

Introgression of clubroot resistance into Canadian *Brassica napus* canola and molecular mapping of the resistances

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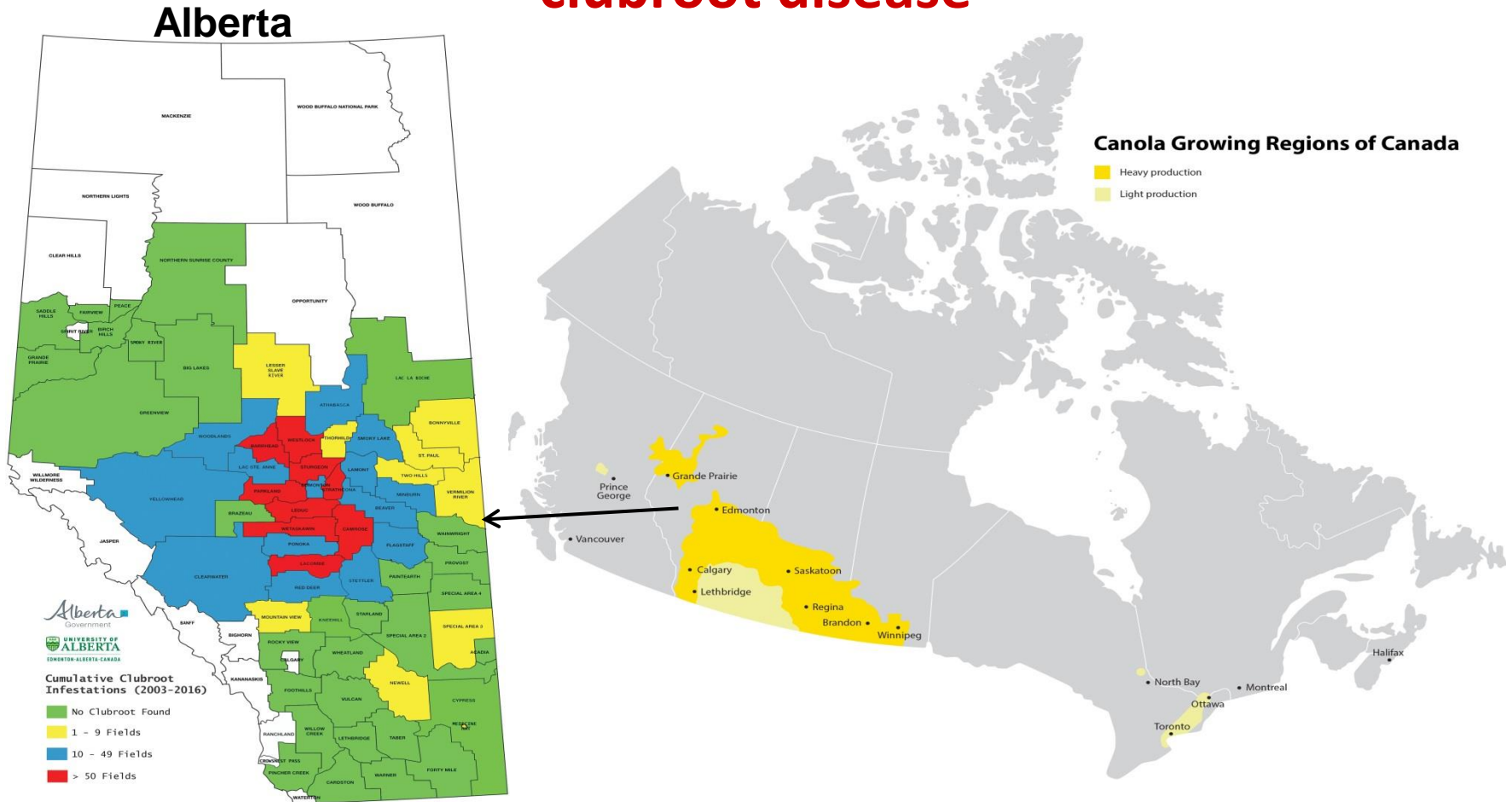
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Overview

- Introduction
- Search for resistance
- Introgression of resistance into canola
- Mapping of resistance

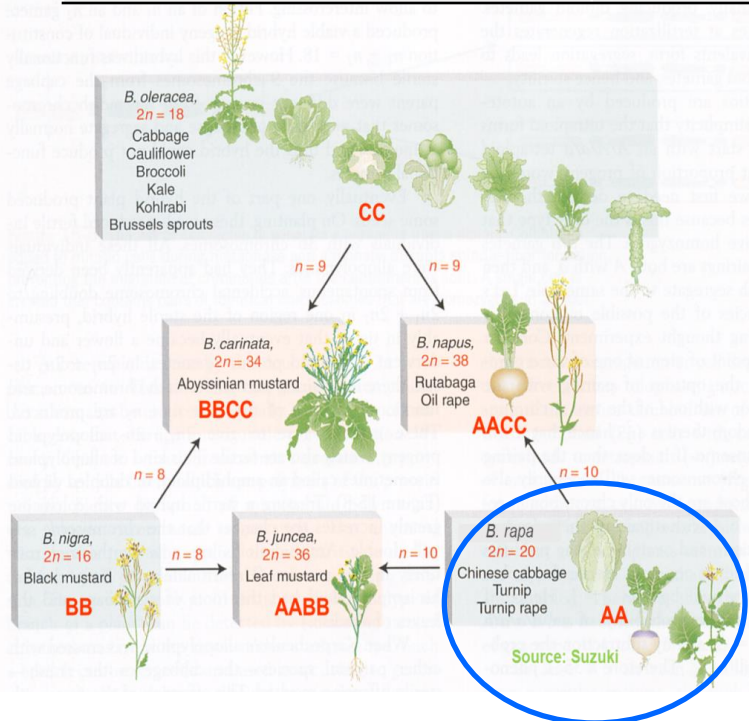
Canola growing regions in Canada and occurrence of clubroot disease



- *Plasmiodiophora brassicae* pathotypes 2, 3, 5, 6 and 8 were initially identified in Canada; among these, pathotype 3 is the most predominant and virulent.
- Additional virulent pathotypes (5x) has been identified in the recent years.

