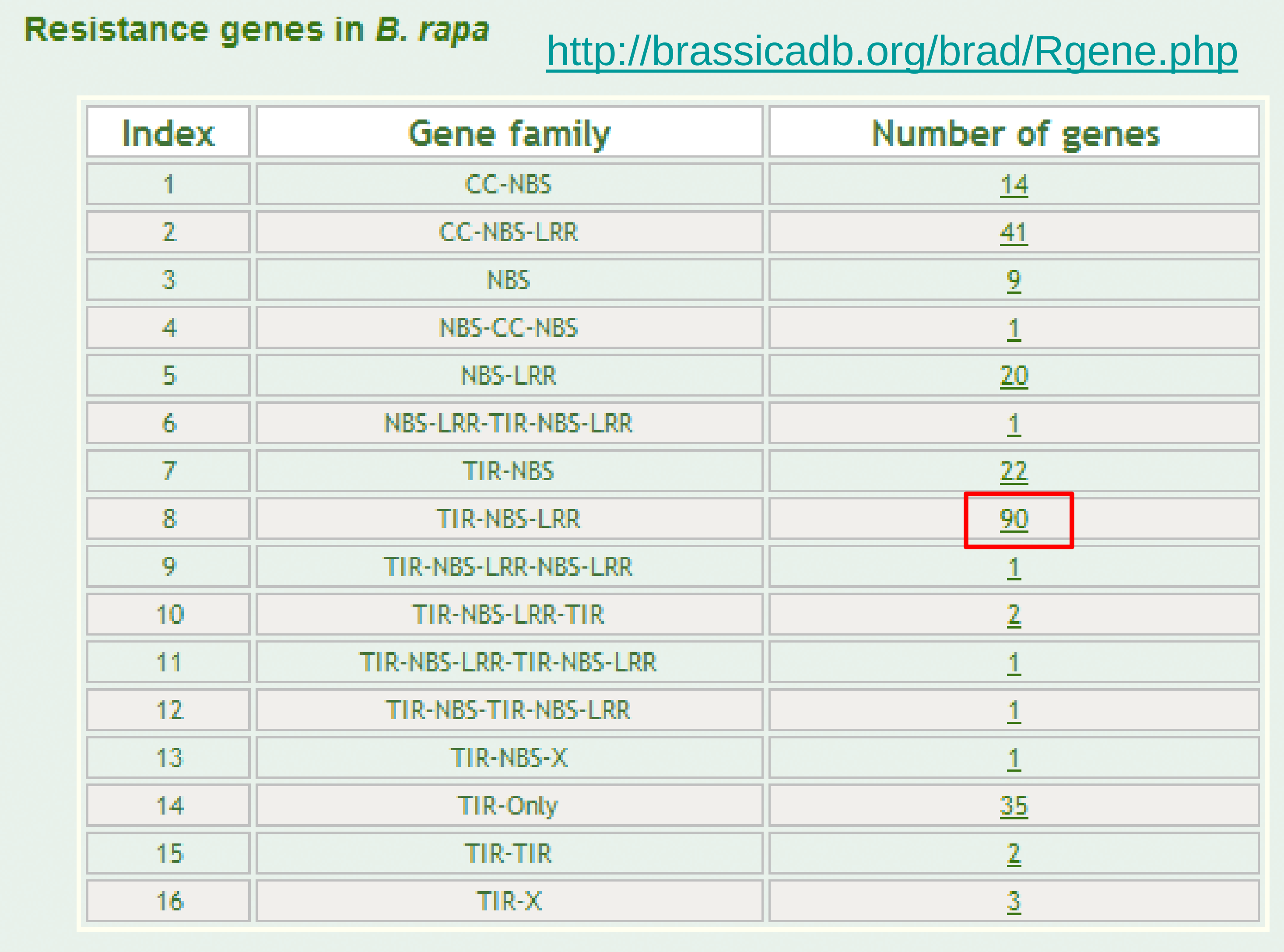
Classes of Resistance Proteins



Chisholm et al. DOI 10.1016/j.cell.2006.02.008 (Review)

