Mapping clubroot resistance genes in Chinese cabbage and ECD accessions of *B. rapa*

Arvind H. Hirani and Genyi Li
Department of Plant Science
University of Manitoba
Winnipeg, Canada

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Outline of presentation

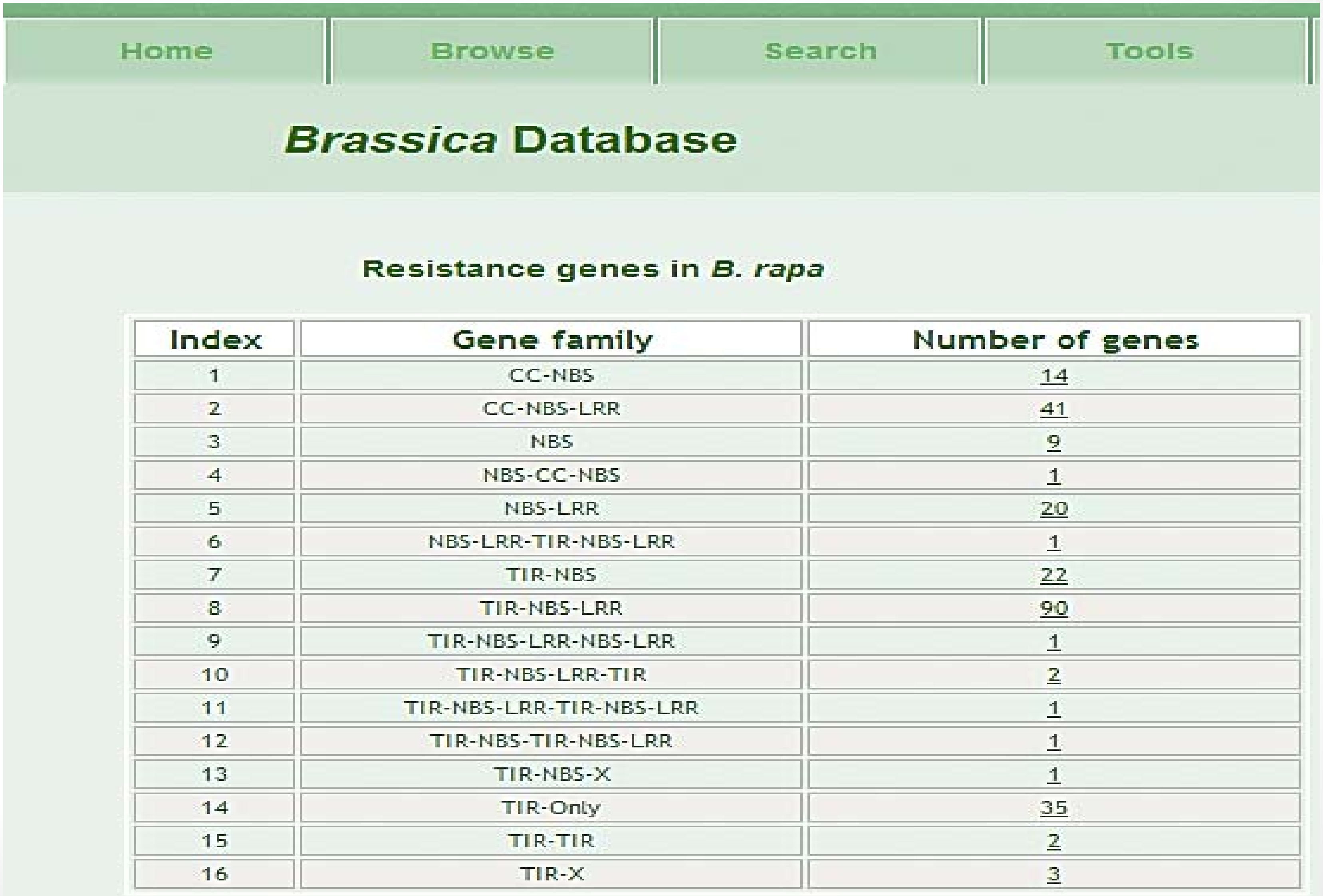
- Genetic of clubroot disease in *Brassica* species
- Mapping clubroot resistance genes in Chinese cabbage and ECD set
- Future research avenue
- Summary

Genetic of clubroot resistance in *Brassica* species

Types of resistance

- **R**ace specific or **Q**ualitative or **V**ertical resistance
  - i.e. Crr1, Crr2, Crr3, CRa, CRk, CRc
- **N**on-race specific or **Q**uantitative or **H**orizontal resistance
- Over 230 R gene sequences revealed in *B. rapa* sequenced genome representing 16 gene families

Resistance genes in *B. rapa* - How many for CR?



