Mechanisms and generational durability of clubroot resistance associated with stacked CR genes

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Resistance is the key to clubroot management

This canola cultivar carries a gene resistant to **pathotype 3** of *Plasmodiophora brassicae*
New *Pb* pathotypes overcame the resistance in commercial varieties (carry a single CR gene?)

Canola varieties resistant to pathotype 3
Some CR sources, mostly *B. rapa*, were identified against old *P. brassica* pathotypes found in Canada.

<table>
<thead>
<tr>
<th>Brassica spp.</th>
<th>Pathotype 2</th>
<th>Pathotype 3</th>
<th>Pathotype 5</th>
<th>Pathotype 6</th>
<th>Pathotype 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (S)</td>
<td>97 e #</td>
<td>99 f</td>
<td>99 d</td>
<td>98 e</td>
<td>98 e</td>
</tr>
<tr>
<td><em>B. nigra</em></td>
<td>0 a</td>
<td>0 a</td>
<td>0 a</td>
<td>1 a</td>
<td>0 a</td>
</tr>
<tr>
<td><em>B. nigra</em></td>
<td>40 d</td>
<td>52 e</td>
<td>39 c</td>
<td>46 d</td>
<td>44 d</td>
</tr>
<tr>
<td><em>B. oleracea</em></td>
<td>0 a</td>
<td>0 a</td>
<td>0 a</td>
<td>0 a</td>
<td>2 ab</td>
</tr>
<tr>
<td><em>B. oleracea</em></td>
<td>0 a</td>
<td>1 ab</td>
<td>1 a</td>
<td>3 ab</td>
<td>0 a</td>
</tr>
<tr>
<td><em>B. rapa</em></td>
<td>3 b</td>
<td>16 cd</td>
<td>18 b</td>
<td>16 bc</td>
<td>10 bc</td>
</tr>
<tr>
<td><em>B. rapa</em></td>
<td>17 c</td>
<td>18 d</td>
<td>25 b</td>
<td>26 c</td>
<td>19 c</td>
</tr>
<tr>
<td><em>B. rapa</em></td>
<td>6 b</td>
<td>5 bc</td>
<td>1 a</td>
<td>2 a</td>
<td>4 ab</td>
</tr>
<tr>
<td><em>B. rapa</em></td>
<td>1 a</td>
<td>1 ab</td>
<td>2 a</td>
<td>1 a</td>
<td>2 ab</td>
</tr>
<tr>
<td><em>B. rapa</em></td>
<td>35 d</td>
<td>43 e</td>
<td>46 c</td>
<td>55 d</td>
<td>56 d</td>
</tr>
<tr>
<td><em>B. rapa</em></td>
<td>43 d</td>
<td>54 e</td>
<td>49 c</td>
<td>48 d</td>
<td>40 d</td>
</tr>
</tbody>
</table>

# Average disease severity index

No single CR gene was effective against all new pathotypes.
Re-synthesizing amphidiploid CR canola species

<table>
<thead>
<tr>
<th>Species</th>
<th>Donor lines</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. napus</em></td>
<td><em>B. oleracea</em> <em>(Rcr7)</em> × <em>B. rapa</em> <em>(Rcr3)</em></td>
<td>Seeds</td>
</tr>
<tr>
<td><em>B. juncea</em></td>
<td><em>B. rapa</em> <em>(Rcr4, Rcr8 or Rcr9)</em> × <em>B. nigra</em> <em>(Rcr6)</em></td>
<td>Seeds</td>
</tr>
</tbody>
</table>

*B. oleracea* × *B. rapa* → *B. napus*
DH *B. napus* lines with 2 or 3 CR genes including *Rcr1* (A3), CRM (A3) and CRB (A8)

<table>
<thead>
<tr>
<th>Crossing</th>
<th>CR gene on</th>
<th># CR genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rcr1</em></td>
<td>A3</td>
<td>1</td>
</tr>
<tr>
<td>CRM</td>
<td>A3</td>
<td>1</td>
</tr>
<tr>
<td>CRB</td>
<td>A8</td>
<td>1</td>
</tr>
<tr>
<td>CRB x <em>Rcr1</em></td>
<td>A3, A8</td>
<td>2</td>
</tr>
<tr>
<td>CRM x CRB</td>
<td>A3, A8</td>
<td>2</td>
</tr>
<tr>
<td>CRB x CRM</td>
<td>A8, A3</td>
<td>2</td>
</tr>
<tr>
<td><em>Rcr1</em> x (CRM x CRB)</td>
<td>A3, A3, A8</td>
<td>3</td>
</tr>
</tbody>
</table>
Key research questions:

- Are these new canola varieties/lives with stacked CR genes effective against 5X (now X)?
- Will stacked CR genes provide more sophisticated resistance mechanisms?
- Will the resistance involving stacked CR genes be durable? (vs. CR gene rotation, to be worked on)
- Deployment strategies - more durable clubroot resistance
<table>
<thead>
<tr>
<th>Pb pathotype population</th>
<th>$A3_M \times A8$ (#5)</th>
<th>$A8 \times A3_1$ (#14)</th>
<th>$A8 \times A3_M$ (#15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X (L-G2)</td>
<td>Susceptible</td>
<td>Partially resistant</td>
<td>Partially resistant</td>
</tr>
<tr>
<td>5X (L-G3)</td>
<td>Susceptible</td>
<td>Partially resistant</td>
<td>Partially resistant</td>
</tr>
</tbody>
</table>
Resistance to pathotype 5X varied among double CR-gene lines resulting from reciprocal crosses.

*P. brassicae* 5x L-G2

*P. brassicae* 5x L-G3

(CRM x CRB)  (CRB x CRM)  (CRB x Rcr1)
I. Transcriptome analysis (RNA-seq): Canola lines with two stacked CR genes (A3, A8) against pathotype 5X
Transcriptome analysis of Rcr1 against pathotype 3 of P. brassicae: RNA-seq

- Regulation of primary metabolic process
- Anatomical structure development
- Cellular component organization

**Defense response**

**Up regulated pathways**
- Jasmonic acid/ethylene
- Deposition of callose

**Down regulation**
- Auxin biosynthesis
- Cell growth/development
Synchrotron-based Fourier transform infrared spectromicroscopy – cell wall modification

- Increased lignin & phenolic biosynthesis
- Supports RNA-seq: Callose deposition
- Via up-regulation of phenylpropanoid pathway – likely activated by *BrPAL1*
Infection can occur in partially resistant lines (A8 x A3, #14), but limited mostly to root hairs/epidermal cells.

*P. brassicae* zoospores in root hairs  
*P. brassicae* plasmodia in root epidermis (confocal)
Characterizing resistance based on epidermal and cortical infection (5X)

Nile red stains intracellular lipid droplets of *P. brassicae*
Resistance erosion

Repeated exposure to the same pathogen population showed reduced resistance on some varieties carrying a single CR gene, even in the 2\textsuperscript{nd} generational cycle.
Mix resting spore into soil (1E +7/g) 
(1st cycle only)

2. Generational resistance durability (repeated exposure)

Seeding

Resting spore quantified (qPCR) on each rep before each cycle

Allow 3 wks for galls to mature before planting

Galls go back to original soil

Cut galls

Repeating in 5 cycles

Clubroot rating 
(6 weeks post inoculation)

Three replicated “tubs” per treatment in each generational cycle

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Disease Severity Index (%) for canola lines carrying single- or multi-CR genes exposed to pathotype 5X (L-G3) in 5 generational rounds.

The disease severity index on Westar was 100%.
Canola lines carrying two CR genes (A8/A3) exposed to the 5X population L-G3 in 5 generational cycles
Canola line carrying *Rcr1* (A3) alone exposed to 5X L-G3 in five generational cycles.
Canola line with CRB (A8) exposed to the same pathotype 5X (L-G3) in five generational cycles
Resting-spore concentration when clubroot galls were all recycled back into the soils continuously

Double genes - CRB (A8)/Rcr1 (A3)

Double genes - CRB (A8)/CRM (A3)
Single CR gene: CRB (A8)

Single CR gene: Rcr1 (A3)
Summary

- CR genes on A3 or A8 are effective against pathotypes 2, 3, 5, 6 and 8 of P. brassicae.

- Stacking these CR genes in certain ways may result in moderate resistance to P. brassicae pathotype 5X.

- This moderate-level resistance appears stable under repeated exposure to a field population of 5X (L-G3).

- The stable resistance coincided with a P. brassicae 5X inoculum decline over the generational cycles.
Acknowledgement

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Collaborators
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• PSI – Quantifying Pb inoculum in soil

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