Mapping and cloning clubroot resistance genes in *B. rapa*

Dr. Genyi Li

University of Manitoba

Gene characterization and marker assisted selection (MAS)

- To map gene positions in the genome
- To distinguish gene interactions with isolates
- To develop molecular markers for each locus
- To perform MAS to accelerate breeding cycles
- To pyramid different resistance genes and develop canola cultivars with durable resistance

Mapped genes of clubroot resistance in *B* . *Rapa* and *B. napus*

Linkage group	**Gene names**	Genetic distance (cM)	**References**
A01	Crr2	2.2	Suwabe et al. 2003, 2006).
A02	CRc	5.1	Sakamoto et al, 2008
A03	Crr3, CRa, CRb, CRk	3-15	Sakamoto et al, 2008
A06	Crr4	2.7	Suwabe et al. 2003, 2006).
A08	Crr1	1.6	Suwabe et al. 2003, 2006).
C2, C3. C7	CR-QTL		Nagaoka et al. (2010)
N3, N5, N8	CR-QTL		Werner et al. (2008)
N13, n15, N16, N19	CR-QTL		Werner et al. (2008)

Many resistance genes in the genomes?

Indoor testing of clubroot resistance in Chinese cabbage cultivars with Canadian isolates

Gene mapping and allelic gene testing

- Five Chinese cabbage cultivars with clubroot resistance
- Three BC1 and two F2 populations were developed using five Chinese cabbage hybrid cultivars
- Gene mapping showed that one Mendelian gene in each population
- All genes were mapped into the same position on A3 chromosome

Gene testing in BC1 and F2 populations of five Chinese cabbage cultivars and a susceptible *B. rapa* rapeseed

Mapping populations	Clubroot symptom scoring*				Expected ratio	X2 test (p value)
	0	1	2	3		
BC1a	29	1	7	30	1:1	0.39 ns
BC1b	28	0	9	23	1:1	0.61 ns
BC1c	41	0	7	39	1:1	0.59 ns
BC1d	38	1	2	11	3:1	1 ns
F2a	44		2	16	3:1	0.46 ns
CK	0	0	0	44		

Fine mapping and identification of candidate clubroot resistance genes

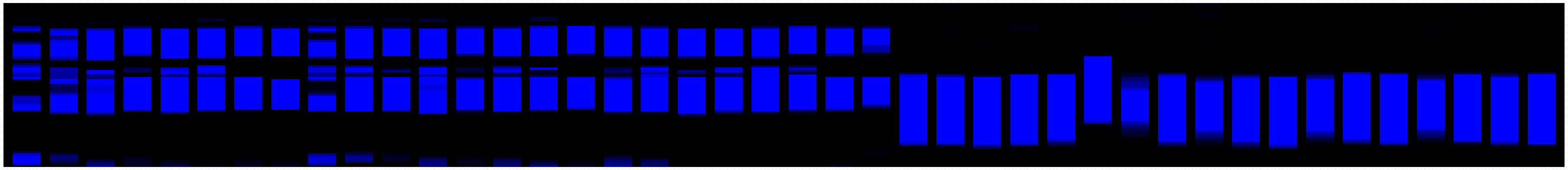
- ❖ 18 SSR, SCAR and SNP molecular markers flanking the clubroot resistance genes on A3 were developed.
- ❖ Two flanking markers were used to find 219 recombinants from 8000 BC2 individuals.
- ❖ There are four recombinants that were used to pinpoint the gene in a 50 kb region.
- ❖ Several clubroot resistant gene candidates in the 50-kb region were identified.

Complemetary and RNAi transformation with candidate genes of clubroot resistance on A3

- A BAC library with 200,000 BAC clones
- Covering approximate 20 times of the whole genome
- Seven positive BAC clones anchoring the molecular markers The selected positive BAC clones were used to make transformation constructs.
- Two constructs with 15 kb and 12 kb fragments
- Two RNA interference (RNAi) constructs
- About 15 transgenic plants for each construct

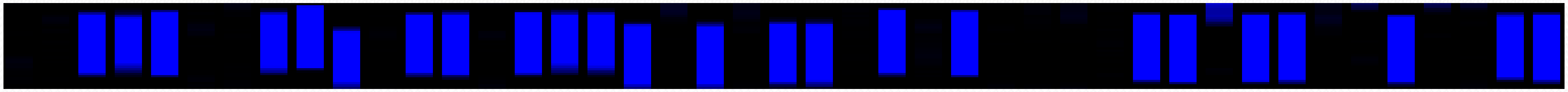
Testing of Monsanto’s breeding materials developed with two clubroot resistant sources*

A



Resistant individuals (R-1 to R-24) Susceptible ones (S-1 to S-18)

B



Resistant individuals (R-1 to R-24) Susceptible ones (S-1 to S-18)

*Molecular marker SCAR3 on N3 co-segregated with a clubroot resistance locus in Monsanto’s breeding lines derived from one clubroot resistant source and did not in another source.

Panel A: Resistant source 1 (perfect matches with phenotypes)

Panel B: Resistant source 2 (Independent segregation)

Validation of molecular markers for clubroot resistance genes on A3 in canola

- DNA of breeding materials from Monsanto were analyzed with the molecular markers on N3
- The molecular markers perfectly matched with phenotypes that were derived from one clubroot resistant source
- No match with phenotypes of the progeny derived from another resistant source
- The resistance genes in Monsanto's breeding materials are diverse
- Gene pyramiding can be performed with two different resistant sources
- Canola cultivars with multiple clubroot resistance gene loci will be developed soon in Monsanto

UNIVERSITY OF MANITOBA

canolacouncil OF CANADA

Testing of ECD accession with a Canadian isolate*

	CR Rating				
Line code	0	1	2	3	
ECD-01	35	2	2	0	Resistant
ECD-02	52	3	4	0	Resistant
ECD-03	39	0	0	0	Resistant
ECD-04	43	0	0	0	Resistant
ECD-05	0	2	2	43	Susceptible
ECD-06	4	0	2	35	Susceptible
ECD-07	0	2	2	29	Susceptible
ECD-08	2	0	1	29	Susceptible
ECD-09	9	3	3	21	Susceptible
ECD-10	24	0	0	4	Resistant
ECD-11	41	0	0	0	Resistant
ECD-12	22	0	0	0	Resistant
ECD-13	12	0	6	0	Resistant
ECD-14	0	2	2	16	Susceptible
ECD-15	18	2	4	4	Resistant
CK	0	0	0	280	Susceptible

*Clubroot ratings: 0, strong resistant and no gall; 1, susceptible with small galls on secondary roots; 2, susceptible with small galls on all roots; 3, totally susceptible with big galls on all roots.

Mapping populations for ECD materials

- ECD1, 2, 3, 4 and 10 were used to develop mapping populations.
- Segregating populations for ECD1, 2, 3, and 4 are being phenotyped
- Candidate genes on different chromosomes are being developed.
- All clubroot resistant *B. rapa* and *B. oleracea* were used to obtain synthetic *B. napus*.
- Mapping populations are being developed with synthetic *B. napus*.

Acknowledgement

Drs. Feng Gao, Arvind Hirani, Zheng Liu, Peter B.E. McVetty at UM

Chunren Wu and Jun Liu at Monsanto

Financial Support

Canola Council of Canada and AAFC