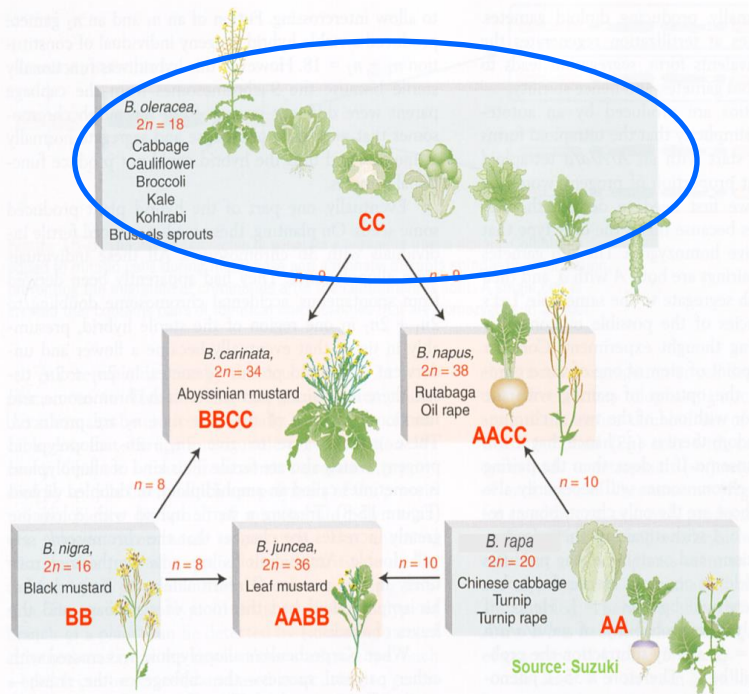
Search for resistance: *B. rapa* (AA, $2n = 20$)

Species	No. accession	No. of accession with resistance				
		Path 2	Path 3	Path 5	Path 6	Path 8
<i>B. rapa</i> var. <i>rapifera</i>	5	5	5	5	5	5
<i>B. rapa</i> var. <i>chinensis</i>	8	1	1	2	1	1
<i>B. rapa</i> var. <i>pekinensis</i>	5	0	0	0	0	0
<i>B. rapa</i> var. <i>oleifera</i>	18	6	9	9	12	12
<i>B. rapa</i> var. <i>oleifera</i>	9	0	0	0	3	3
Total	45	12	15	16	21	21



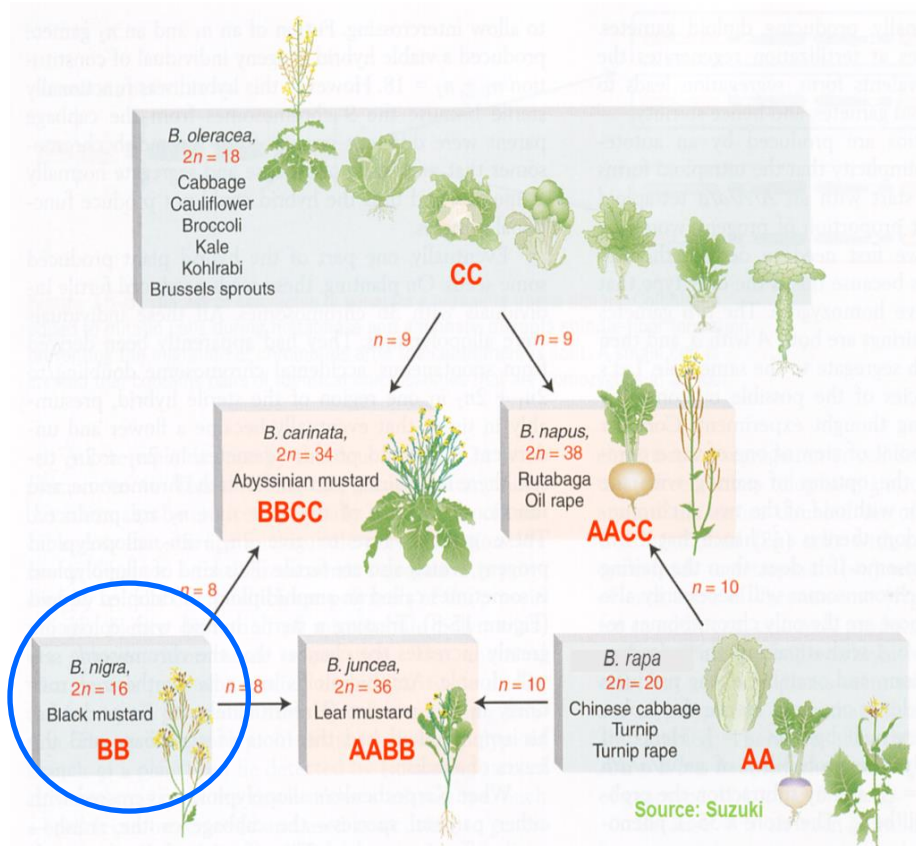
Search for resistance: *B. oleracea* (CC, $2n = 18$)

Species	No. accession	No. of accession with resistance				
		Path 2	Path 3	Path 5	Path 6	Path 8
<i>B. oleracea</i> var. <i>capitata</i>	16	1	3	2	1	2
<i>B. oleracea</i> var. <i>botrytis</i>	14	0	0	0	0	0
<i>B. oleracea</i> var. <i>italica</i>	13	0	0	0	0	1
<i>B. oleracea</i> var. <i>gemmifera</i>	3	0	2	1	0	0
<i>B. oleracea</i> var. <i>alboglabra</i>	2	0	0	0	0	0
<i>B. oleracea</i> var. <i>villosa</i>	1	0	0	1	0	0
Total	49	1	5	4	1	3



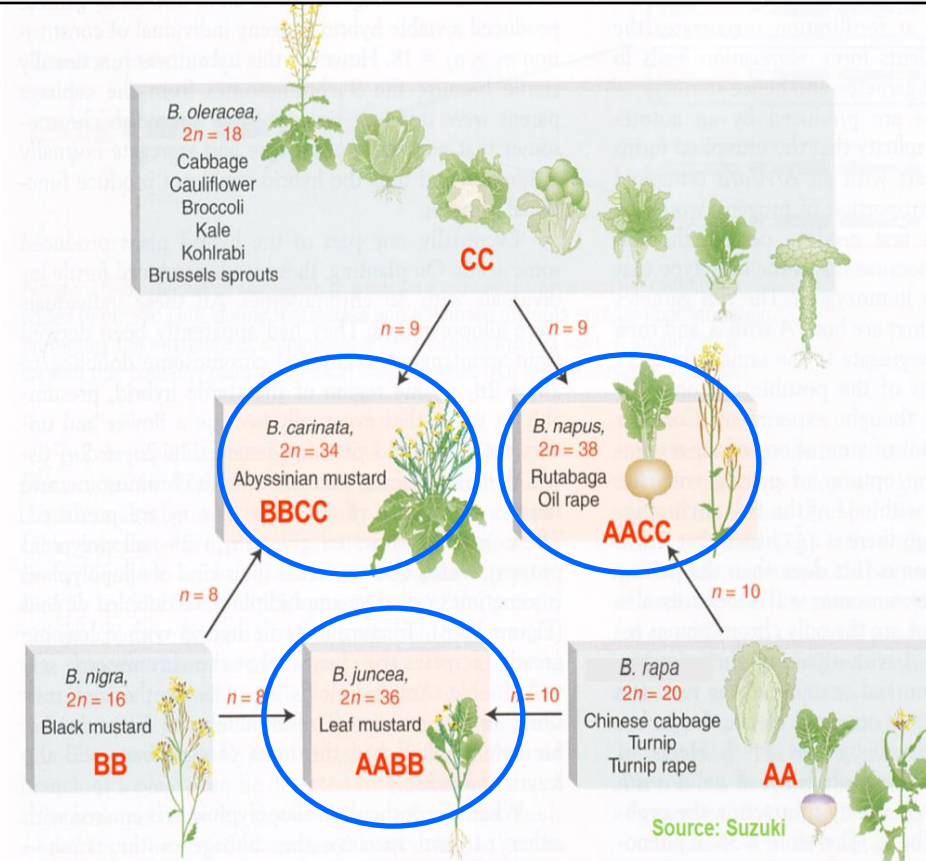
Search for resistance: *B. nigra* (BB, $2n = 16$)

