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Mechanisms and generational durability of clubroot resistance mediated by two genes

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Genetic resistance: Key to clubroot management

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



Resistant

Susceptible

Photo courtesy of A. Van Beers

“New” *Pb* pathotypes: highly virulent to most of resistant canola cultivars

Inoculation with the new pathotype 5X (5X)



cv. X

cv. Y

cv. Z

Canola varieties resistant to pathotype 3H

The loss of resistance happened within 3-4 years; so possibly it was similar single CR gene(s) in these cultivars ...

DH *B. napus* lines carrying 2 resistance genes, *Rcr1* (A3) and CRB (A8) were produced

Lines	Crossing	CR gene on	# CR genes
CPS13	<i>Rcr1</i> x <i>Rcr1</i>	A3	1
CPS20	CRB x CRB	A8	1
CPS14	CRB x <i>Rcr1</i>	A8, A3	2

Inoculated
with 5X



(A8)

(A3)

(A8/A3)

By Nutrien Ag Solutions

Research objectives:

- ❖ Understand resistance mechanisms with two stacked CR genes against **5X** vs single CR genes.
- ❖ Will the resistance involving two CR genes be durable against **5X** (MR) or **3H** (R)?
- ❖ Deployment strategy for CR durability

Mechanisms of two CR genes against 5X -transcriptome analysis

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

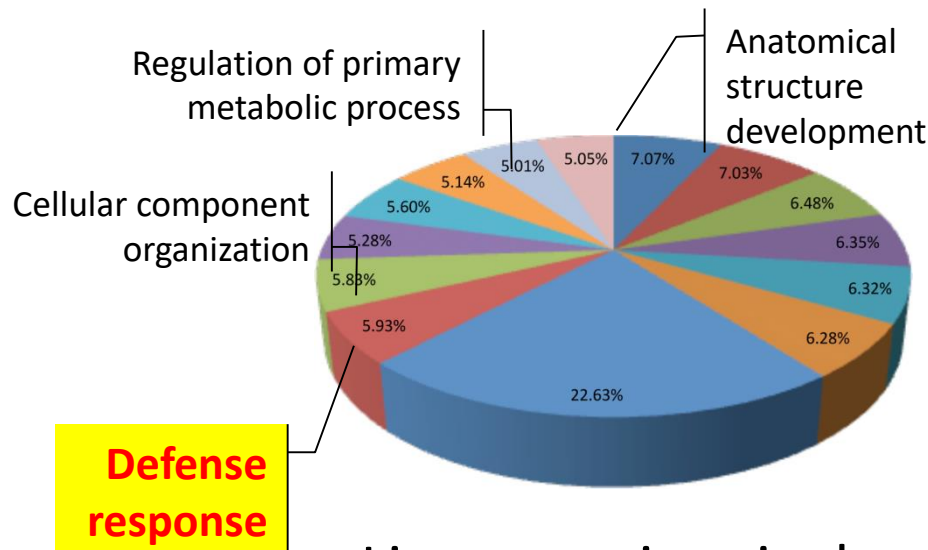


RESEARCH ARTICLE

Open Access

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

Mingguang Chu[†], Tao Song[†], Kevin C. Falk, Xingguo Zhang, Xunjia Liu, Adrian Chang, Rachid Lahlali, Linda McGregor, Bruce D. Gossen, Fengqun Yu[†] and Gary Peng^{*}



Differentially expressed genes (DEGs, up or down regulation) in R & S plants

Defense-related DEGs and biological processes –bioinformatics

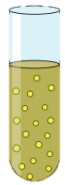
Lines carrying single vs. two CR genes against 5X

Pathway	DEG	CPS15 (A8/A3)	CPS16 (A3)	CPS20 (A8)
PTI	Receptor-like protein 12	760.2	2.1	36.6
	Wrky transcription factor 70	177.2	2.7	30.6
	Wrky transcription factor 51 isoform x 1	43.0	1.7	7.5
	CRR-like protein kinase 5	18.8	3.3	4.8
ETI	Probable disease resistance protein 1	85.6	1.6	5.9
	Probable disease resistance protein 2	23.0	3.7	5.1
	Enhanced disease susceptibility 1	44.9	1.4	13.1

Generational CR durability

(repeated exposure) -3 reps, 2 repeats

Pb resting spores ($10^7/g$)
(1st cycle only)

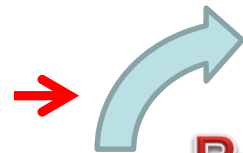


Seeding



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

Repeated in
5 cycles



Clubroot rating

(6 weeks post inoculation)



