Genomic tools for the management of clubroot of canola (*Brassica napus*)

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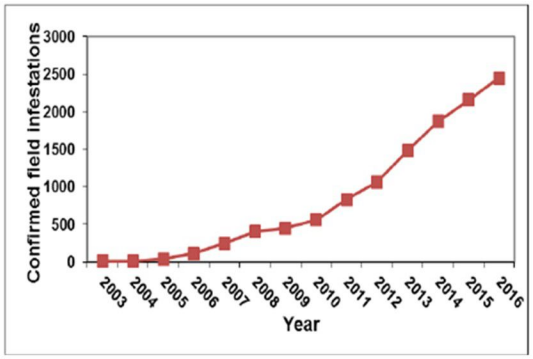
Clubroot disease is caused by P. brassicae and affects the 26.7 billion canola industry in Canada.

Clubroot infestations

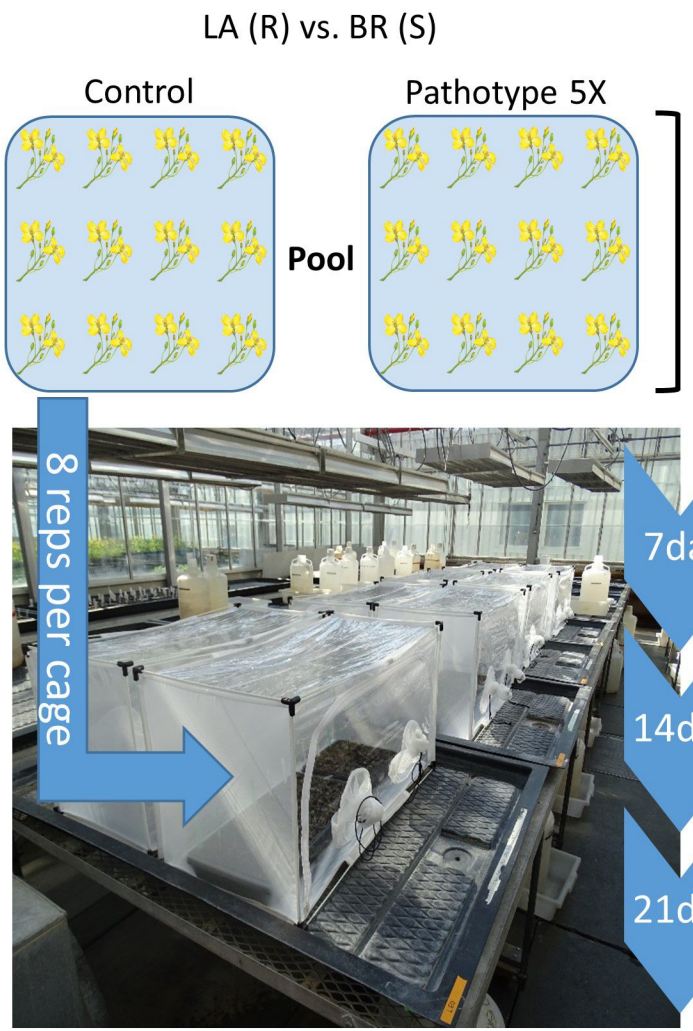
P. Brassicae has spread rapidly for a soilborne pathogen.

Currenty there are over 3000 cases across Alberta.

Resistance was broken by pathotype 5x.