Species	No. accession	No. of accession with resistance				
		Path 2	Path 3	Path 5	Path 6	Path 8
<i>B. nigra</i>	77	67	70	67	68	75



Search for resistance: Amphidiploid species

Species	No. accession	No. of accession with resistance				
		Path 2	Path 3	Path 5	Path 6	Path 8
<i>B. napus</i> (AACC, $2n = 38$)	41	34	3	5	32	37
<i>B. juncea</i> (AABB, $2n = 36$)	48	0	0	0	0	0
<i>B. carinata</i> (BBCC, $2n = 34$)	24	0	0	0	0	0
Total	163	34	3	5	32	37



Search for resistance: Summary

- A total of 275 accessions of six *Brassica* species evaluated.
- Resistance frequent in:
 - Turnip (*B. rapa* var. *rapifera*) and
 - *B. nigra*
- No resistance was found in *B. juncea* and *B. carinata*.

➤ *Resistance used in breeding*

Introgression of clubroot resistance (CR) into canola and mapping of the resistance genes

- CR of winter *B. napus* canola cv. Mendel
- CR of Rutabaga (*B. napus* var. *napobrassica*)



Mendel-resistance: Introgression into canola



Mendel-resistance — × — Spring canola



F₁



F₂



Selection for resistance to pathotype 3



Introgression of Mendel-resistance into canola: F₃ families

Segregation for resistance to pathotype 3

Cross	Number plants				χ^2 test	
	Total	R	S	Ratio	χ^2	<i>p</i>
Mendel × A04-71NA	173	124	49	3:1	1.02	0.31
Mendel × A04-75NA	70	56	14	3:1	0.93	0.33
Total	243	180	63	3:1	0.11	0.74

➤ *A major gene controls resistance to pathotype 3*



➤ *Selection continued for clubroot resistance and agronomic and seed quality traits*

Introgression of Mendel-resistance into canola: F₅ families

	Clubroot resistance ¹	Days to flower	Oil (%)	Sat. FA (%)
Mendel × A04-71NA	0.43	47.1	48.9	7.26
Mendel × A04-75NA	0.40	46.0	51.7	6.85
46A65 (Check)	3.00	44.5	52.5	6.90

¹Clubroot resistance:

<1 = Resistant

>1 = Susceptible

➤ *Further improvement for agronomic and seed quality traits was needed*

Introgression of Mendel-resistance into canola: CR in test hybrids

Hybrids developed by crossing of Susceptible (♀) and Resistant (♂) lines

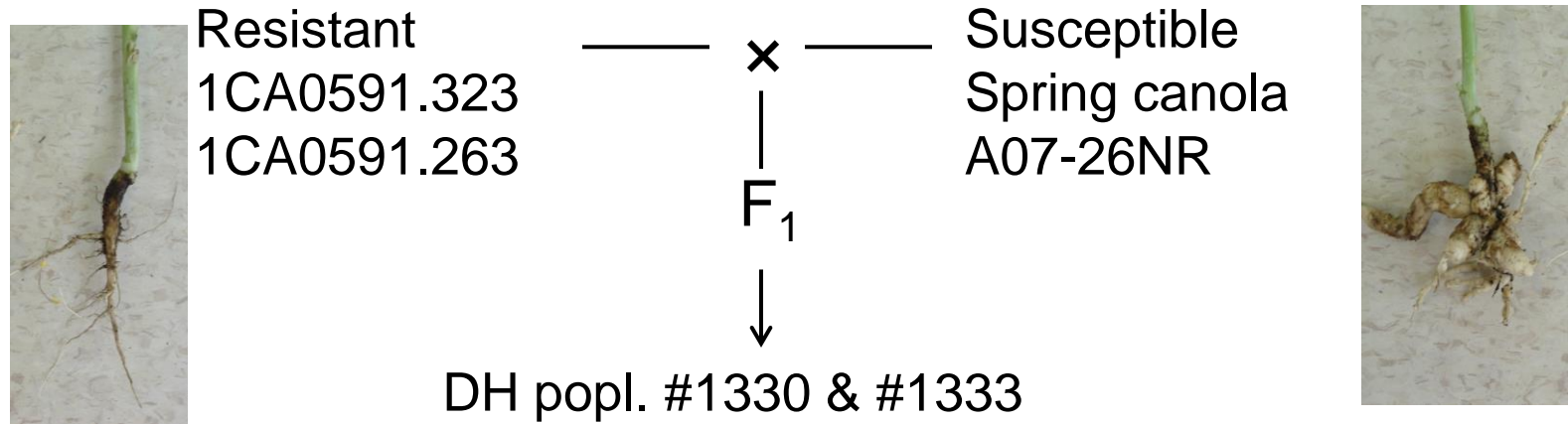
Line/Hybrid	DSI Path 3	DSI Path 5	DSI Path 6
591.165 Line	0	0	0
591.165 Hybrid	0	0	7.4
591.186 Line	0	0	0
591.186 Hybrid	0	0	0
591.225 Line	27.8	0	0
591.225 Hybrid	22.2	0	9.3
591.232 Line	0	0	0
591.232 Hybrid	0	2.8	0