Cut galls

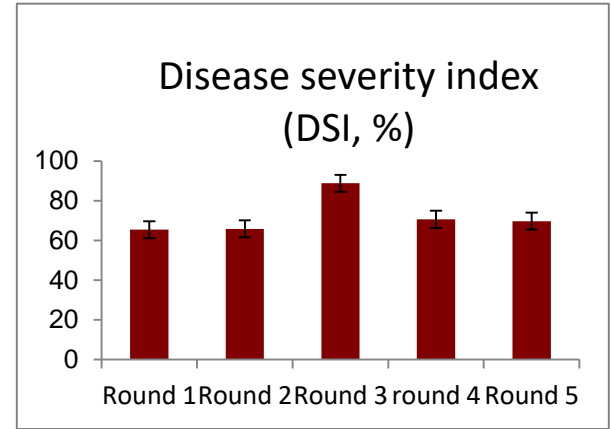
P. brassicae inoculation

1. **5X** (variant -new) –Experiment 1
2. **3H** (common) –Experiment 2
-Low ($10^4/g$) -High ($10^7/g$)

CR canola lines

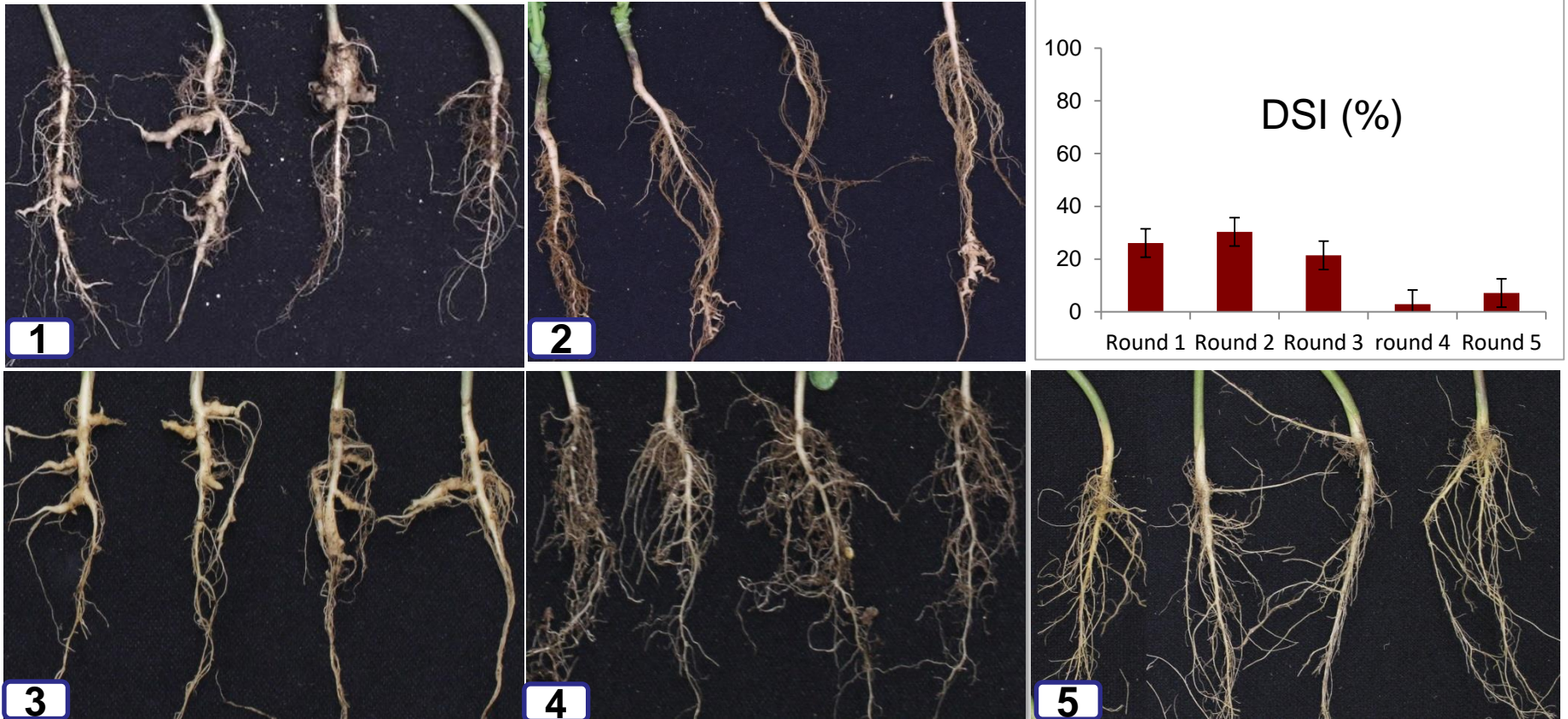
1. CPS13 (*Rcr1*, A3)
2. CPS20 (*CRB*, A8)
3. CPS 15 (*CRB/Rcr1*, A8/A3)