- Two main classes of R proteins
  - nucleotide binding leucine-rich repeat (NB-LRR)
  - extracellular LRR (eLRR)
- NB-LRR class is the most abundant
  - coiled-coil (CC)
  - Toll-interleukin-1 receptor (TIR)
- eLRRs
  - RLPs
  - RLKs
  - PGIP
- Novel R proteins
  - Xa27
  - RRS1-R protein

http://brassicadb.org/brad/Rgene.php

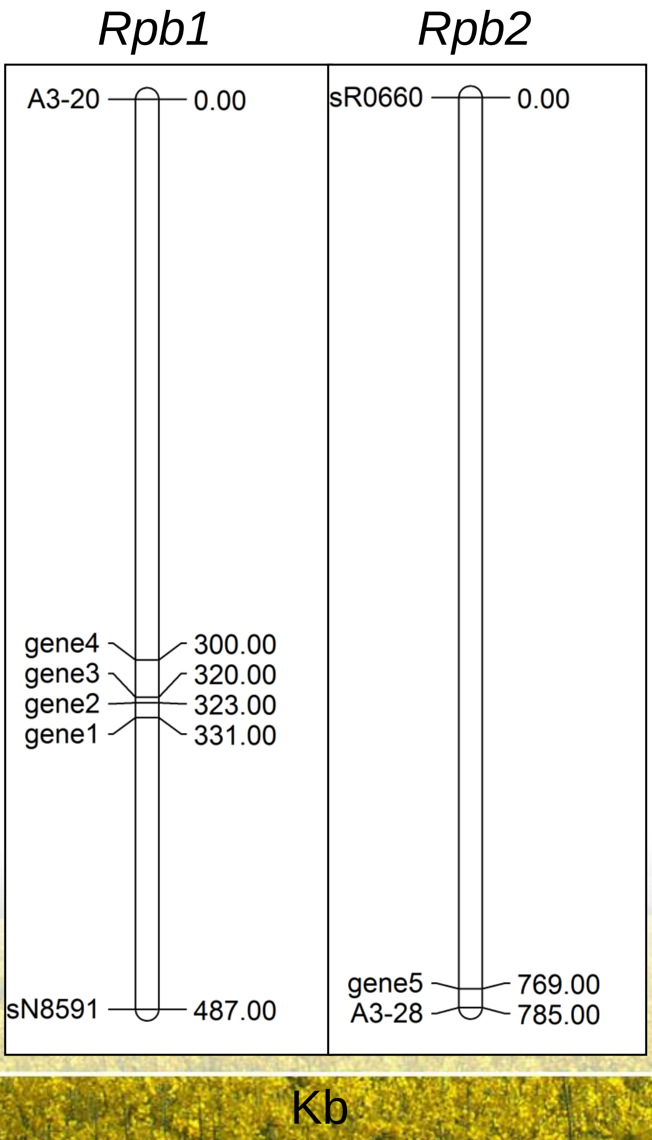


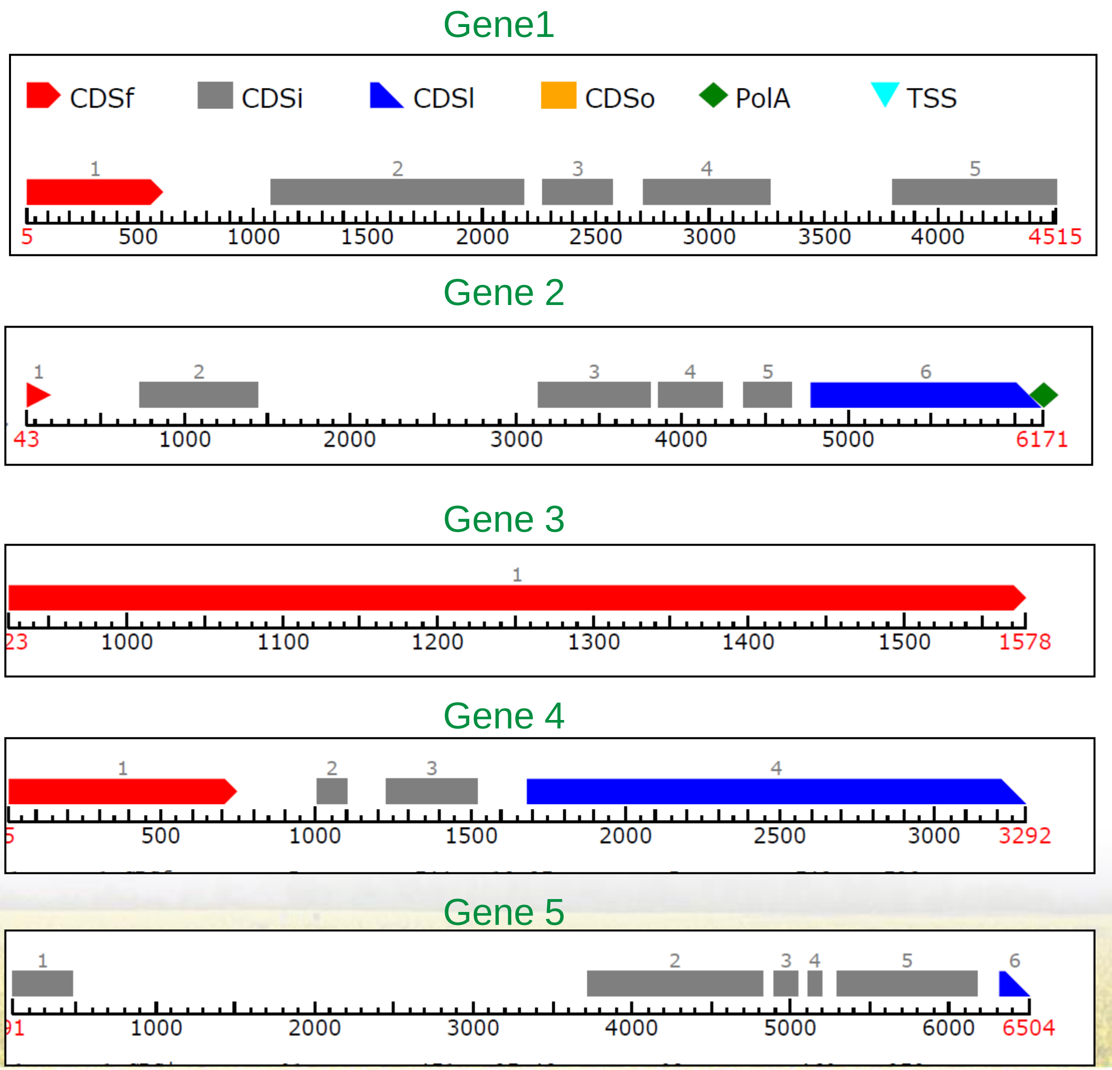
Resistance genes in *B. rapa*

Index	Gene family	Number of genes
1	CC-NBS	14
2	CC-NBS-LRR	41
3	NBS	9
4	NBS-CC-NBS	1
5	NBS-LRR	20
6	NBS-LRR-TIR-NBS-LRR	1
7	TIR-NBS	22
8	TIR-NBS-LRR	90
9	TIR-NBS-LRR-NBS-LRR	1
10	TIR-NBS-LRR-TIR	2
11	TIR-NBS-LRR-TIR-NBS-LRR	1
12	TIR-NBS-TIR-NBS-LRR	1
13	TIR-NBS-X	1
14	TIR-Only	35
15	TIR-TIR	2
16	TIR-X	3

Two cloned CR genes *CRa* and *Crr1* encode TIR-NBS-LRR proteins.