DSI = Disease severity index

0 = no disease, i.e. highly resistant; 100 = highly susceptible

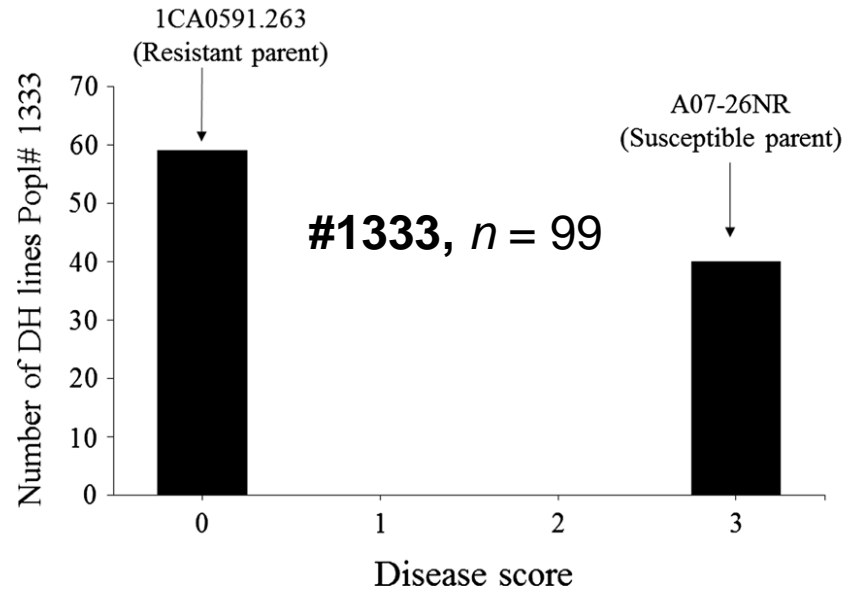
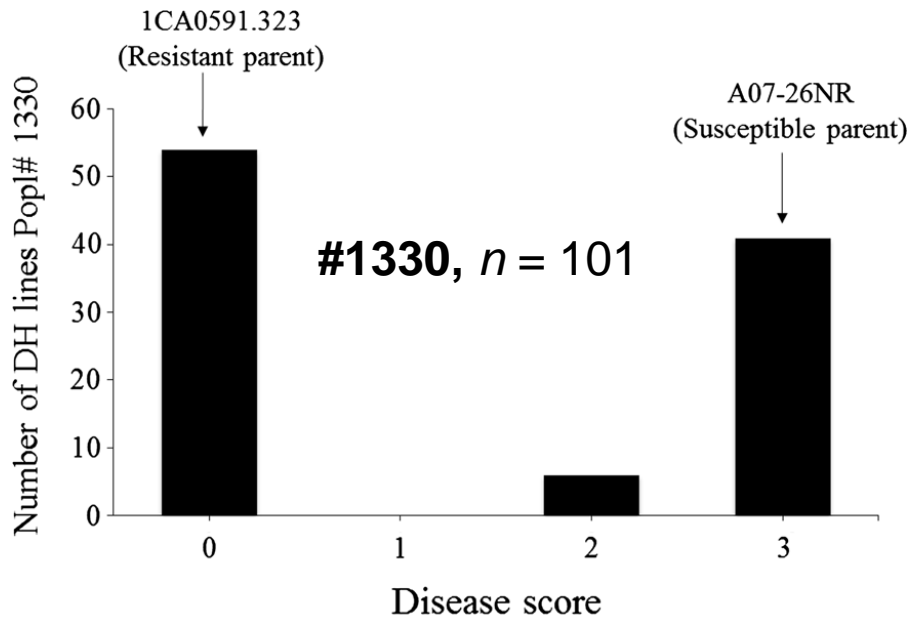


Mapping Mendel-resistance: Development of mapping population



Mapping Mendel-resistance:

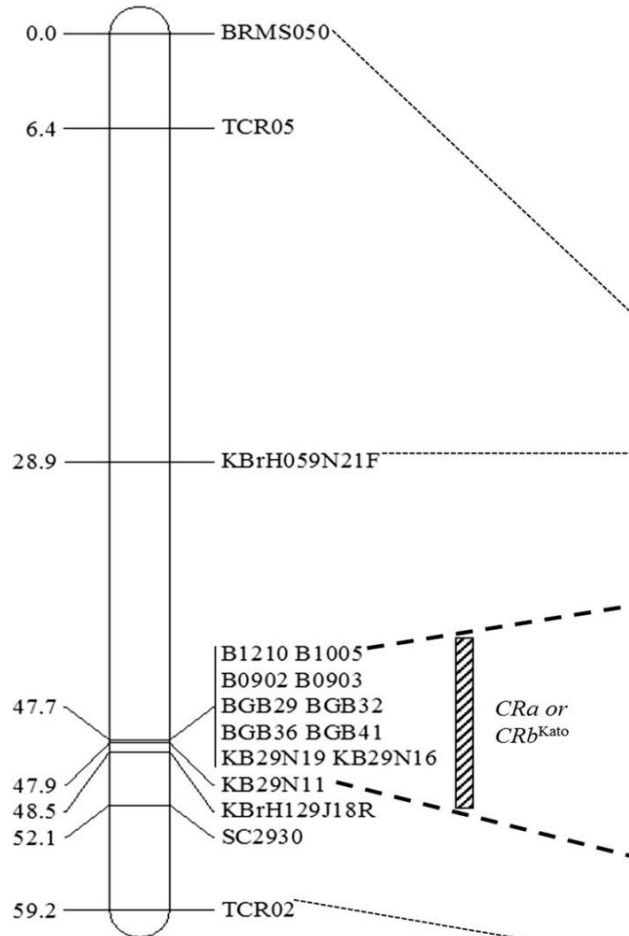
Segregation of the mapping (DH) populations for resistance to pathotype 3



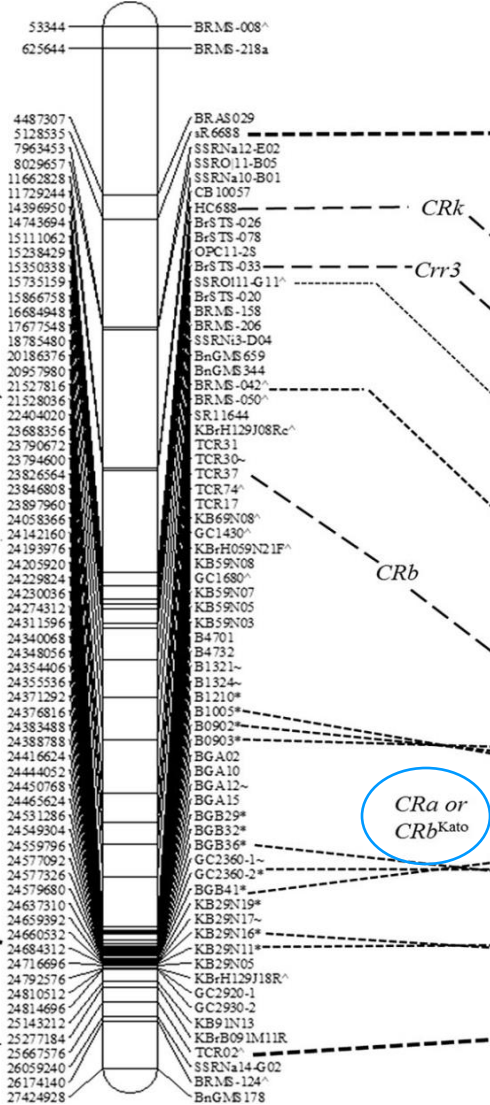
DH population	Total DH	Resistant	Susceptible	χ^2 (1:1)	<i>P</i>
#1330	101	54	47	0.485	0.4861
#1333	99	59	40	3.646	0.0562