Canola line carrying *CRB* (A8) exposed to 5X for five “generation cycles” in controlled environment



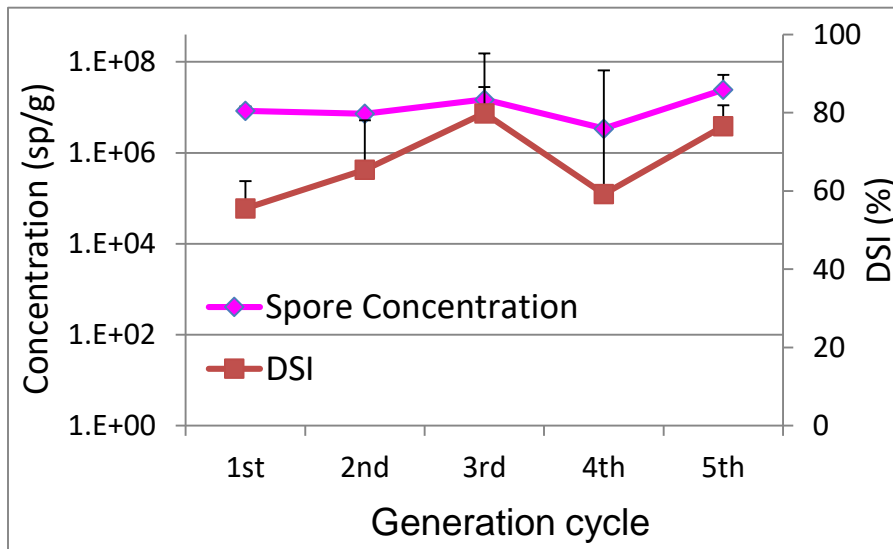
The canola line carrying *CRB* (A8) or *Rcr1* (A3, data not shown) was susceptible with >60% DSI in each of the 5 generational cycles

Canola line carrying two CR genes (A8/A3) exposed to 5X for 5 “generational cycles”

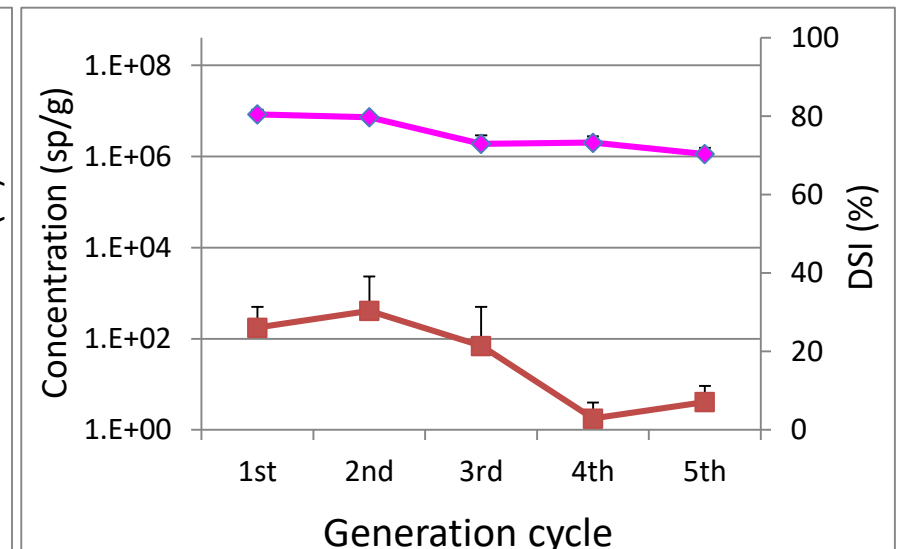


There was no increase in DSI after 5 generational cycles of exposure to the same 5X population.

