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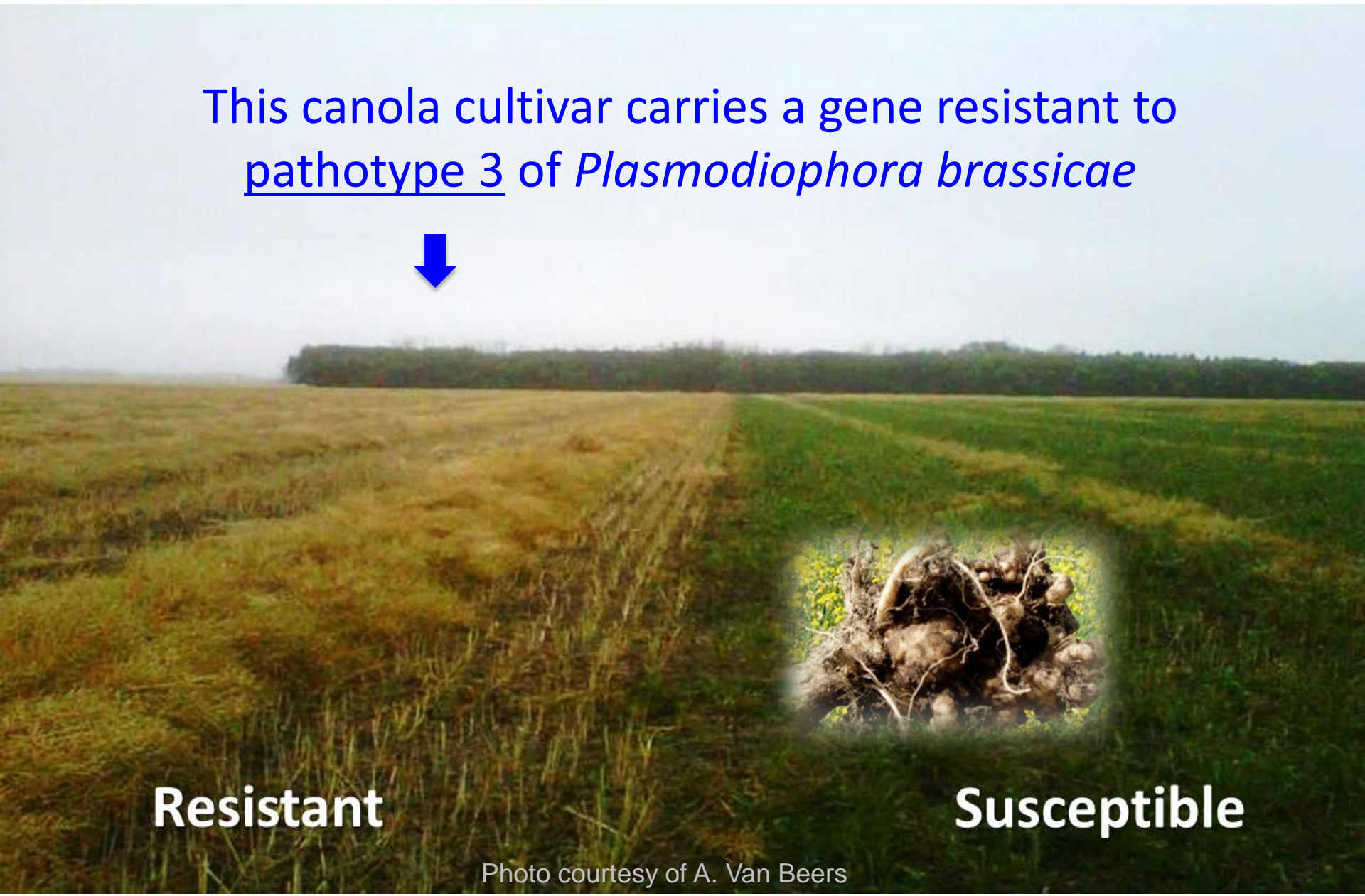
# **Mechanisms and generational durability of clubroot resistance associated with stacked CR genes**

*Song T, Tonu M, Wen R, Yu F, Peng G*



# Resistance is the key to clubroot management

This canola cultivar carries a gene resistant to pathotype 3 of *Plasmodiophora brassicae*



**Resistant**



**Susceptible**

# New *Pb* pathotypes overcame the resistance in commercial varieties (carry a single CR gene?)

5x L-G2



5x L-G3



cv. W

cv. X

cv. Y

cv. Z

Canola varieties resistant to pathotype 3

Some CR sources, mostly *B. rapa*, were identified against old *P. brassica* pathotypes found in Canada

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

# Average disease severity index

**No single CR gene was effective against all new pathotypes**



# Re-synthesizing amphidiploid CR canola species

Species	Donor lines	Stage
<i>B. napus</i>	<i>B. oleracea</i> ( <i>Rcr7</i> ) x <i>B. rapa</i> ( <i>Rcr3</i> )	Seeds
<i>B. juncea</i>	<i>B. rapa</i> ( <i>Rcr4</i> , <i>Rcr8</i> or <i>Rcr9</i> ) x <i>B. nigra</i> ( <i>Rcr6</i> )	Seeds