Mapping of clubroot resistance of cv. Mendel

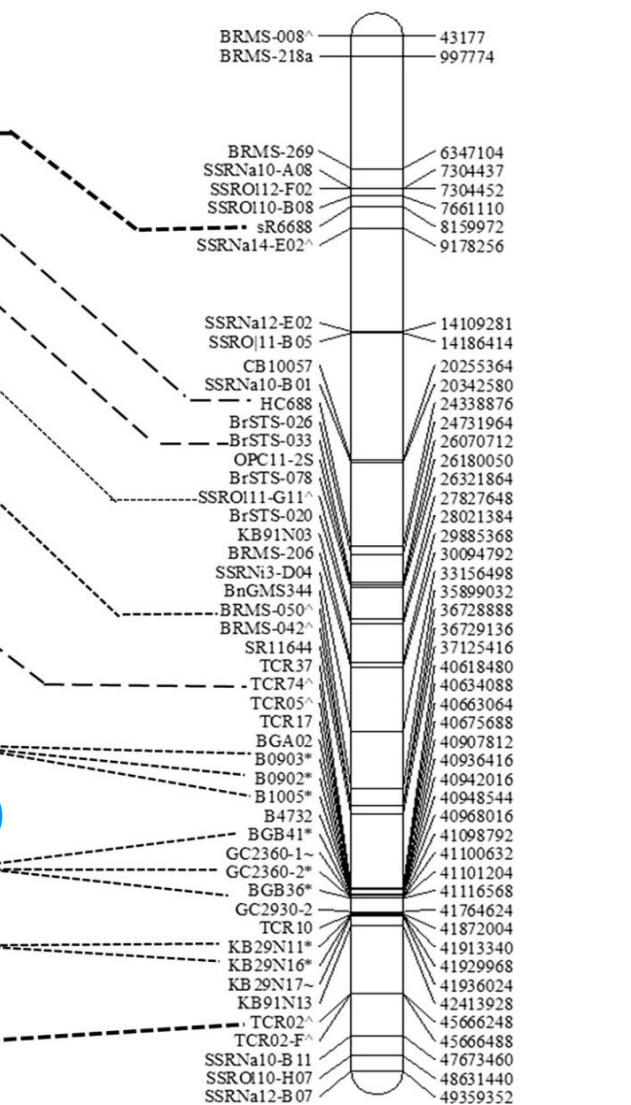
Genetic map: *B. napus* A3



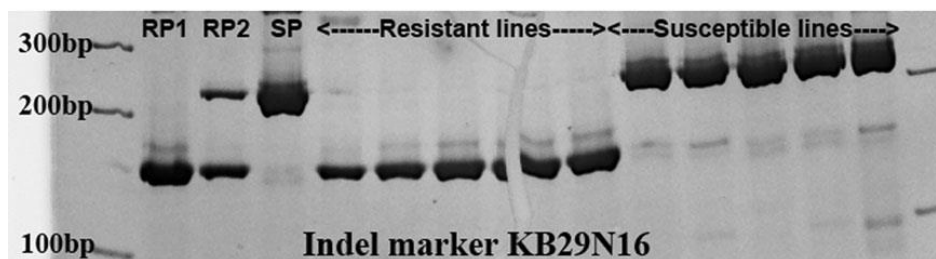
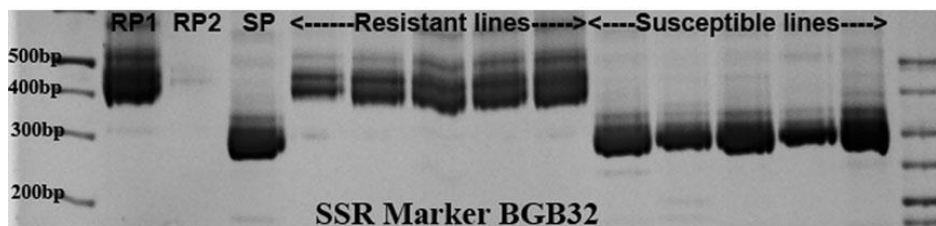
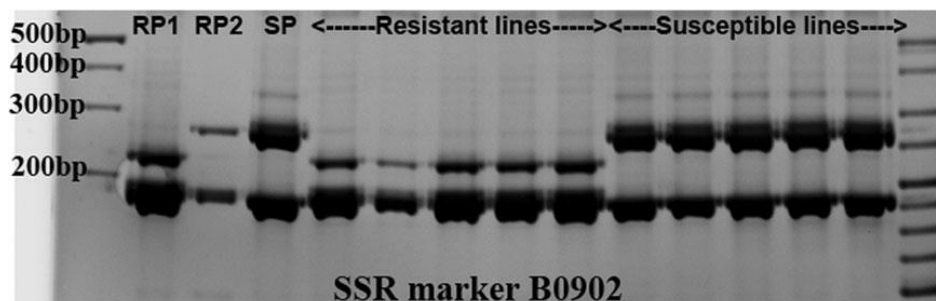
Physical map: *B. rapa* A3