Lines with two CR genes (**A8/A3**) and moderate CR also reduced **5X** inoculum (qPCR quantified) in the generational cycles

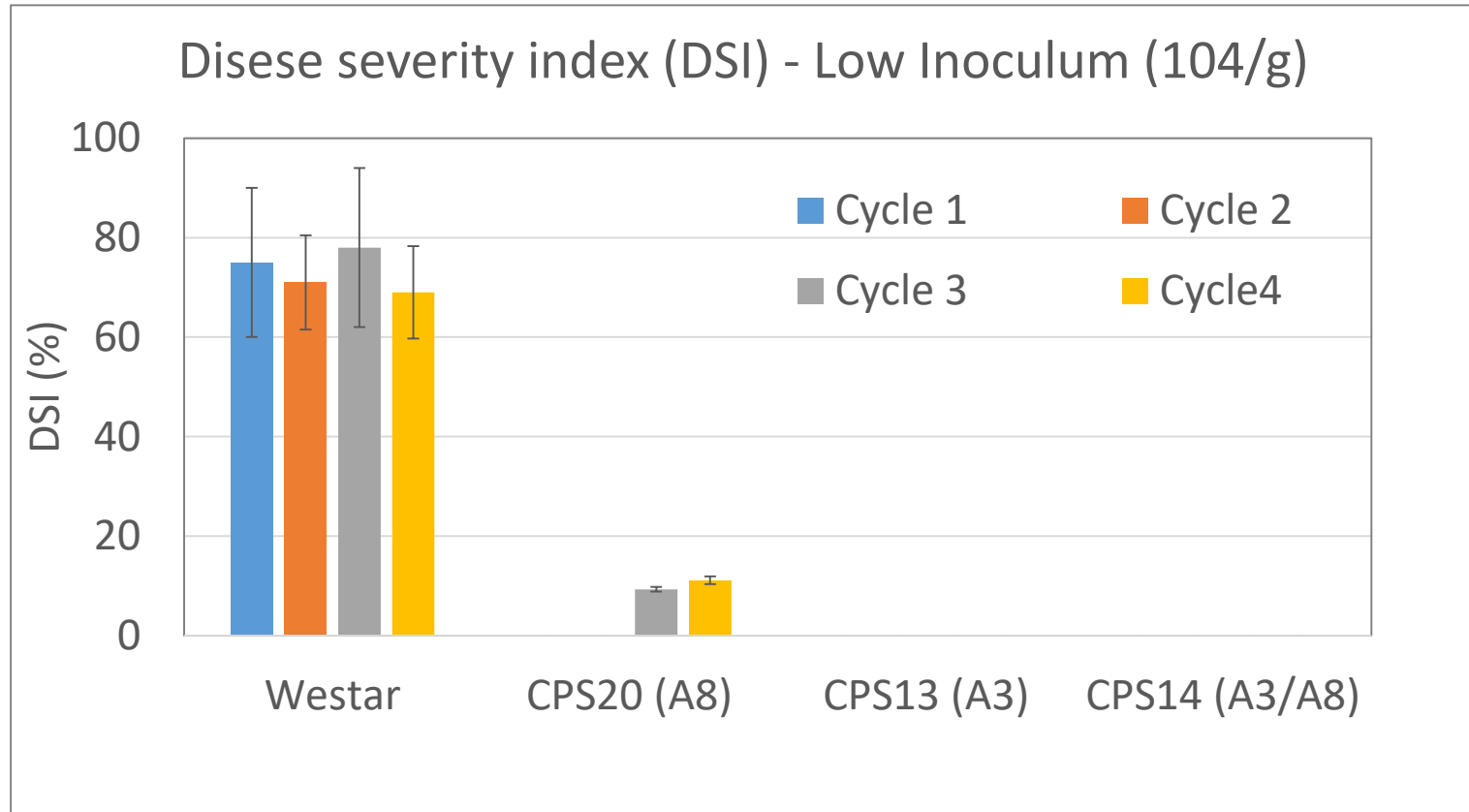


Single CR gene: (**A8**)