*B. oleracea* x *B. rapa*



x *B. napus*

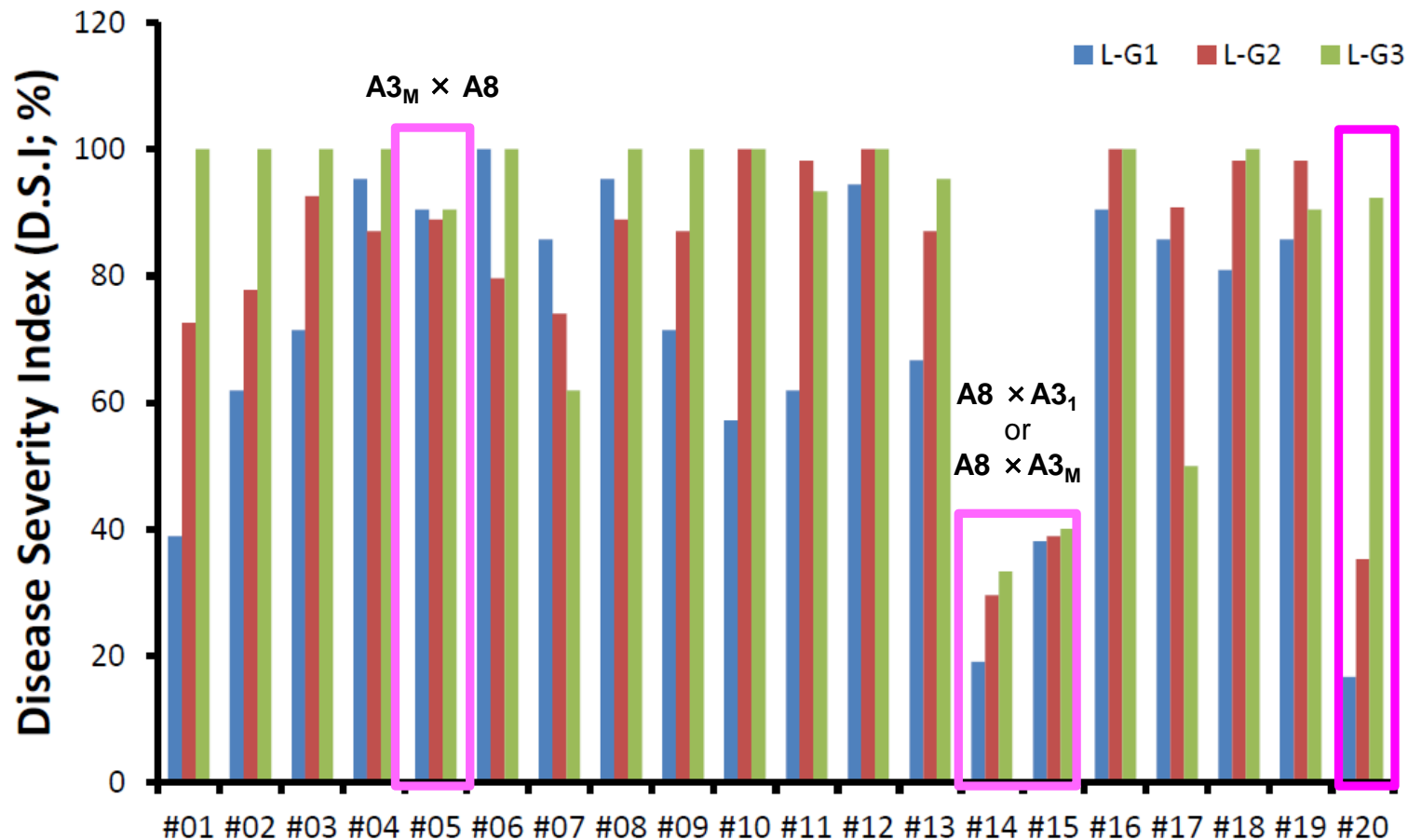


**DH *B. napus* lines with 2 or 3 CR genes including *Rcr1* (A3), CRM (A3) and CRB (A8)**

Crossing	CR gene on	# CR genes
<i>Rcr1</i>	A3	1
CRM	A3	1
CRB	A8	1
CRB x <i>Rcr1</i>	A3, A8	2
CRM x CRB	A3, A8	2
CRB x CRM	A8, A3	2
<i>Rcr1</i> x (CRM x CRB)	A3, A3, A8	3

# Key research questions:

- ❖ Are these new canola varieties/lines 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



<i>Pb</i> pathotype population	$A3_M \times A8$ (# 5)	$A8 \times A3_1$ (#14)	$A8 \times A3_M$ (#15)
5X (L-G2)	Susceptible	<b>Partially resistant</b>	<b>Partially resistant</b>
5X (L-G3)	Susceptible	<b>Partially resistant</b>	<b>Partially resistant</b>



# Resistance to pathotype **5X** varied among double CR-gene lines resulting from reciprocal crosses

*P. brassicae*  
**5x L-G2**



*P. brassicae*  
**5x L-G3**

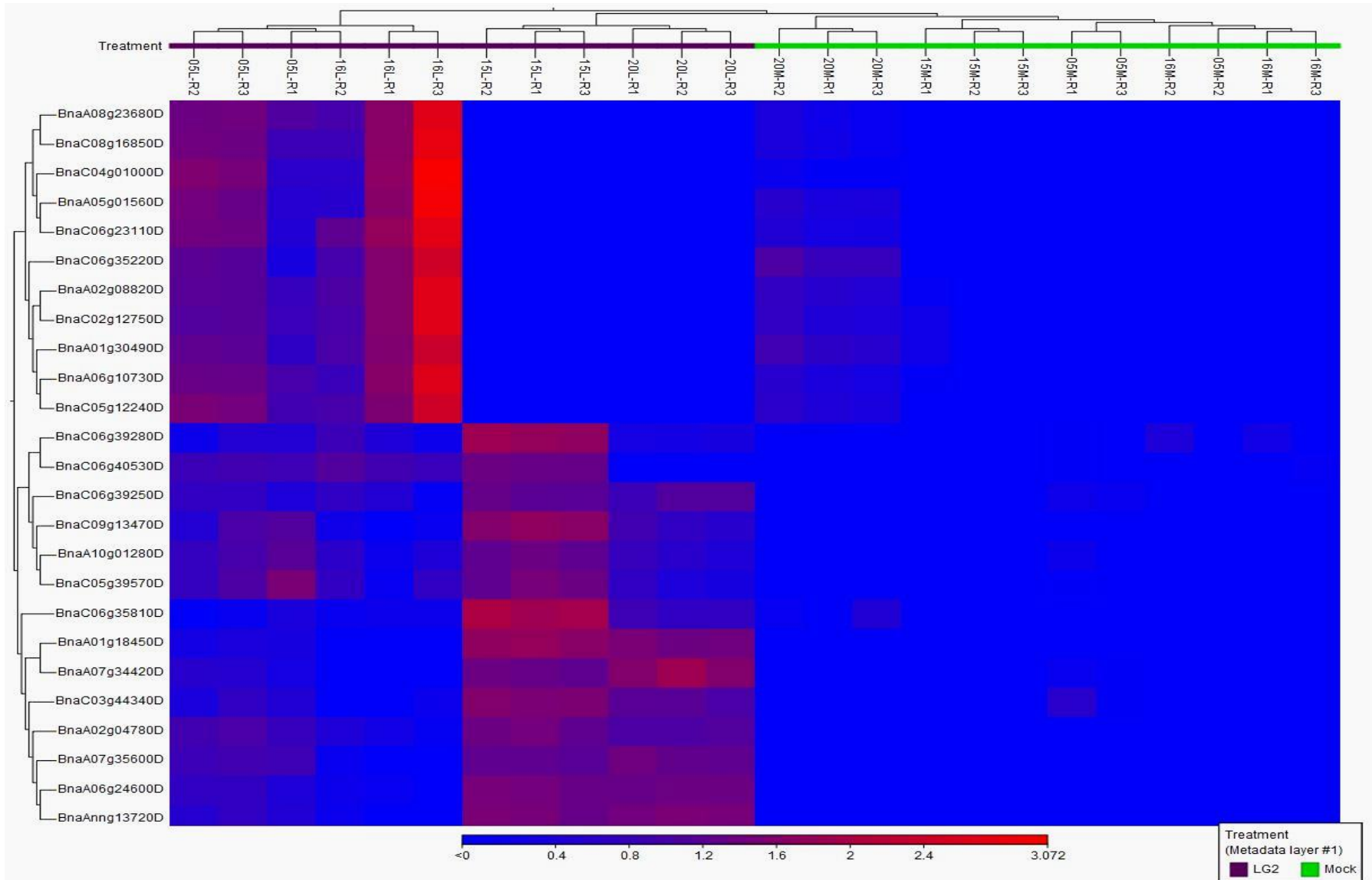


**(CRM x CRB)**

**(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

Chu et al. BMC Genomics 2014, 15:1166  
<http://www.biomedcentral.com/1471-2164/15/1166>