Molecular cloning of CR genes



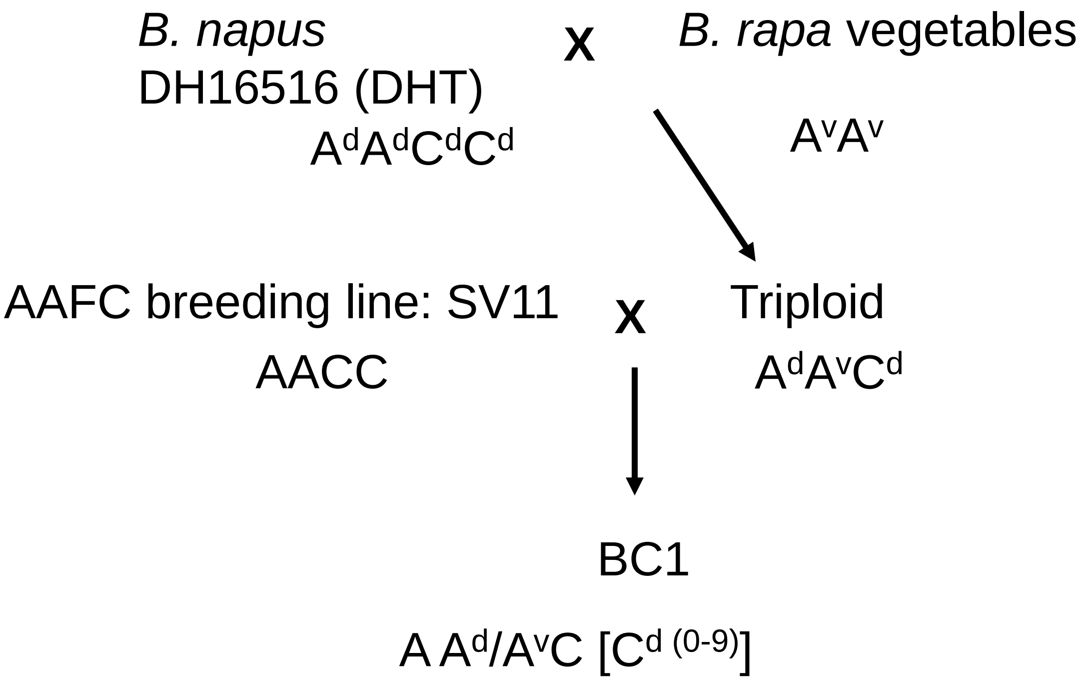


Generation of transgenic canola lines - complementation analysis

- Difficult to generate *B. rapa* transgenic plants
- *B. napus* DH12075
- Agrobacterium-mediated transformation
- Seeds of T1 will be obtained in three months.

TIR-NBS-LRR	CR candidate	No. of transformants
Gene1	*Rpb1*	137
Gene2	*Rpb1*	44
Gene3	*Rpb1*	21
Gene4	*Rpb1*	15
Gene5	*Rpb2*	61

Starting materials



B. napus DH16516 (DHT) x *B. rapa* vegetables

$A^dA^dC^dC^d$ A^vA^v

AAFC breeding line: SV11 x Triploid

AACC $A^dA^vC^d$

BC1

$A\ A^d/A^vC\ [C^{d\ (0\text{-}9)}]$

- Transfer resistance genes from *B. rapa* vegetables into *B. napus*
- Recover full set of C-genome chromosomes (9 pairs)
- Eliminate unnecessary genetic background from the vegetables