courtesy of: Stephen Strelkov



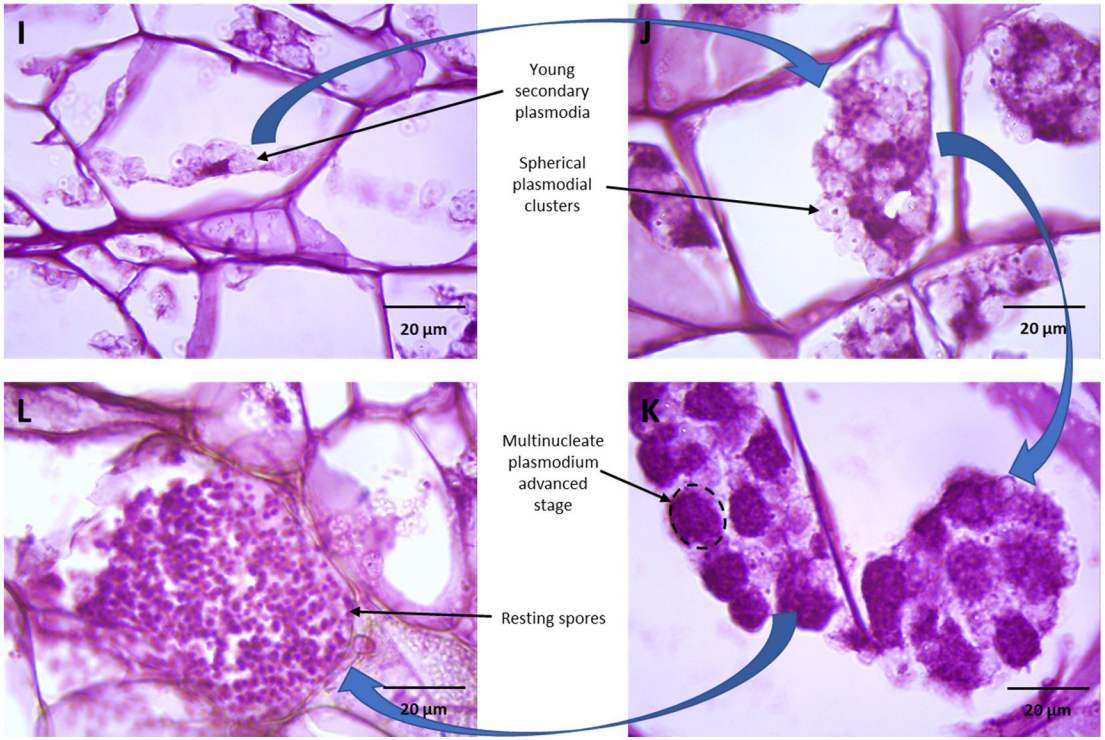
We are testing responses of cultivars to distinct P. brassicae pathotypes to find candidate genes that can be manipulated to increase resistance.

On our first experiment the cultivar Laurentian was moderately resistant against pathotype 5x, while Brutor was susceptible.