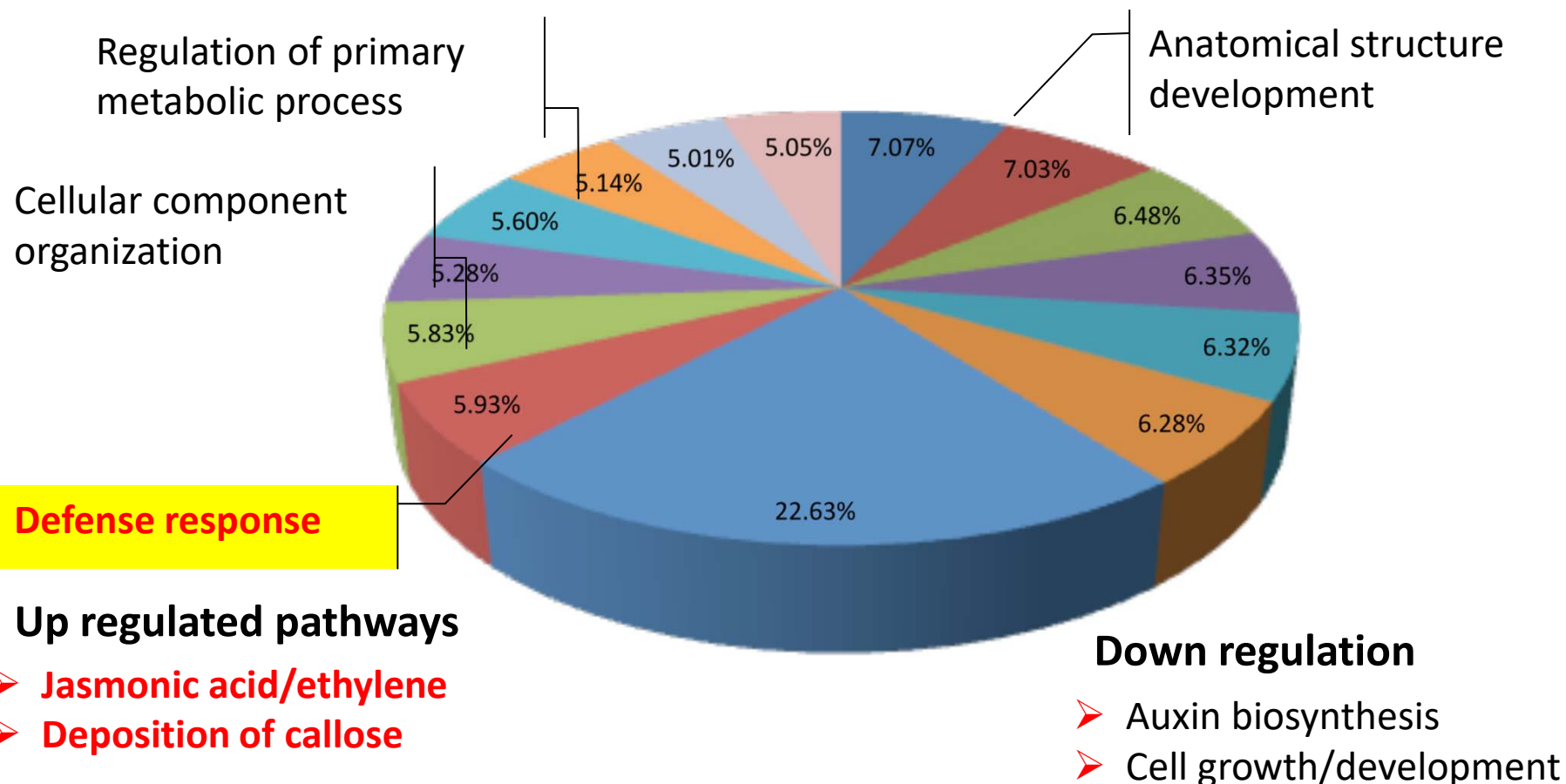


## RESEARCH ARTICLE

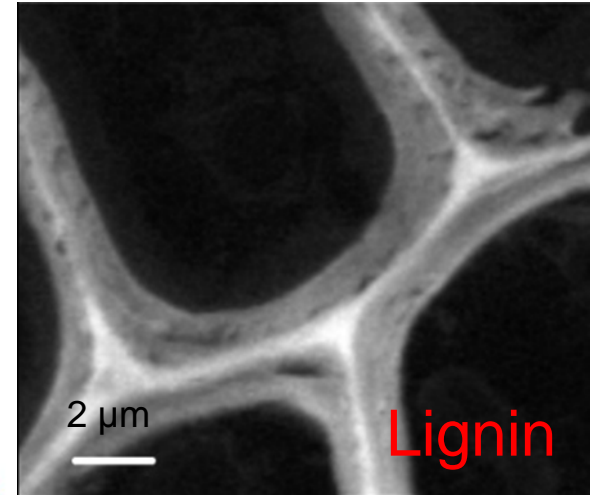
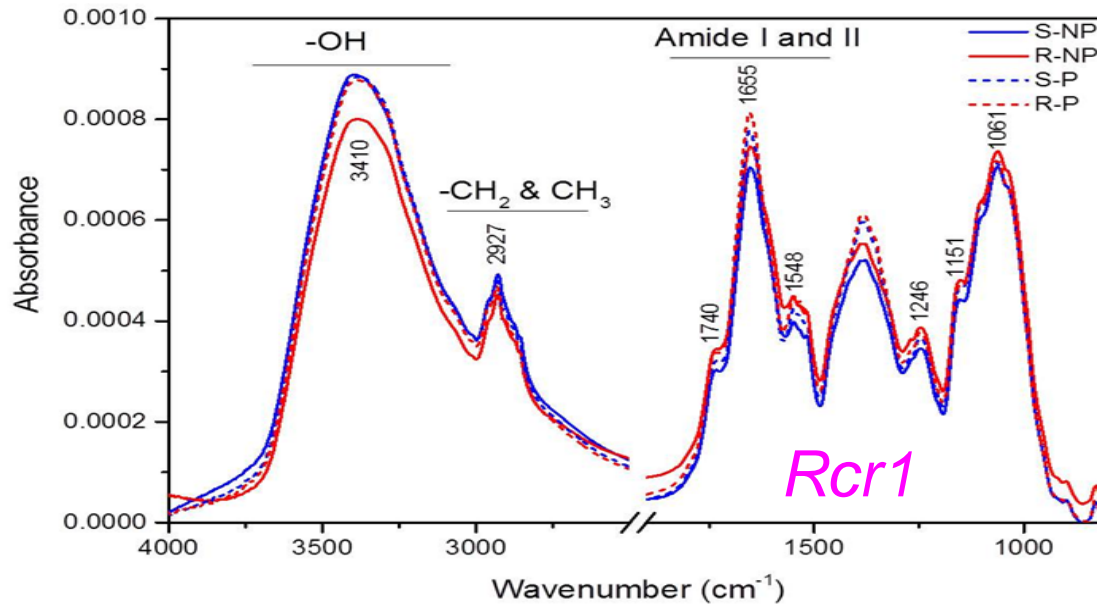
Open Access

Fine mapping of *Rcr1* and analyses of its effect on transcriptome patterns during infection by *Plasmodiophora brassicae*

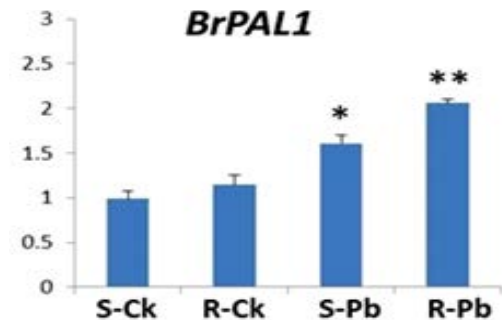
Mingguang Chu<sup>†</sup>, Tao Song<sup>†</sup>, Kevin C Falk, Xingguo Zhang, Xunjia Liu, Adrian Chang, Rachid Lahlali, Linda McGregor, Bruce D Gossen, Fengqun Yu<sup>\*</sup> and Gary Peng<sup>\*</sup>