Physical map: *B. napus* A3



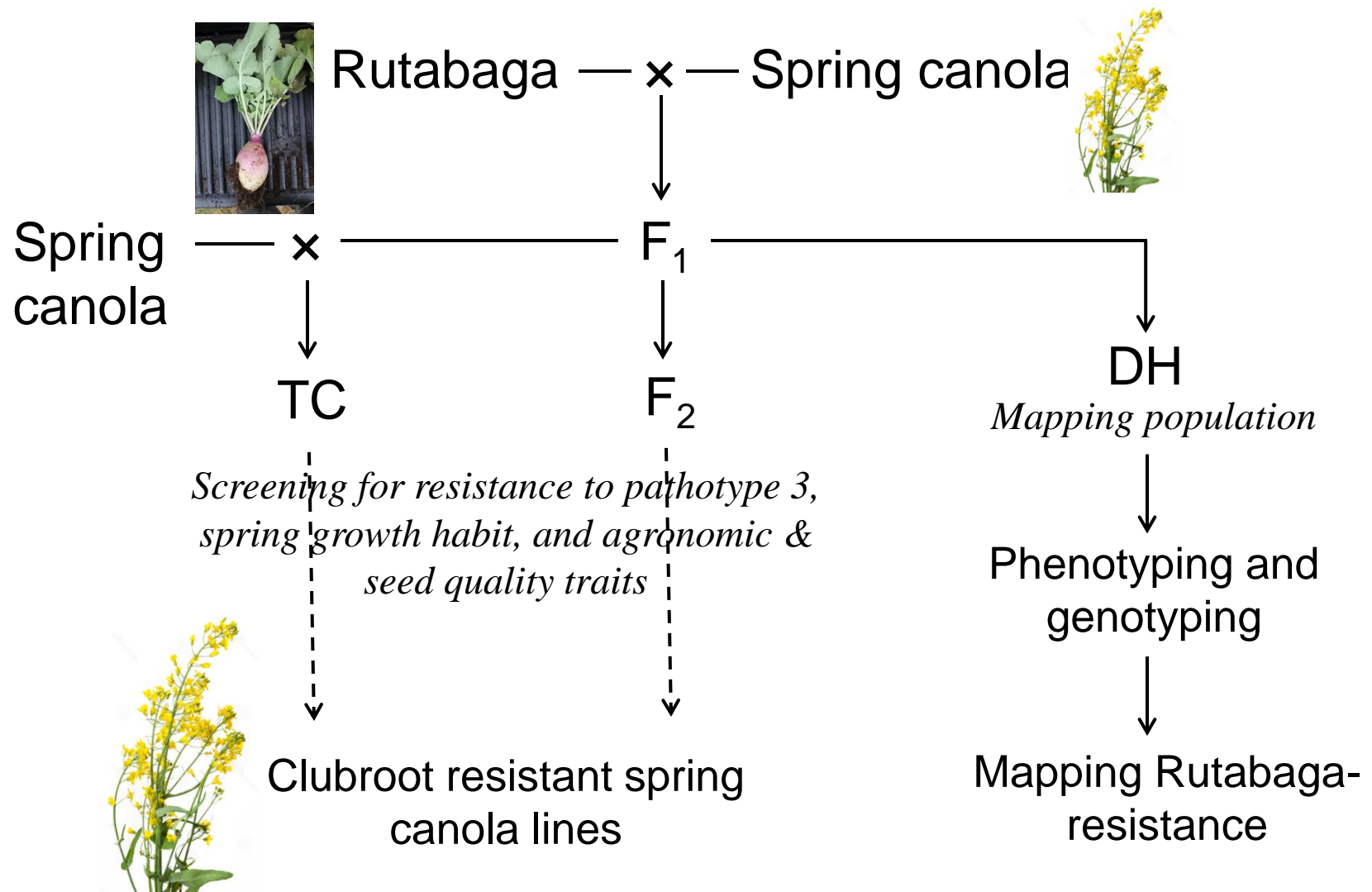
Co-segregation of SSR and Indel markers of *CRb^{Kato}* with Mendel-CR



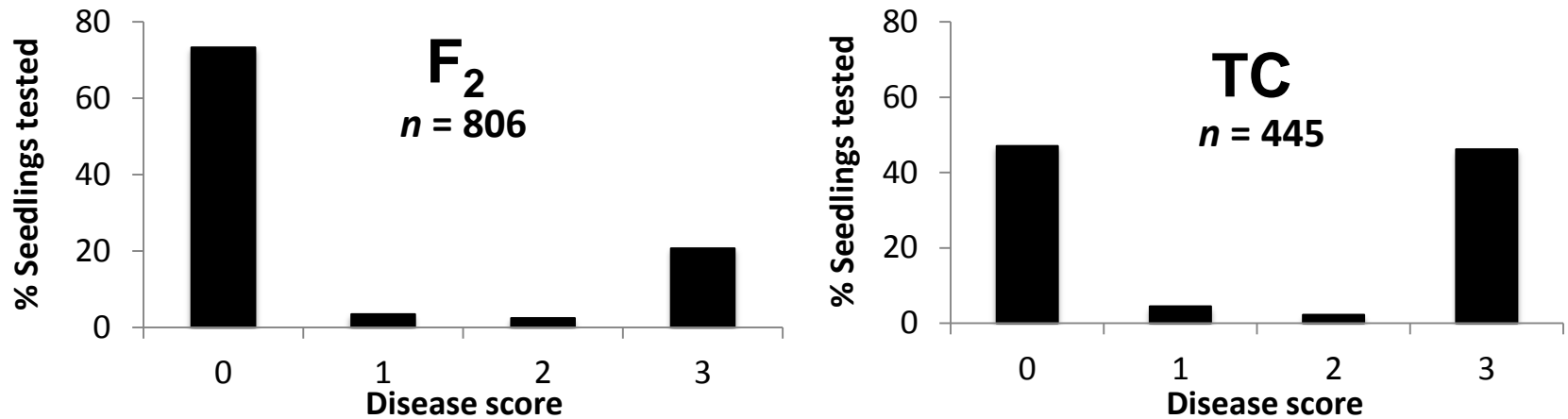
Mendel-CR: Summary

- The major dominant gene of cv. Mendel, conferring resistance to pathotype 3, is located in a genomic region of chromosome A3 where the clubroot resistance genes *CRa* and *CRb^{Kato}* are located.
- Mendel-resistance can be used for the development of F₁ hybrid cultivars conferring resistance to pathotype 3.

Rutabaga-resistance: Genetic study, mapping and introgression of resistance

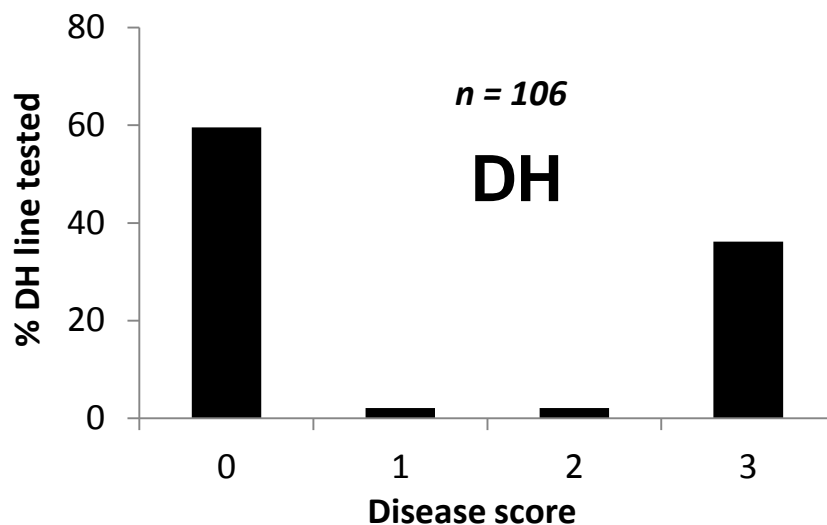


Segregation for resistance to pathotype 3 in F₂ and backcross (TC) populations of Rutabaga × Spring canola cross