Laurentian 21 dai

Brutor 21 dai

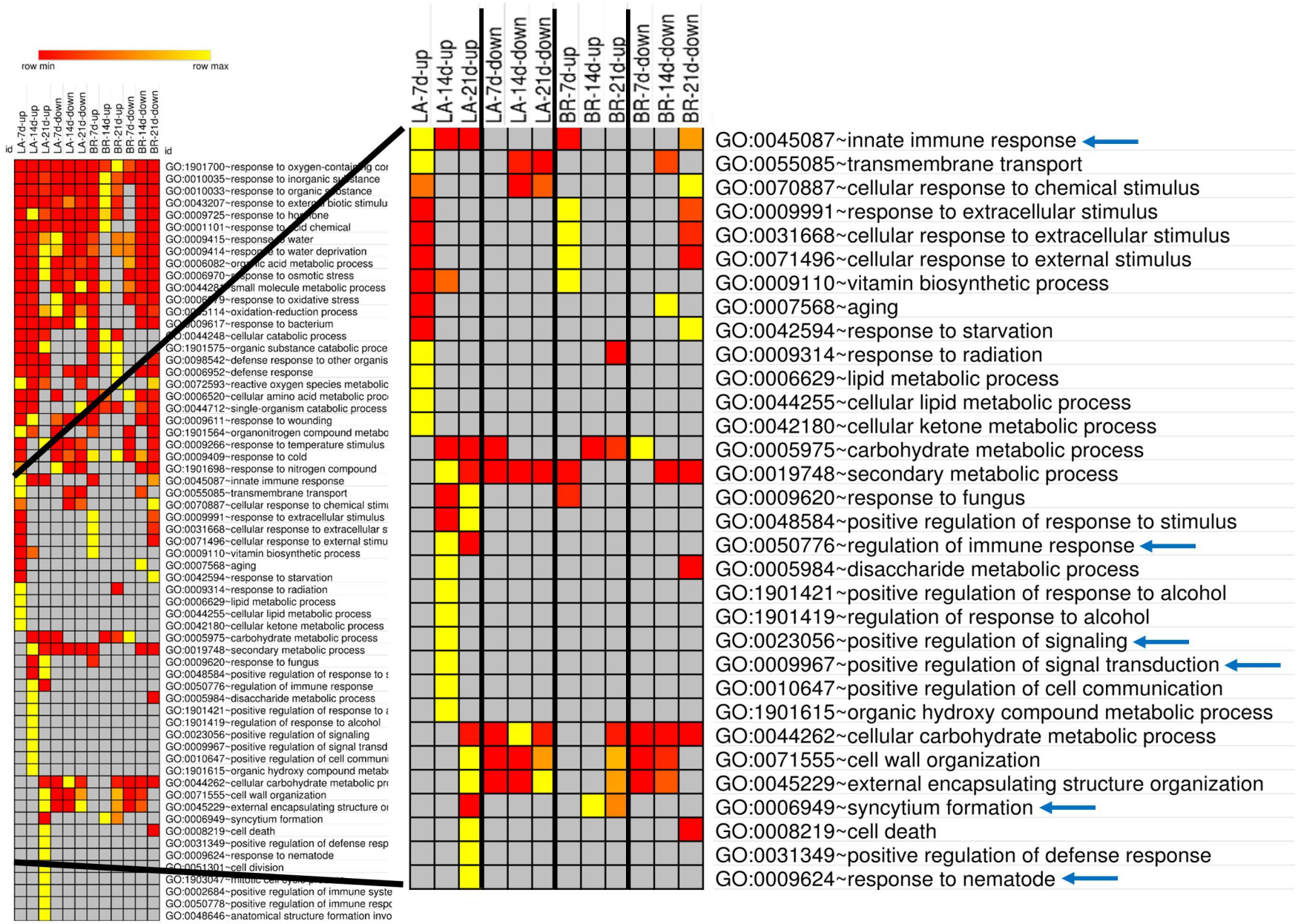
Brutor 21 dai



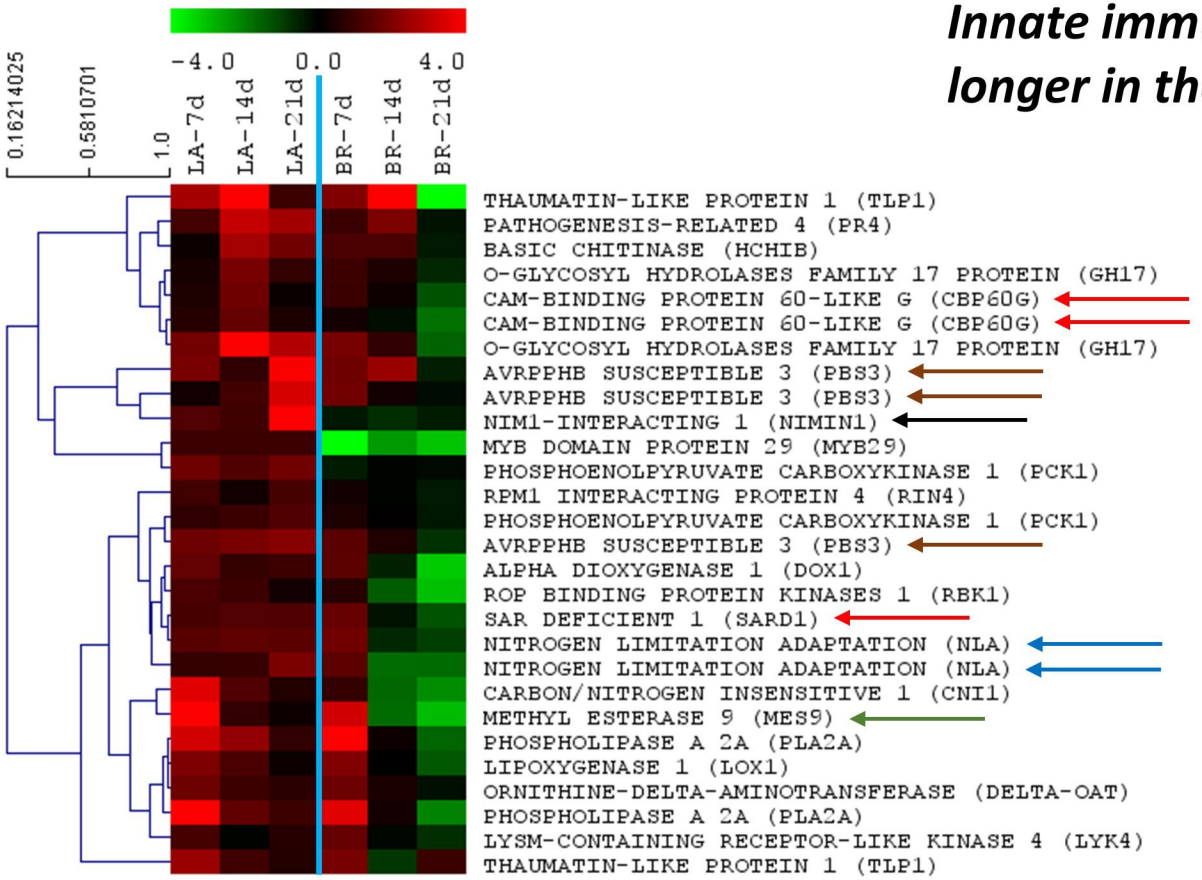
Disease was detected at 14 dai for Brutor and until 21 dai for Laurentian.

All stages of secondary infection are evident 21 dai.

Functional categories enrichment from RNAseq differentially expressed genes shows contrasting patterns between cultivars.