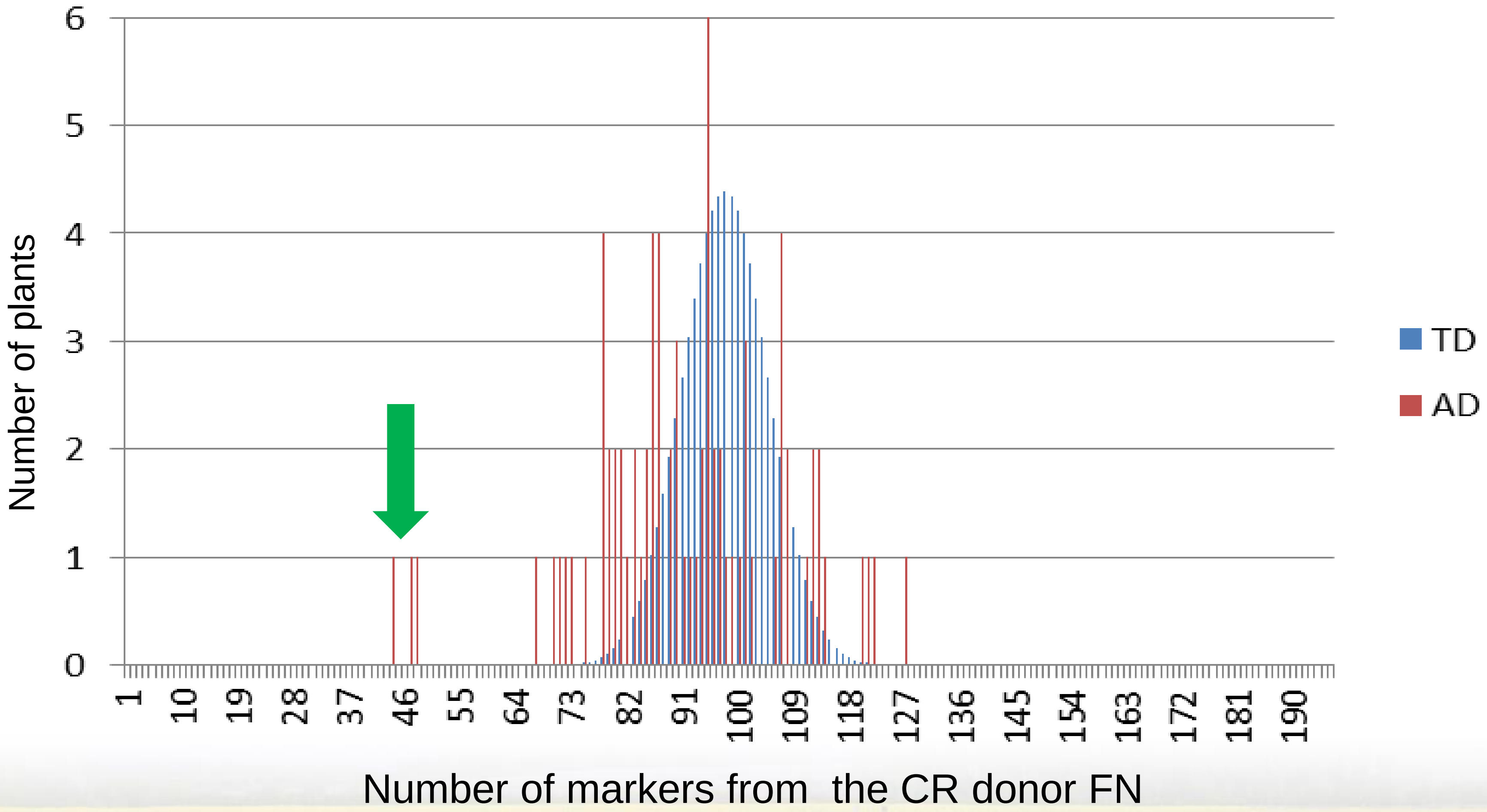
Introgression of CR genes into canola

FN (*Rpb1*)
CR donor

SV11
Canola

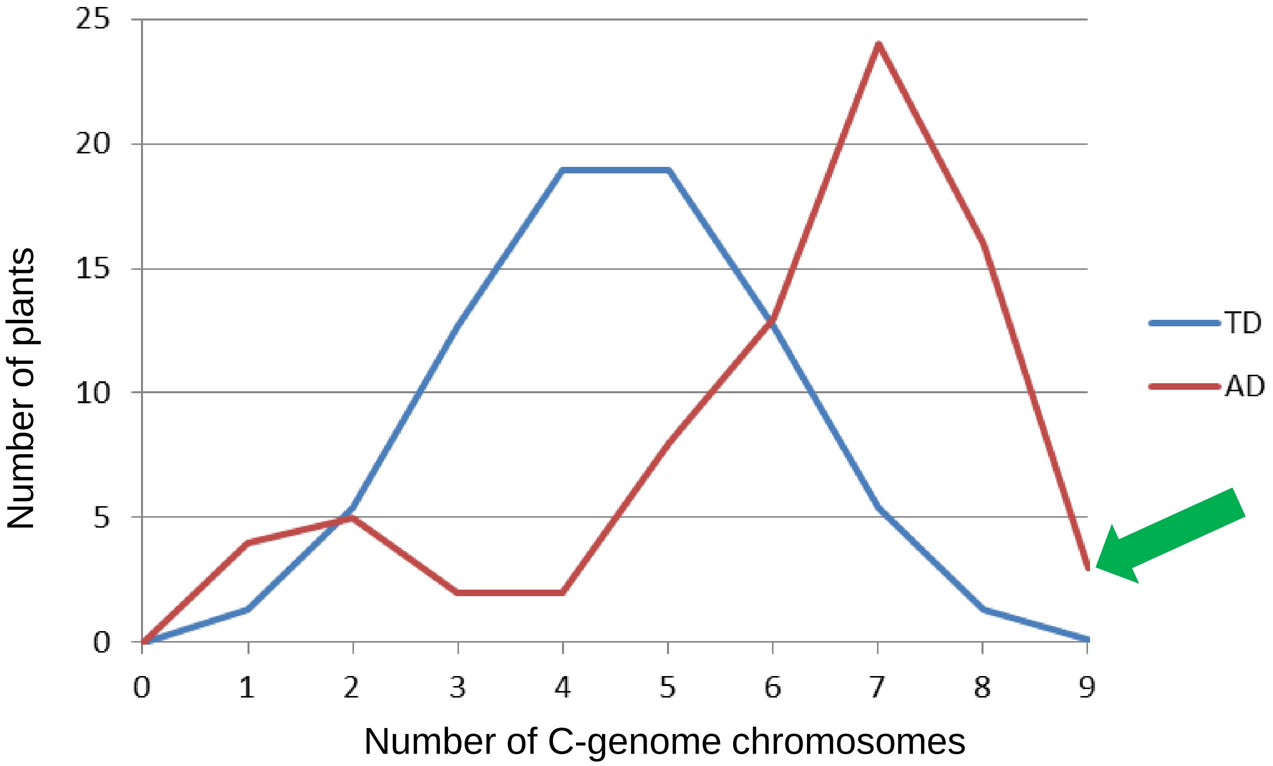
- A total of 173 BC_1 were tested for resistance to clubroot
- 94R:79S; fit 1:1 (X^2= 1.30, P= 0.254)
- 77R plants were selected for genome-wide SNP marker analysis using 6K Illumina SNP assay

Analysis of A-genome markers



- A total of 77 CR plants from SV11 x (DHT x FN)
- 198 polymorphic robust markers almost evenly distributed on A-genome chromosomes
- Theoretical distribution (TD): binomial; parameters *N* = 198 and *P* = 50%
- Actual distributions (AD)

Analysis of C-genome markers



- Theoretical distribution (TD): binomial; parameters *N* = 9 and *P* = 50%; average = 4.5
- Actual distributions (AD): More plants with 6 to 9 C-genome chromosomes; average = 6.1
- High frequency of C-genome transmission

Introgression of CR genes into canola

	AAFC elite line	**Topas**	**CR donor**	**SV11 x (DHT x FN)**					
	SV11	DHT	FN	BC1-33	BC1-135	BC1-126	BC1-66	BC1-19	BC1-48
No of A-genome markers from FN	0	0	198	44	47	48	78	92	112
% A-genome markers from FN	0.0	0.0	100.0	22.4	24.0	24.5	39.8	46.9	57.1
No of C genome chromosomes	9	9	0	8	6	7	9	9	9