***Brassica* Database**

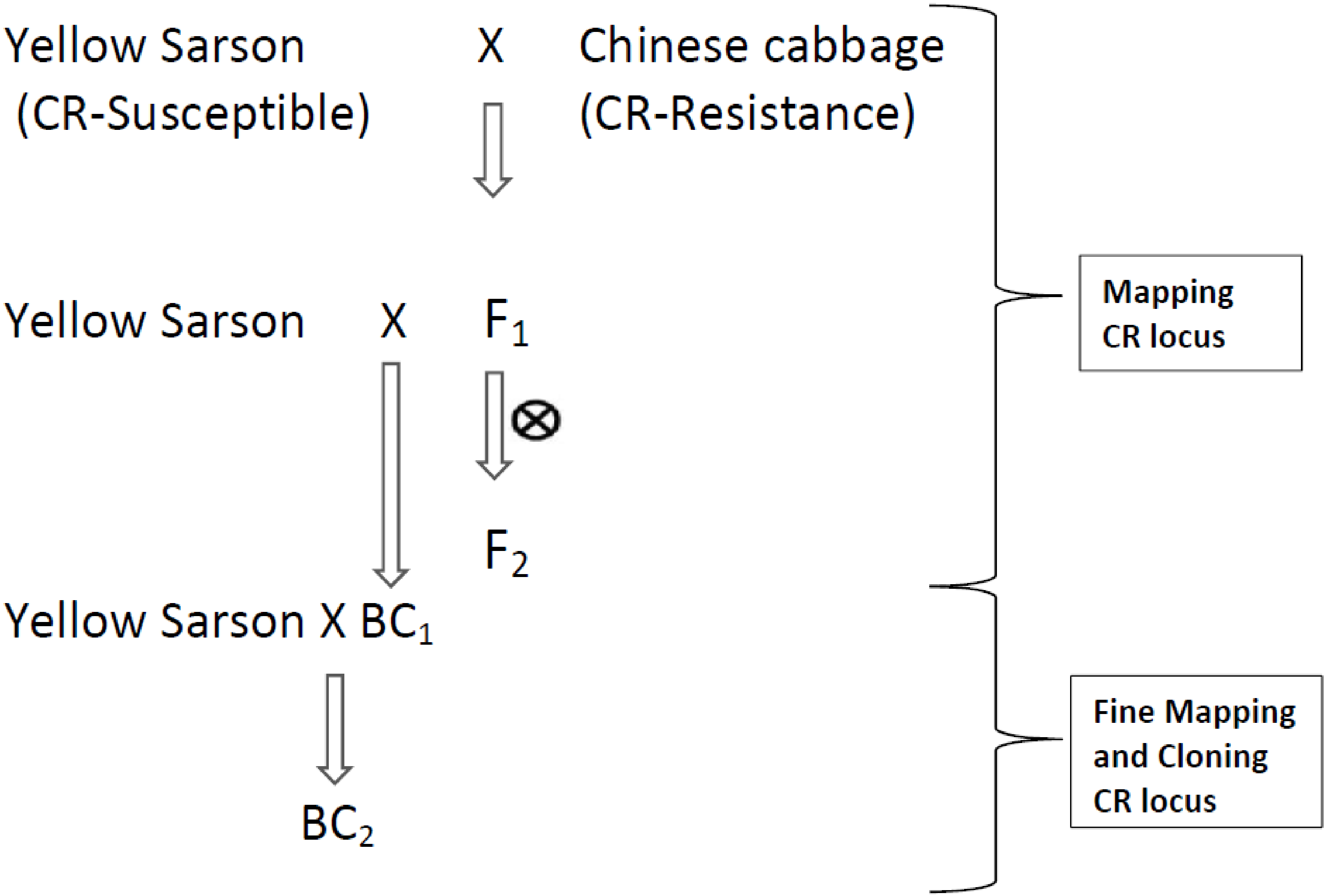
Resistance genes in *B. rapa*

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

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

Mapping and fine mapping a clubroot resistance gene in Chinese cabbage

Population development for mapping and fine mapping



Yellow Sarson (CR-Susceptible) X Chinese cabbage (CR-Resistance)