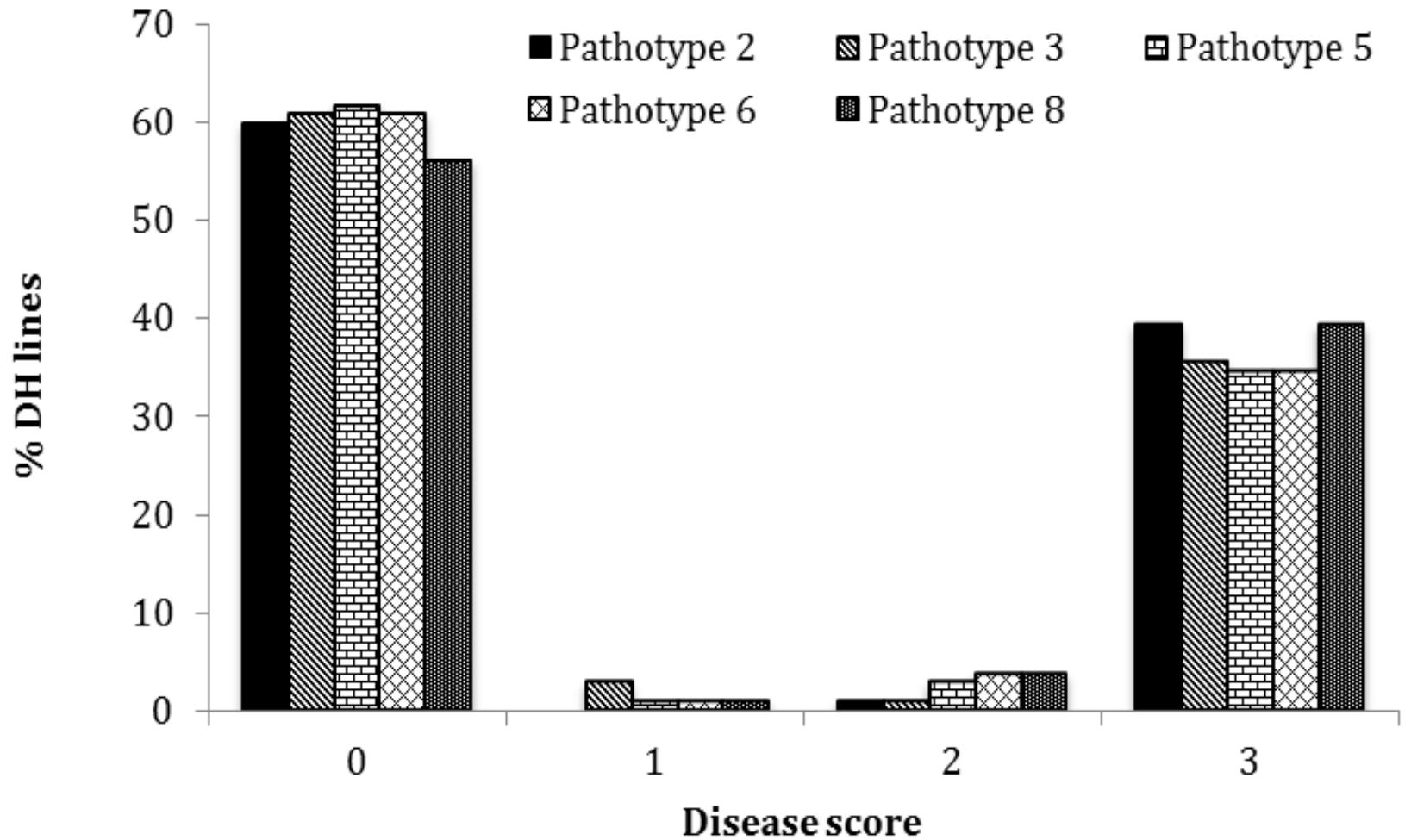
Generation	Family	Total plants	No. resistant	No. suscep.	Segregation		
					Ratio	χ^2	p-value
F ₂	Fam. 1	223	169	54	3:1	0.073	0.79
	Fam. 2	165	121	44	3:1	0.244	0.62
	Fam. 3	206	155	51	3:1	0.006	0.94
	Fam. 4	212	174	38	3:1	5.66	0.02
Total	All	806	619	187	3:1	1.39	0.24
TC	Fam. 1	105	64	41	1:1	5.04	0.025
	Fam. 2	113	59	54	1:1	0.22	0.64
	Fam. 3	113	49	64	1:1	1.99	0.16
	Fam. 4	114	56	58	1:1	0.04	0.85
Total	All	445	228	217	1:1	0.27	0.60

Segregation for resistance to pathotype 3 in DH population of Rutabaga × Spring canola cross



Cross	Total DH	No. Resistant	No. Susceptible	Segregation		
				Ratio	χ^2	p-value
Fam. 1	26	18	8	1:1	3.85	0.05
Fam. 2	8	5	3	1:1	0.5	0.48
Fam. 3	34	19	15	1:1	0.47	0.49
Fam. 4	13	9	4	1:1	1.92	0.16
Fam. 5	13	8	5	1:1	0.69	0.40
Total	94	59	35	1:1	6.13	<0.05