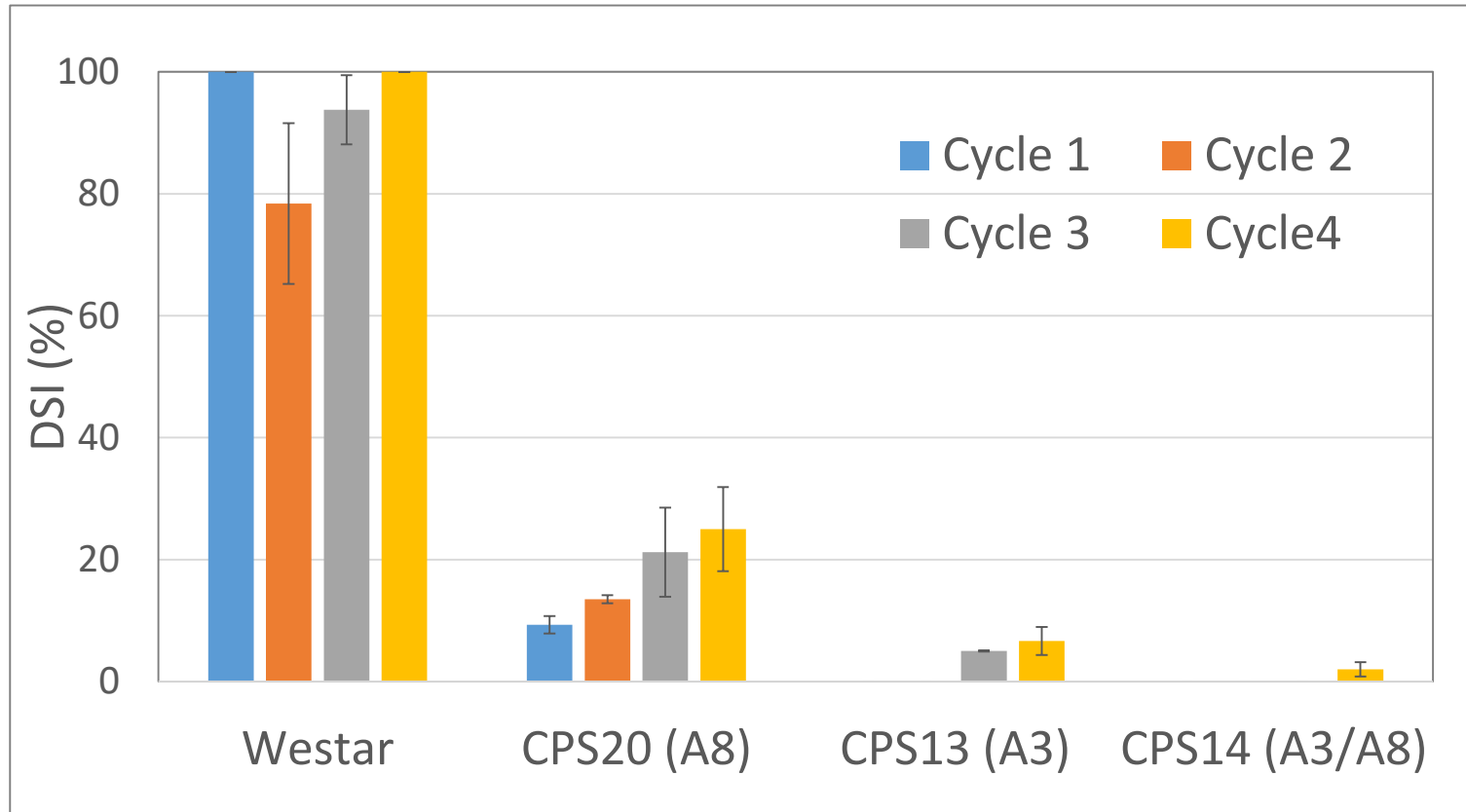
Two CR genes: (**A8/A3**)

Lines with single (*CRB*, **A8; *Rcr2*, **A3**) and double (**A8/A3**) CR genes exposed to low inoculum ($10^4/g$) of **3H** for 4 “generational cycles”**



Canola line carrying double CR genes (**A8/A3**) or single CR gene on **A3** showed no sign of resistance erosion, while the line carrying the single CR gene on **A8** showed low DSI in 3rd and 4th generation cycles

Lines with single (*CRB*, **A8**; *Rcr2*, **A3**) and double (**A8/A3**) CR genes exposed to high inoculum ($10^7/g$) of **3H** for 4 “generation cycles”



Canola lines carrying a single CR gene on **A3** or **A8** showed low DSI from 1st and 3rd generation cycles, respectively, while the line carrying double CR genes (**A8/A3**) showed low DSI and only in 4th cycle.

The 5th generation is being completed

Conclusions

- ❖ Stacking two CR genes (**A8, A3**) in canola varieties resulted in moderate resistance to **5X**
- ❖ This resistance to a field population of **5X** appeared stable during repeated exposure
- ❖ Against the common **3H**, CR lines were all effective initially, but resistance seemed to erode with repeated exposure, especially under high inoculum pressure for lines with a single CR gene (more so for **A8**)

Acknowledgement

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Canola lines carrying different CR genes are produced
by Nutrien Ag Solutions, Saskatoon