Yellow Sarson X F_1

⊗

F_2

Yellow Sarson X BC_1

BC_2

Mapping CR locus

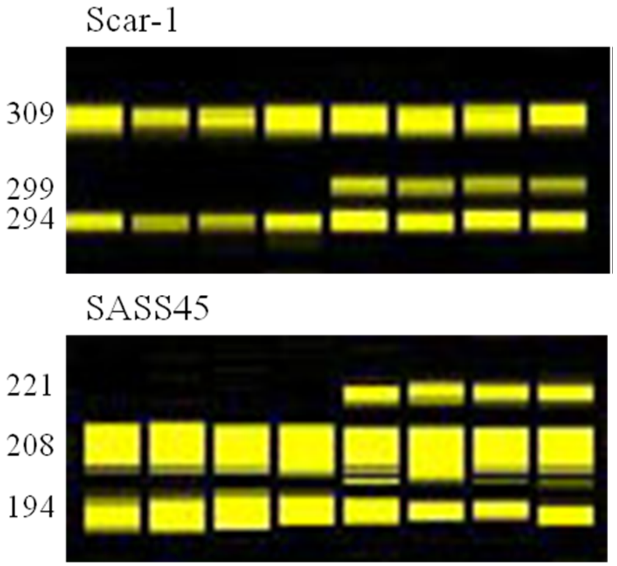
Fine Mapping and Cloning CR locus

Fine mapping clubroot resistance locus in Chinese cabbage

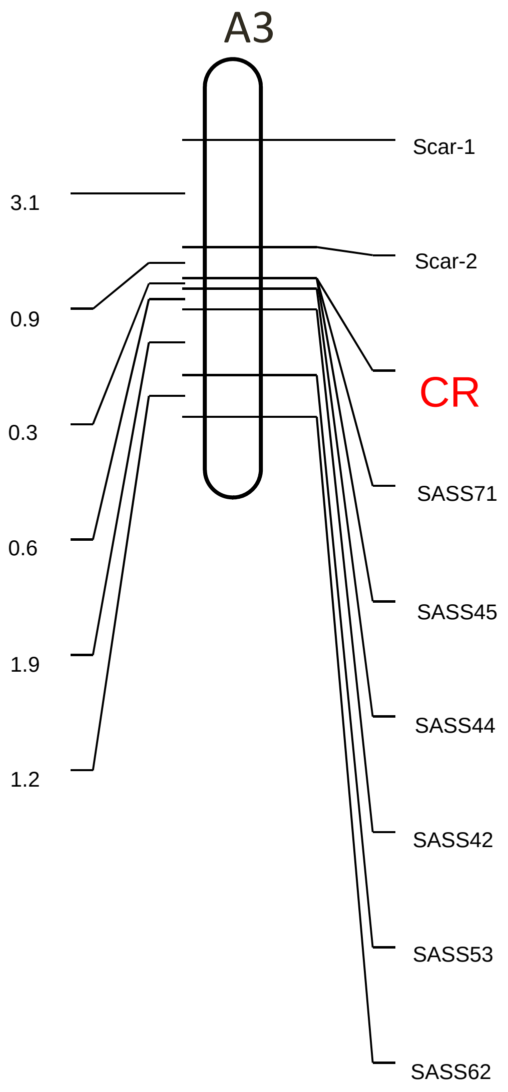
- Phenotype evaluation revealed single Mendelian locus for clubroot resistance in Chinese cabbage
- SRAP markers found to be co-segregated with clubroot resistance phenotype
- These SRAP markers were located on linkage group R3 on ultra-dense genetic linkage map (Sun et al. 2007)
- Over 8,000 BC_2 individual plants were used for fine mapping this locus
- 219 recombinant individuals were used for cloning and markers development
- This CR locus was introgressed from *B. rapa* to *B. napus* by modified backcross breeding
- Molecular markers were developed in canola for marker assisted selection

Mapping clubroot resistance locus in Chinese cabbage

Generations	Total	CR Resistance	CR Susceptible	Ratio
F_2	85	67	18	3:1
BC_1	67	30	37	1:1



Molecular markers developed for clubroot resistance loci for MAS in canola



Mapping clubroot resistance genes in ECD set

Mapping clubroot resistance loci in ECD set

- The **E**uropean **C**lubroot **D**ifferential (ECD) set was evaluated for clubroot resistance by Canadian field isolate of *P. brassicae*
- Clubroot resistance accessions of ECD set, ECD1, ECD2, ECD3 and ECD4 were used as resistance parents
- BC_1 populations were evaluated for clubroot disease by Canadian field isolate of *P. brassicae*
- One or two gene segregation ratio observed in BC_1 populations of ECDs
- All the BC_1 populations were screened by markers developed for R3 specific CR locus based on Chinese cabbage
- For additional locus, over 150 SRAP primer pairs were used to find co-segregation
- New SSR markers developed based on SRAP marker linkage on ultra-dense genetic map

Clubroot disease evaluation of ECD set by Canadian field isolate

ECD accessions	Clubroot ratings			
	0	1	2	3
ECD1	35	0	0	0
ECD2	43	0	0	0
ECD3	39	0	0	0
ECD4	43	0	0	0
ECD5	0	2	2	43
ECD6	4	0	2	35
ECD7	0	2	2	29
ECD8	2	0	1	29
ECD9	9	3	3	21
ECD10	24	0	0	4
ECD11	41	0	0	0
ECD12	22	0	0	0
ECD13	12	0	6	13
ECD14	0	2	2	16
ECD15	18	2	4	9