# 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*



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Molecular Sciences 2018



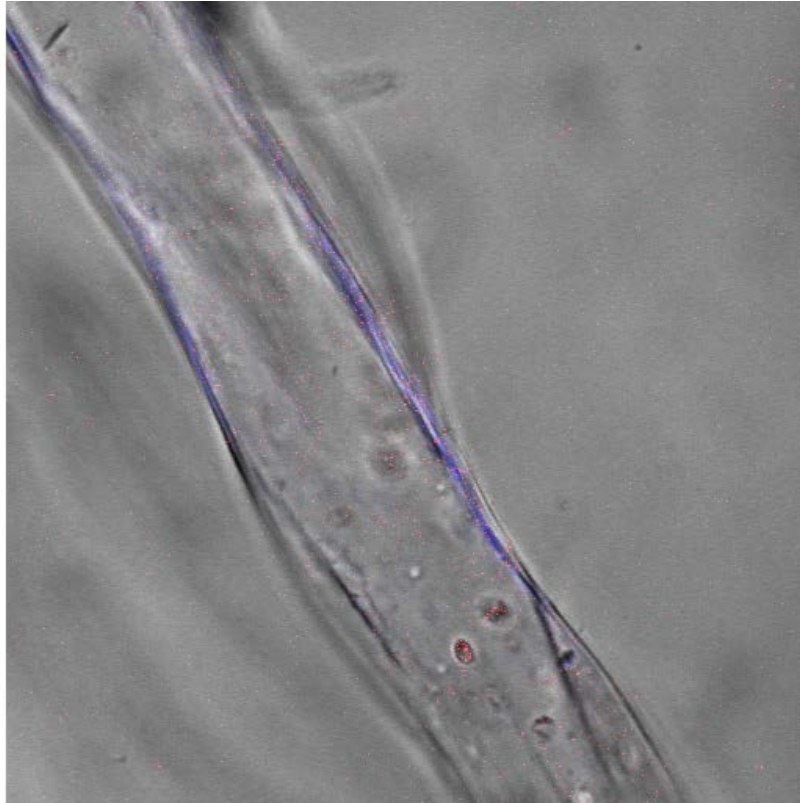
Article

Evaluating Changes in Cell-Wall Components Associated with Clubroot Resistance Using Fourier Transform Infrared Spectroscopy and RT-PCR

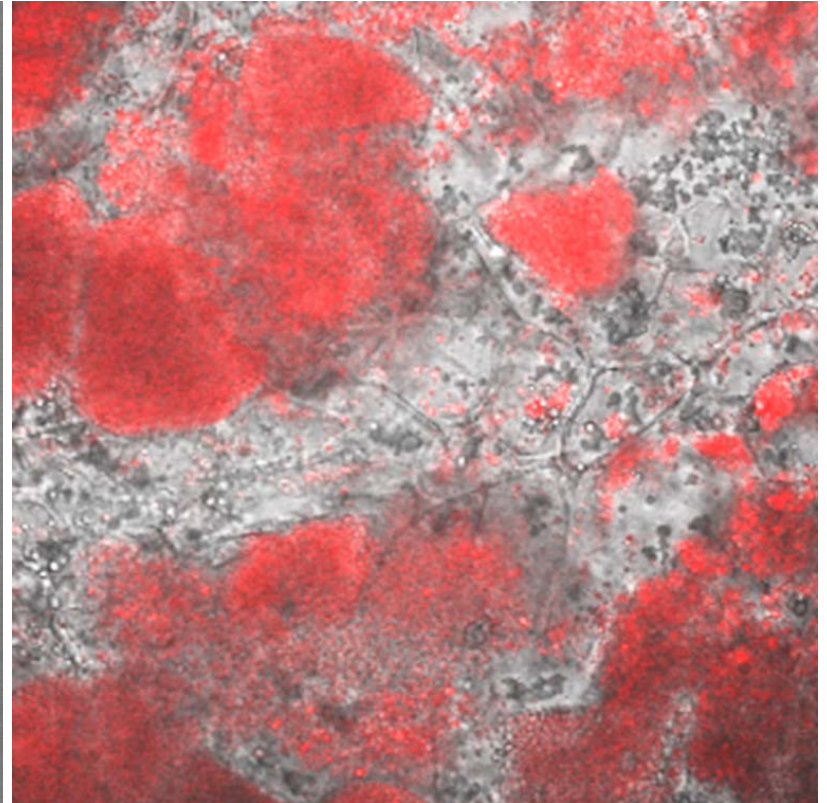
Rachid Lahlali <sup>1,2</sup>, Tao Song <sup>3</sup>, Mingguang Chu <sup>3</sup>, Fengqun Yu <sup>3</sup>, Saroj Kumar <sup>1,4</sup>, Chithra Karunakaran <sup>1</sup> and Gary Peng <sup>3,\*</sup>



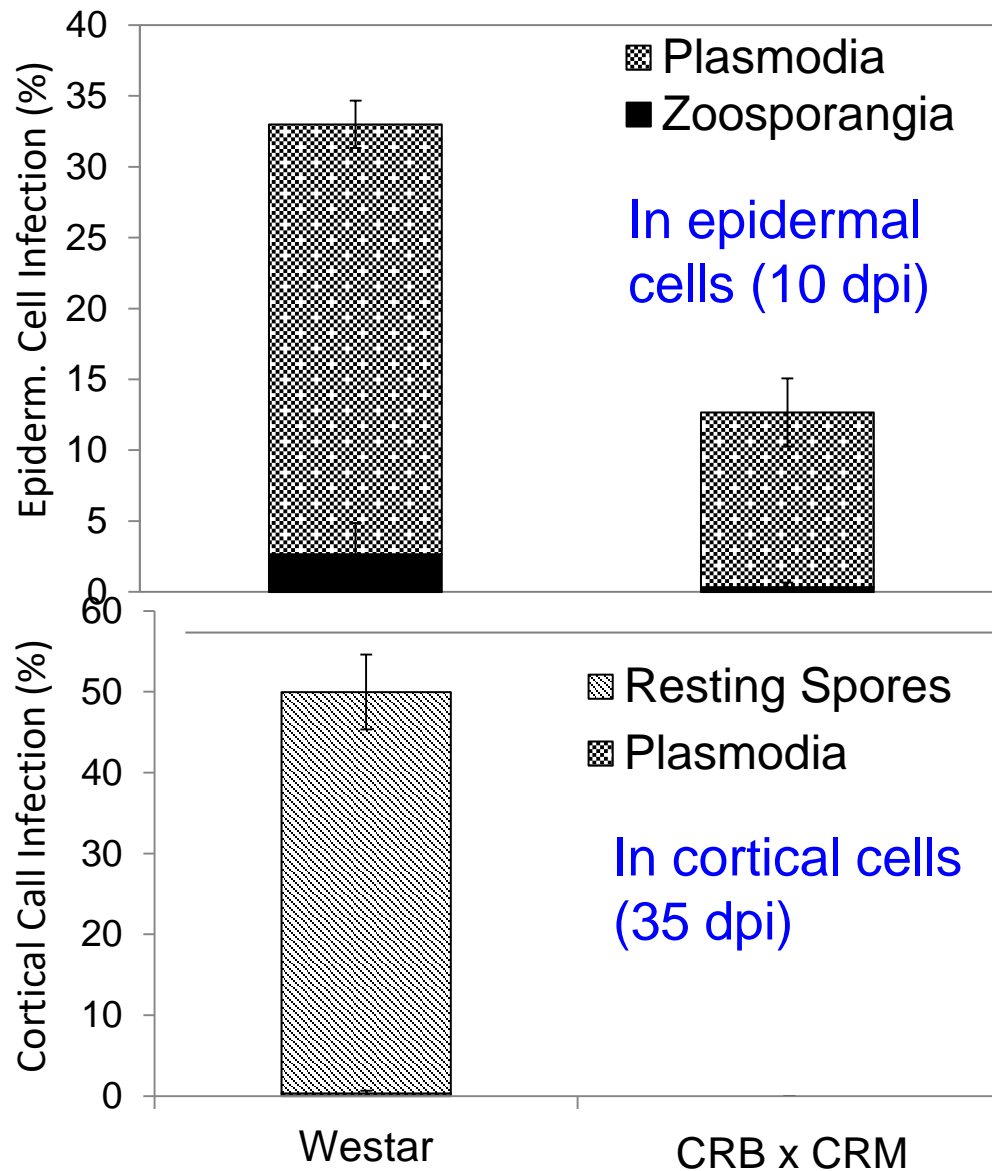
Infection can occur in partially resistant lines (A8 x A3, #14), but limited mostly to root hairs/epidermal cells