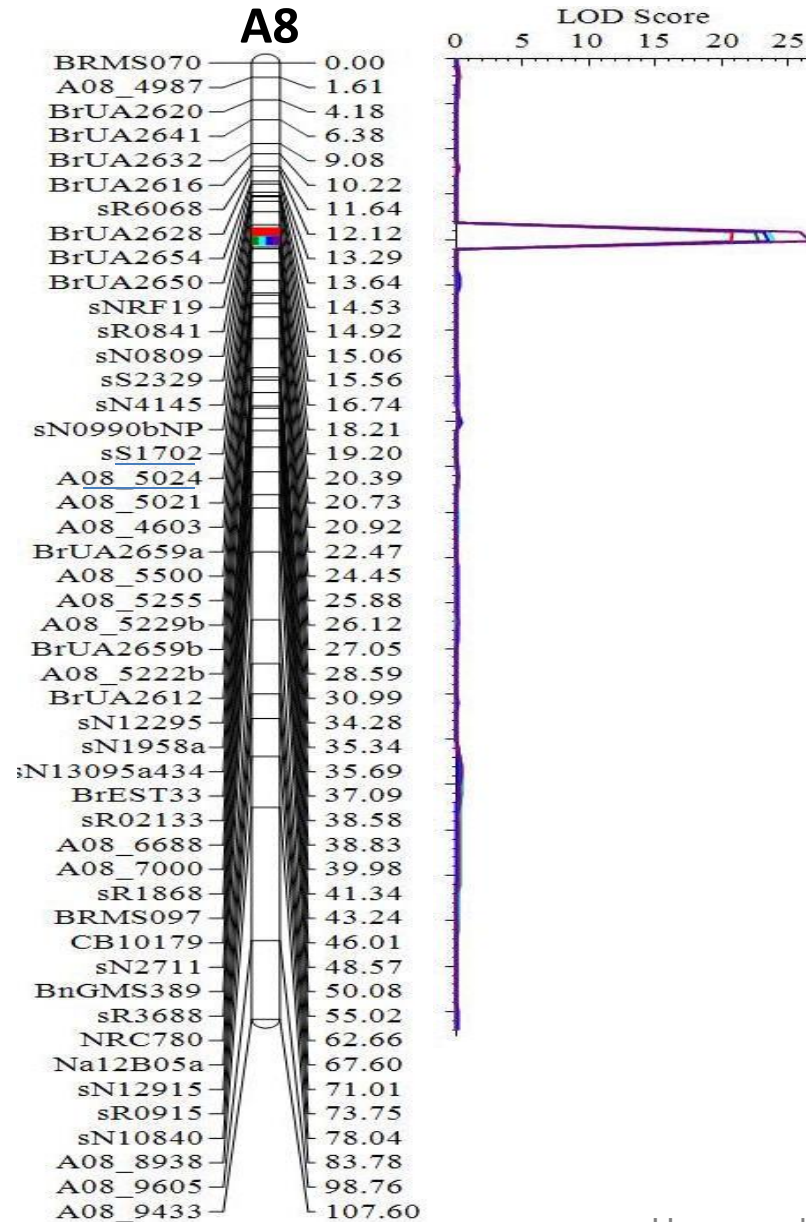
Frequency distribution of the DH population derived from Rutabaga × Spring canola cross for resistance to five pathotypes



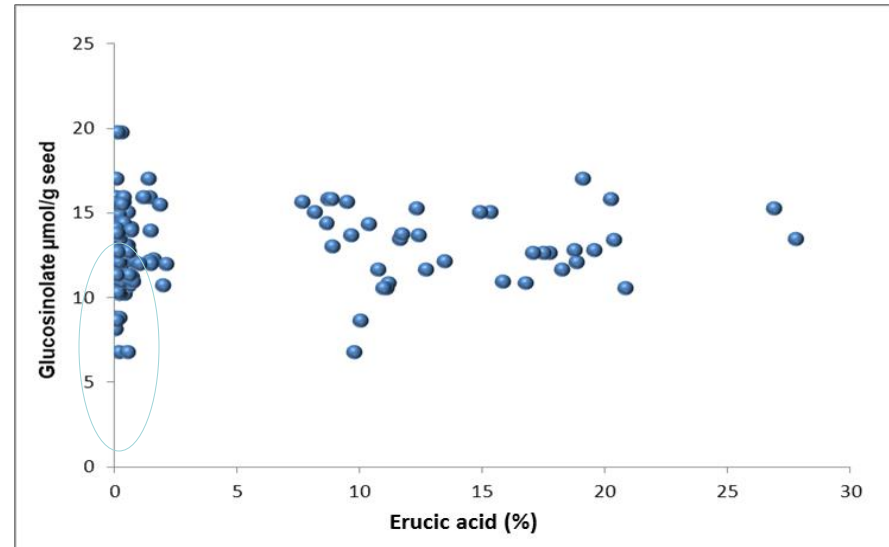
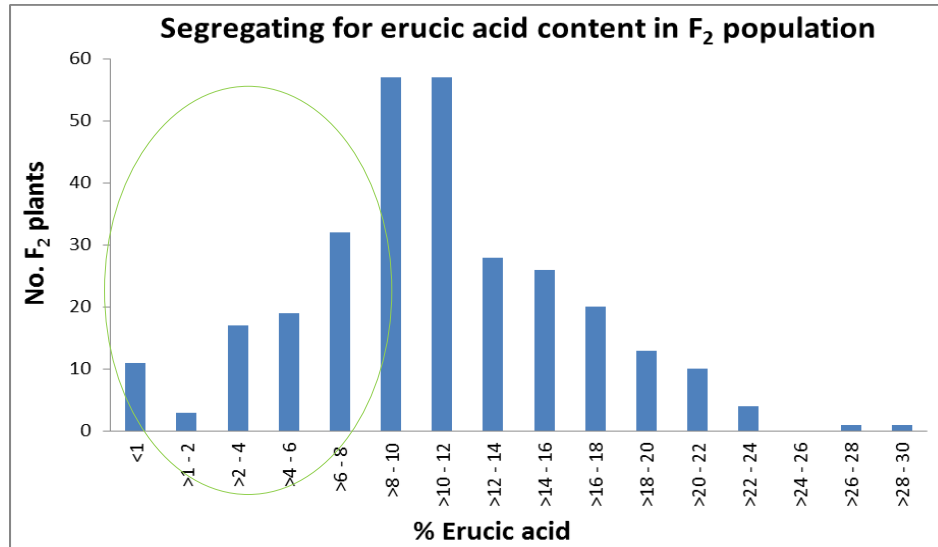
Coefficient of correlation for resistance to different pathotypes in the DH population of Rutabaga × Spring canola cross

	Path. 3	Path. 5	Path. 6	Path. 8
Path. 2	0.947	0.957	0.942	0.922
Path. 3		0.899	0.946	0.887
Path. 5			0.896	0.878
Path. 6				0.903

Linkage map of chromosome A8 of *Brassica napus* carrying resistance to different pathotypes



Canola quality lines derived from spring canola × rutabaga cross



- Introgression of resistance from rutabaga was more challenging than introgression from winter canola due to involvement of non-canola quality traits. Intensive breeding was needed to develop elite canola lines.

Advanced generation lines derived from spring canola × rutabaga cross

	<i>n</i>	Days to flower	Days to maturity	Oil (%)	Protein (%)	GSL (μmol/g)	Sat FA (%)
F ₆	249	50.6	103.1	47.6	24.3	19.9	6.6
A07-26NR	6	48.4	97.4	48.6	25.2	15.1	6.7

GSL = Glucosinolates; Sat FA = Saturated fatty acids.

N.B. All are zero-erucic acid type; most of the lines had low GSL in seed.

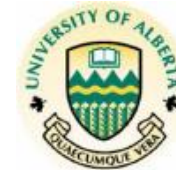
Rutabaga-CR: Summary

- The major dominant gene of rutabaga, conferring resistance to pathotype 3, is located in a genomic region of the chromosome A8.
- This chromosome region also confer resistance to other pathotypes, such as pathotype 2, 5, 6 and 8.
- Several canola quality spring *B. napus* lines carrying clubroot resistance of rutabaga were developed.

Acknowledgements



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