Single locus segregating BC_1 families derived from ECDs

BC_1 Families	Resistant	Susceptible	Total	χ^2	P value	Ratio	DF
BC_1-A1	24	22	46	0.09	0.77	1:1	1
BC_1-A2	39	37	76	0.05	0.82	1:1	1
BC_1-A3	14	15	29	0.03	0.85	1:1	1
BC_1-A4	22	18	40	0.40	0.53	1:1	1

Two CR loci segregating BC_1 families derived from ECDs

Pedigree	Resistant	Susceptible	Total	P value	Ratio	DF
BC_1-B1	76	22	98	0.56	3:1	1
BC_1-B2	40	15	55	0.70	3:1	1
BC_1-B3	30	6	36	0.25	3:1	1
BC_1-B4	14	5	19	0.89	3:1	1
BC_1-B5	27	9	36	1.00	3:1	1

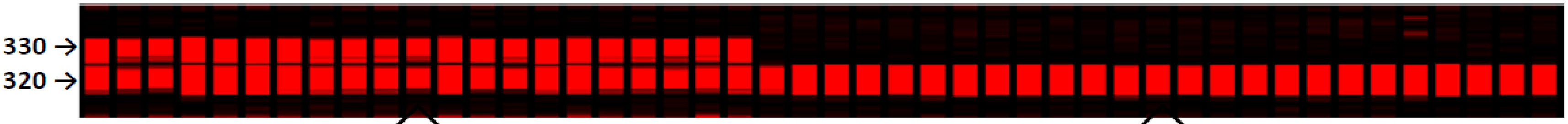
Segregation of clubroot phenotypes in ECD derived BC_1

1:1

3:1

Microsatellite markers linked with CR genes

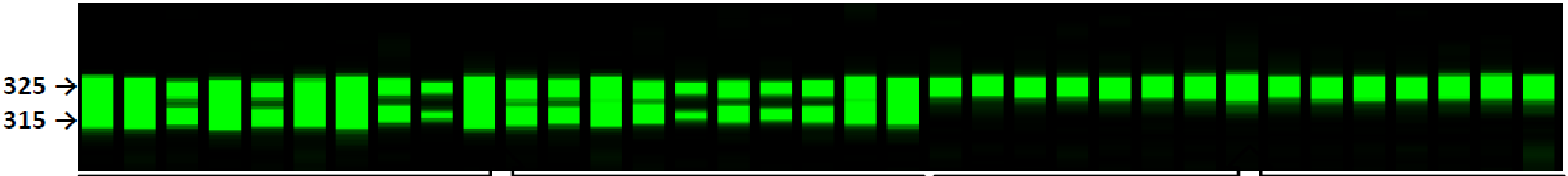
S14R14



Clubroot Resistance

Clubroot Susceptible

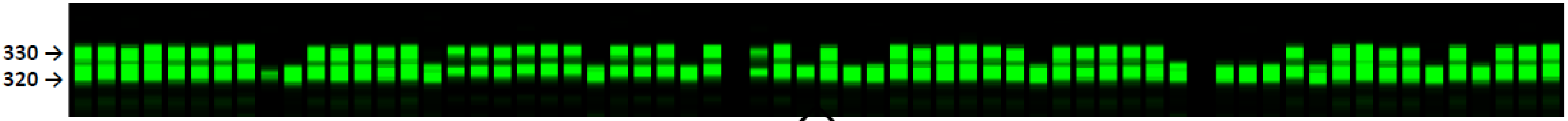
S17R17



Clubroot Resistance

Clubroot Susceptible

S14R14



Two loci segregating in ECD3 derived BC1 clubroot resistance families

Future research avenues

- Integration of clubroot resistance loci from *B. rapa* to *B. napus*
- Clubroot disease evaluation on NIL with single locus in same genetic background with different isolates of *P. bassicae*
- Functional characterization of all the loci through canola transformation
- Determination of structural and functional variations of resistance loci between and within ECD accessions
- Development of SCAR and SNP markers (functional markers) for MAS in canola
- Pyramiding of resistance loci for durable resistance to clubroot disease

Summary

- Single clubroot resistance locus mapped and fine mapped in five Chinese cabbage cultivars
- This locus was introgressed from Chinese cabbage to canola through MAS
- Two clubroot resistance loci mapped in ECD derived BC_1 populations
- ECD accessions (1 to 4) are segregating for one or two loci for clubroot resistance
- Two loci are mapped and mapping of the third clubroot resistance locus is underway
- NIL with single clubroot resistance locus developed in same genetic background
- Molecular markers developed for MAS in canola

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Thank You for your attention!