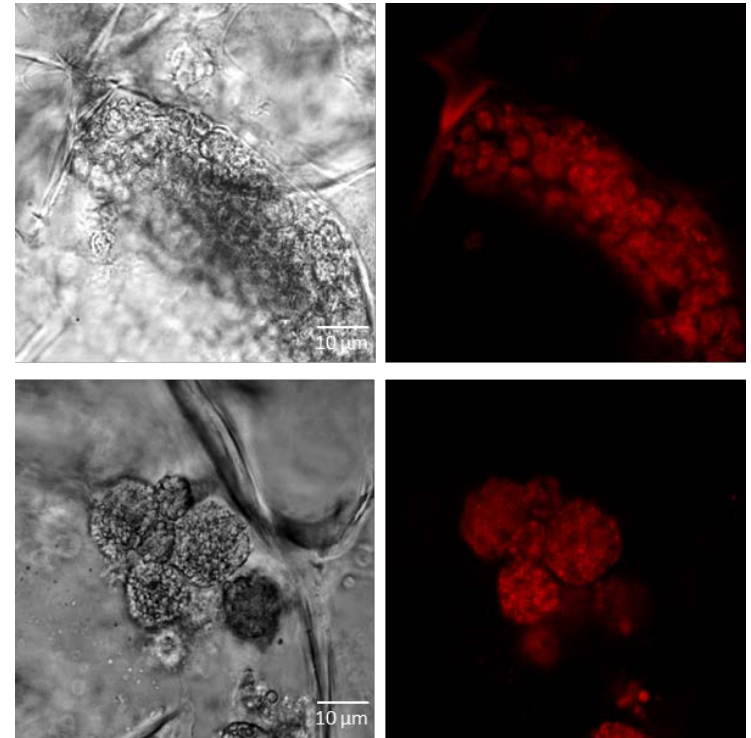
*P. brassicae* zoospores in root hairs



*P. brassicae* plasmodia in root epidermis (confocal)



Characterizing resistance based on epidermal and cortical infection (5X)



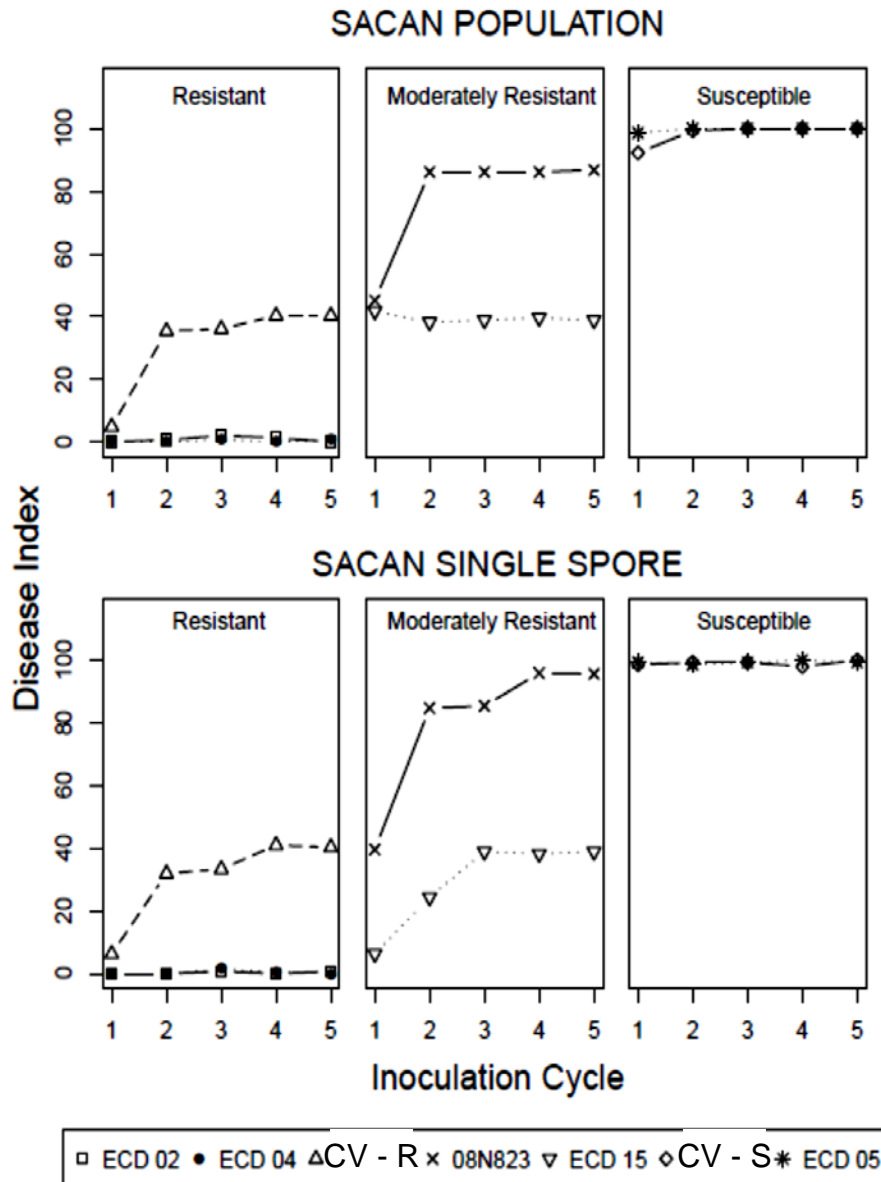
Nile red stains intracellular lipid droplets of *P. brassicae*

