Clubroot Disease

- 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

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

- CRa
- CRk
- Crr3
- Pb-Bn1
- Crr2
- CRc
- Crr4
- Crr1

QTL

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

*Plasmodiophora brassicae* pathotype 3

- B. carinata
- B. juncea
- B. napus
- B. nigra
- B. oleracea
- B. rapa
- Other
Resistant Brassica germplasm identified at AAFC - Saskatoon

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of lines</th>
<th>Number of R lines</th>
<th>R%</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. carinata</td>
<td>10</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>B. juncea</td>
<td>91</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>B. napus</td>
<td>137</td>
<td>3</td>
<td>2.2</td>
</tr>
<tr>
<td>B. nigra</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>B. oleracea</td>
<td>33</td>
<td>3</td>
<td>9.1</td>
</tr>
<tr>
<td>B. rapa</td>
<td>698</td>
<td>24</td>
<td>3.4</td>
</tr>
<tr>
<td>Other</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1039</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>
Development of segregating populations

B. rapa ACDC  \( \times \)  B. rapa lines resistant to clubroot

self-compatible  \( \downarrow \)  self-incompatible

B. rapa ACDC  \( \times \)  F1

Non-segregation  \( \downarrow \)  segregated

BC1  \( \rightarrow \)  Mapping

Phenotyping F1

\[ \text{ACDC10-9 x Jnc-2} \]
\[ \text{Jnc-1 x ACDC} \]
Inheritance of clubroot resistance in *B. rapa*

Cross | CR/CS plants | Expected ratio | X$^2$ | 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 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 (Chiflu-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.
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
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
Map-based cloning of *Rpb1*

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

**Diagram:**

- **B. napus** DH16516 $A^dA^dC^dC^d$
- **B. rapa** JNC $A^*A^*$
- **B. napus** triploid $A^dA^*C^d$
- **BC1** $A^d/A^*C^{[C^d (0-9)]}$
Genome-wide marker assisted selection in BC1

B. napus DHT (A genome) x B. rapa JNC (C genome) = triploid

B. napus DHF (A genome) x (C genome) = 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

- $B.\ napus \times BC1$
- $B.\ napus \times BC2$
- $B.\ napus \times BC3 \rightarrow BC3S1 \rightarrow BC3S2$

- 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

<table>
<thead>
<tr>
<th>Cross</th>
<th>CR/CS plants</th>
<th>Expected ratio</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1830 x BRA</td>
<td>11/14</td>
<td>1:1</td>
<td>0.36</td>
<td>0.549</td>
</tr>
<tr>
<td>1768 x BRA</td>
<td>17/8</td>
<td>1:1</td>
<td>3.24</td>
<td>0.072</td>
</tr>
<tr>
<td>1830 x PI 21</td>
<td>36/35</td>
<td>1:1</td>
<td>0.01</td>
<td>0.906</td>
</tr>
<tr>
<td>1768 x PI 21</td>
<td>13/12</td>
<td>1:1</td>
<td>0.04</td>
<td>0.841</td>
</tr>
</tbody>
</table>

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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