- BC_1 plants carrying limited genetic background from the CR donor and a full set of C-genome chromosomes were identified through genome wide marker selection
- The recipients of CR genes: *B. napus*, *B. rapa and B. juncea* canola
- BC_2 CR plants have been obtained

Developing CR *B. carinata* – a crop for biofuel

- Two elite *B. carinata* lines
  - 080798EM-086 and 080798EM-148
  - Doubled haploid
  - Yellow seeded
- *Two B. nigra* CR lines
  - BRA and PI
  - Single genes control CR
- Introgression of CR genes from the *B. nigra* into the *B. carinata*
  - Determined the number of C-genome chromosomes in BC_1 by analysis of SSR markers
  - Obtained BC_2 plants

Further work

- Map CR genes in *B. rapa* (two turnips) , *B. oleracea* (two cabbage and three cauliflowers) and two *B. nigra* lines
- Develop robust SNP markers for MAS
- Introgress CR genes identified into *B. napus*, *B. rapa*, *B. carinata* and *B. juncea* breeding lines
- Developing near-isogenic lines for differentiating pathotypes of *P. brassicae* and canola resistance to clubroot
- Re-synthesize amphidiploid species highly resistant to clubroot using CR diploid species
- Molecular cloning and characterization of CR genes

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AAFC Growing Forward I
SaskCanola

Thank you!