# The durability of clubroot resistance

*LeBoldus et al. (2012)*

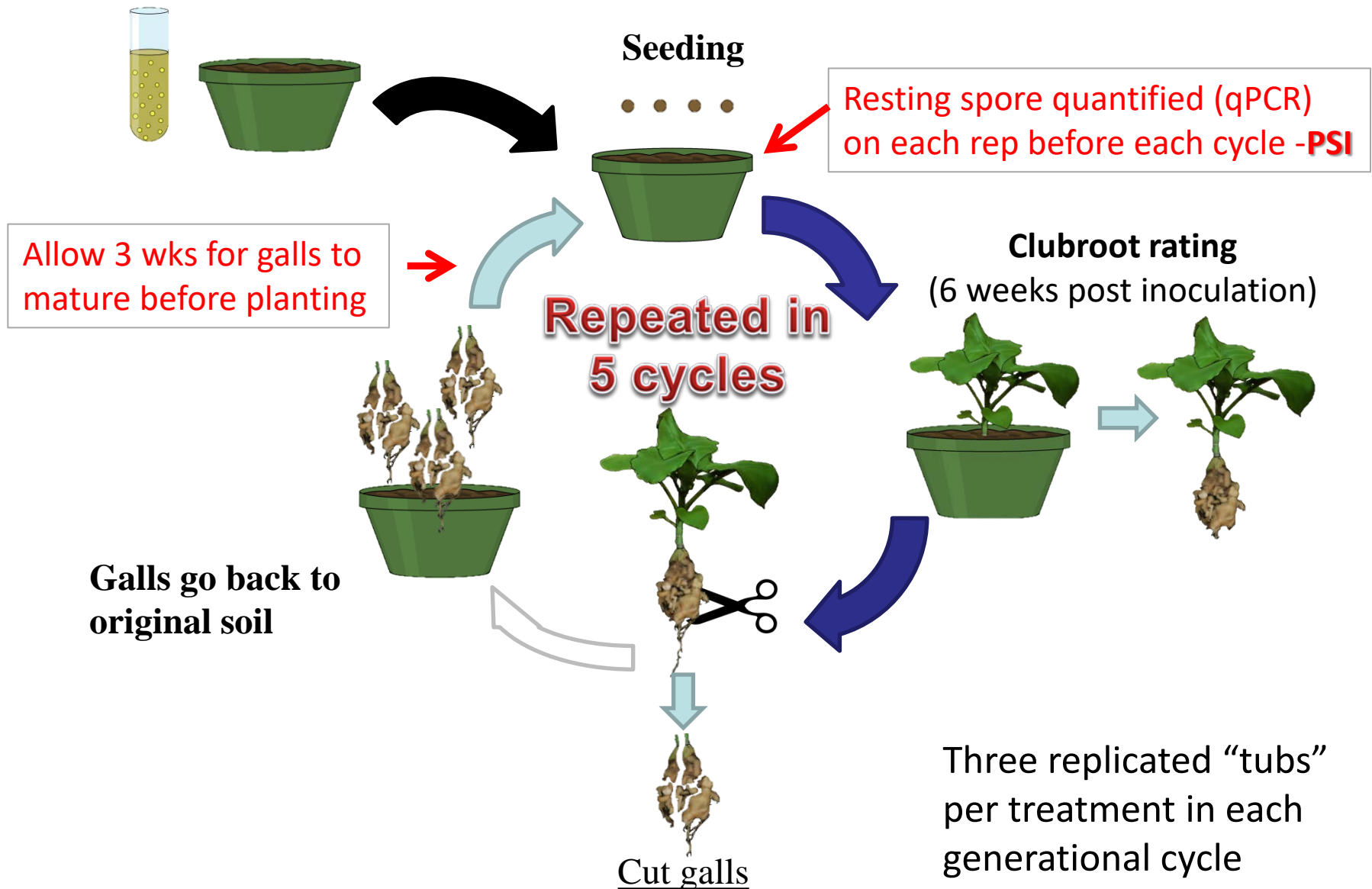
## Resistance erosion

Repeated exposure to the same pathogen population showed reduced resistance on some varieties carrying a single CR gene, even in the 2<sup>nd</sup> generational cycle.



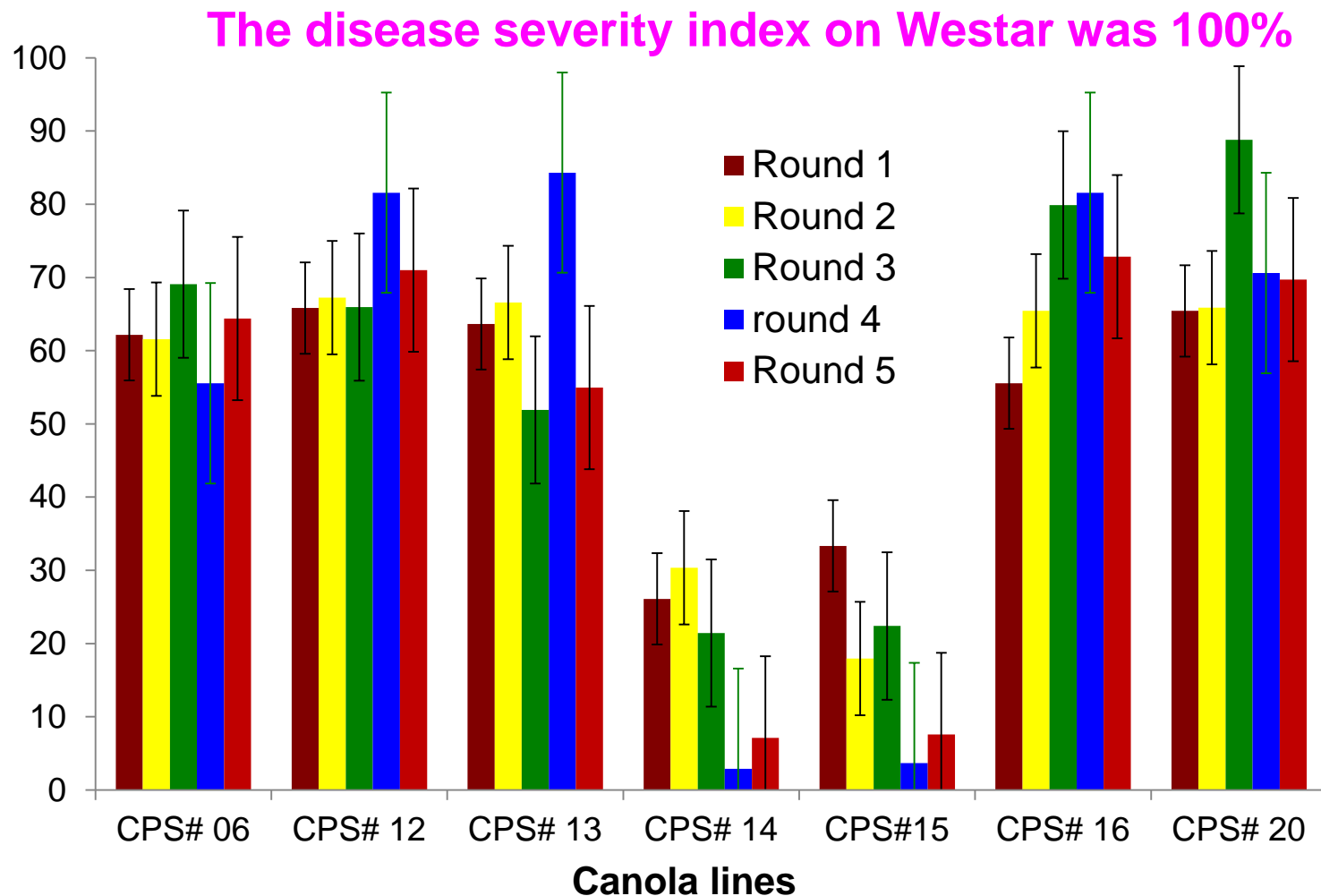
Mix resting spore into soil (1E +7/g)  
(1<sup>st</sup> cycle only)

## 2. Generational resistance durability (repeated exposure)

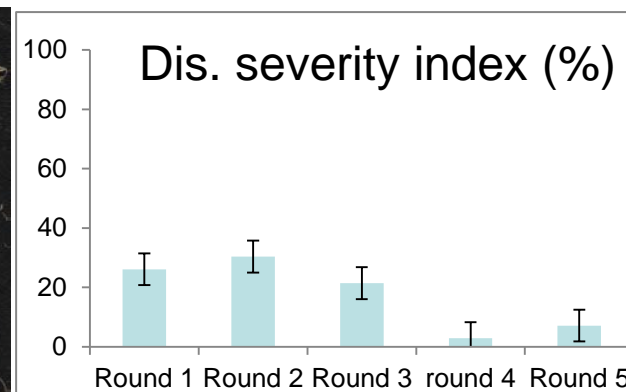




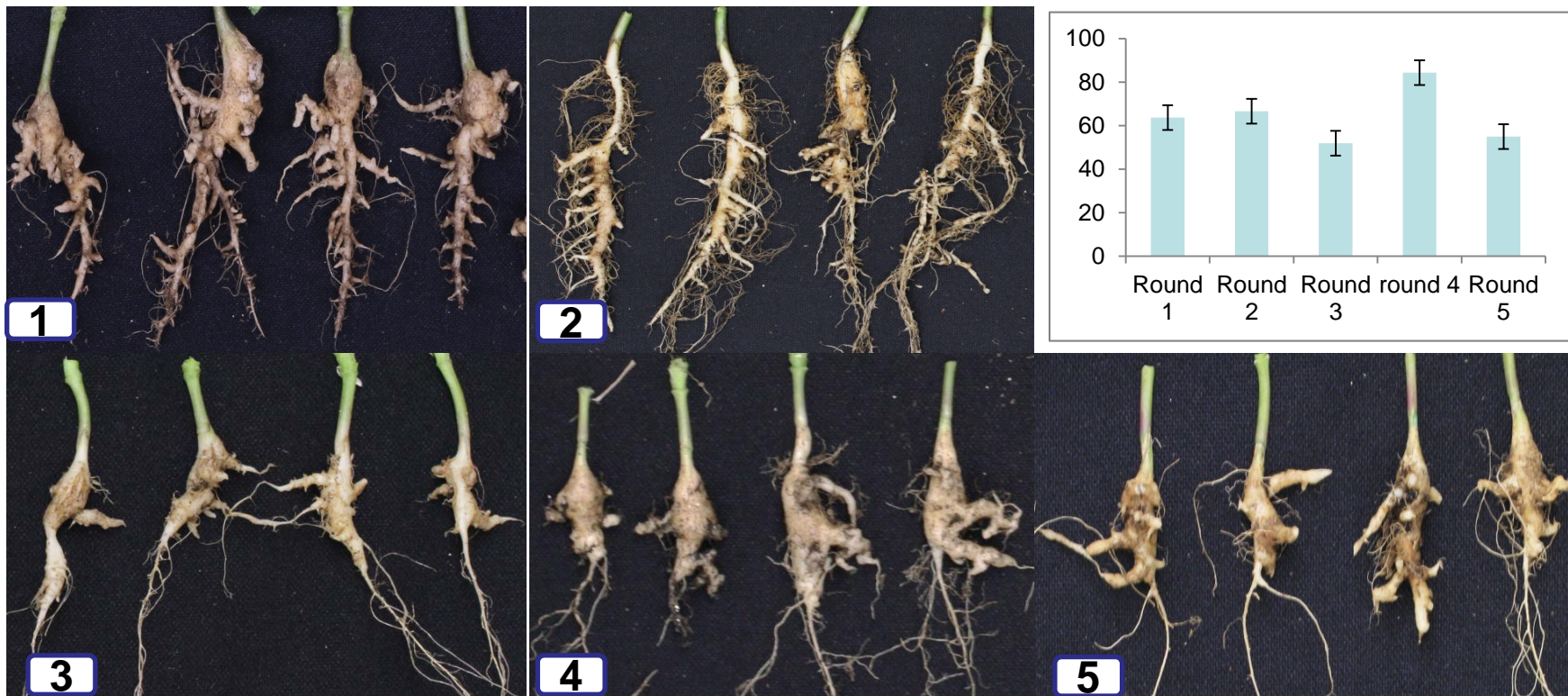
# Disease Severity Index (%) for canola lines carrying single- or multi-CR genes exposed to pathotype 5X (L-G3) in 5 generational rounds



# Canola lines carrying two CR genes (**A8/A3**) exposed to the **5X** population L-G3 in 5 generational cycles

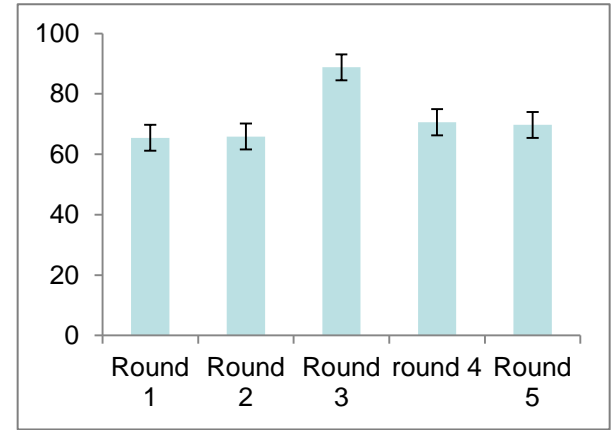


# Canola line carrying *Rcr1* (A3) alone exposed to **5X** L-G3 in five generational cycles



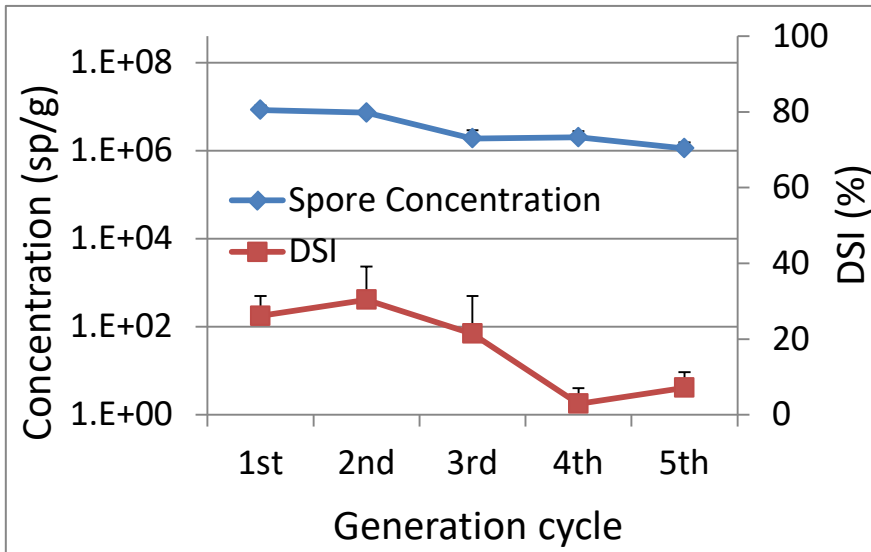


# Canola line with CRB (A8) exposed to the same pathotype 5X (L-G3) in five generational cycles

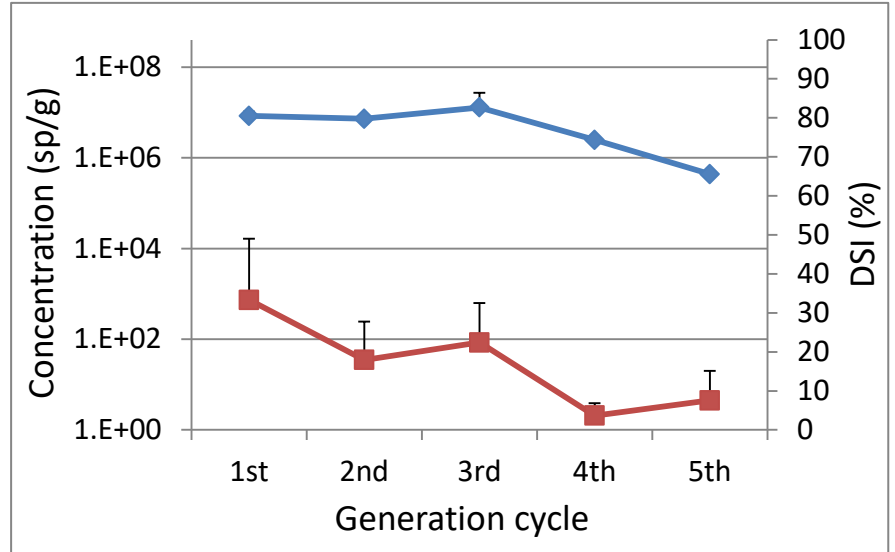
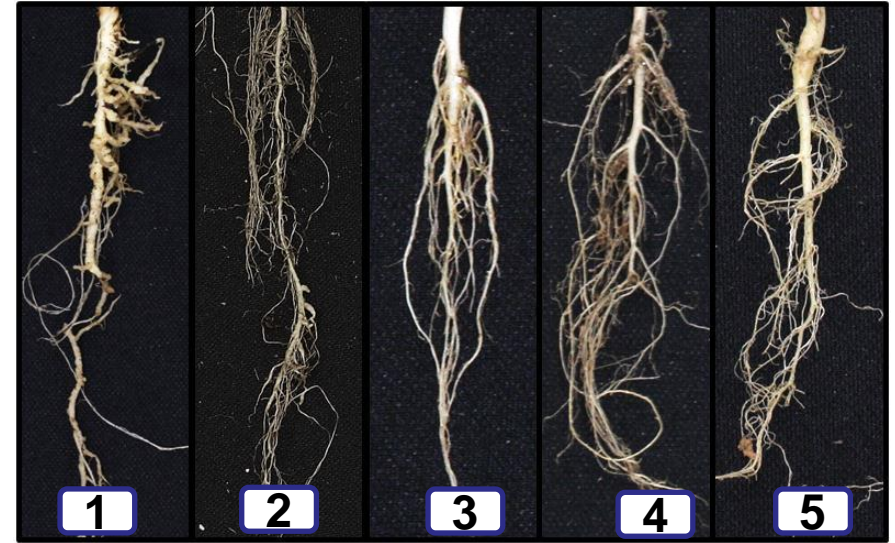




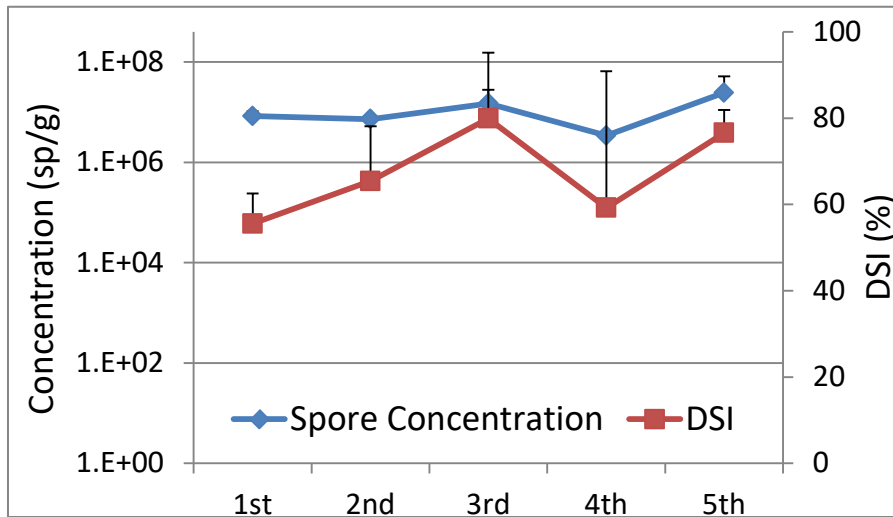
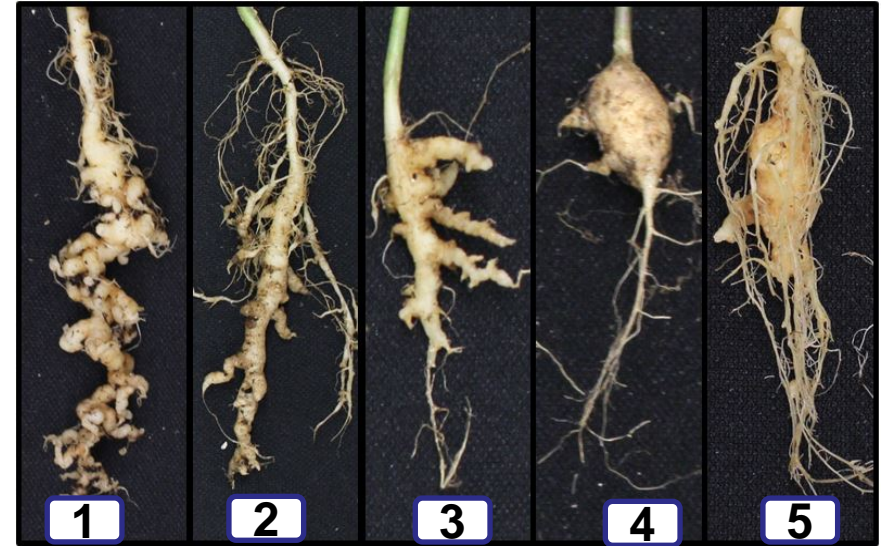
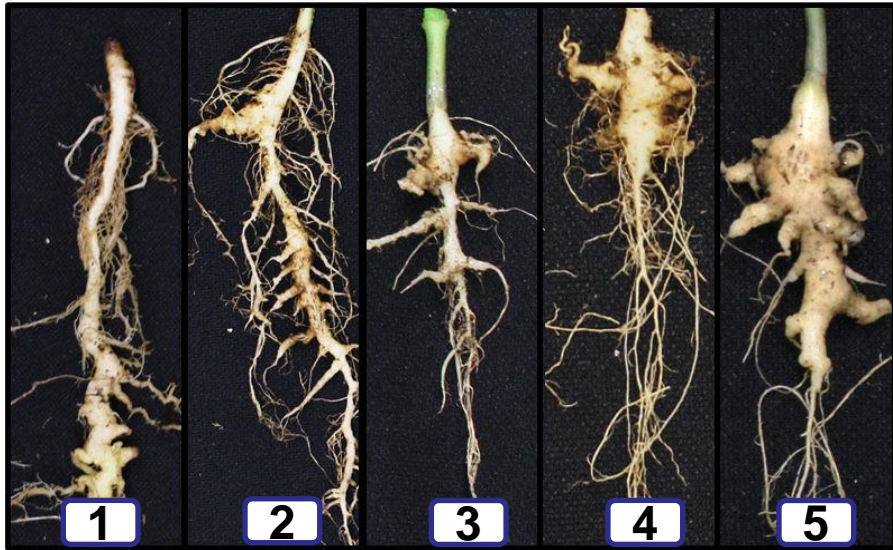
# Resting-spore concentration when clubroot galls were all recycled back into the soils continuously



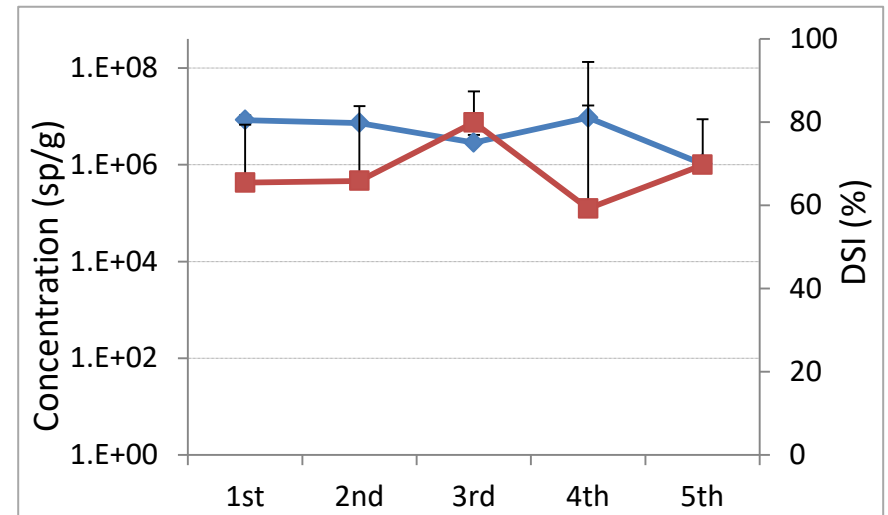
Double genes -CRB (A8)/Rcr1 (A3)



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

## Technical assistance      Collaborators

Hornaday K, Lee J, Bush J,  
McGregor L

- Franke C –Nutrin Ag Solutions
- PSI –Quantifying Pb inoculum in soil

Funding support: SaskCanola, WGRF

A photograph of a light green textured surface with a yellow sticky note attached to it. The sticky note has the words "THANK You!" written on it in a casual, handwritten style. The word "THANK" is in all caps and "You!" is in title case with an exclamation mark.

THANK  
You!