Innate immune responses are activated longer in the resistant cultivar.



SA-mediated response is fully deployed

Mutations on CBP60g and SARD1 genes increase susceptibility to *Pseudomonas syringae* in *Arabidopsis thaliana* (Wang et al., 2011).

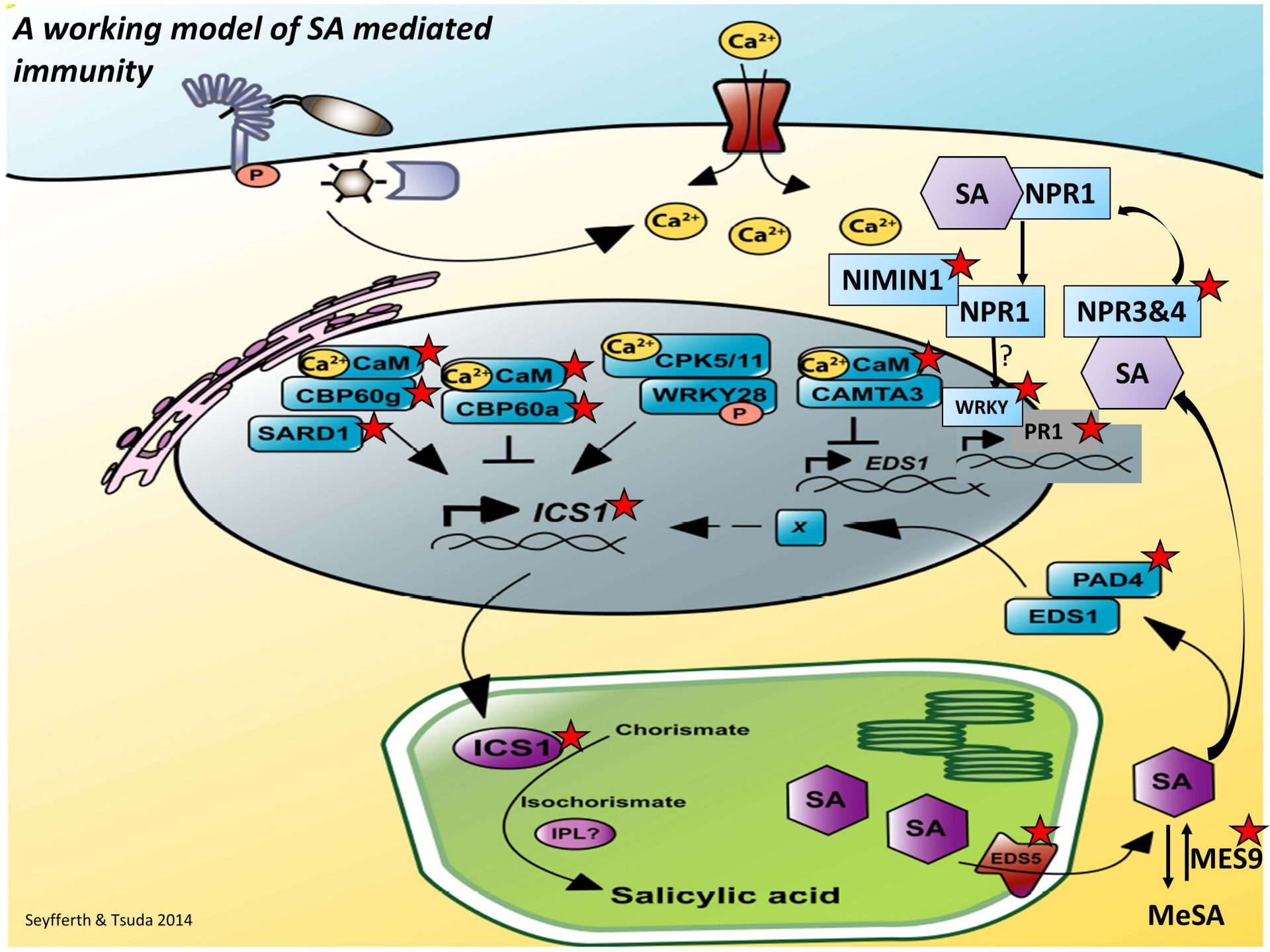
When NLA is suppressed by miRNA in At, susceptibility to nematodes increases (Hewezi et al., 2016). Controller of SA-mediated immunity to *Pseudomonas syringae* in At (Yaeno and Iba 2008).

PBS3, also know as *WIN3* (HOPW1-1-Interacting3), confers resistance to the biotrophic pathogen *Pseudomonas syringae* (Lee et al., 2007; Nobuta et al., 2007).

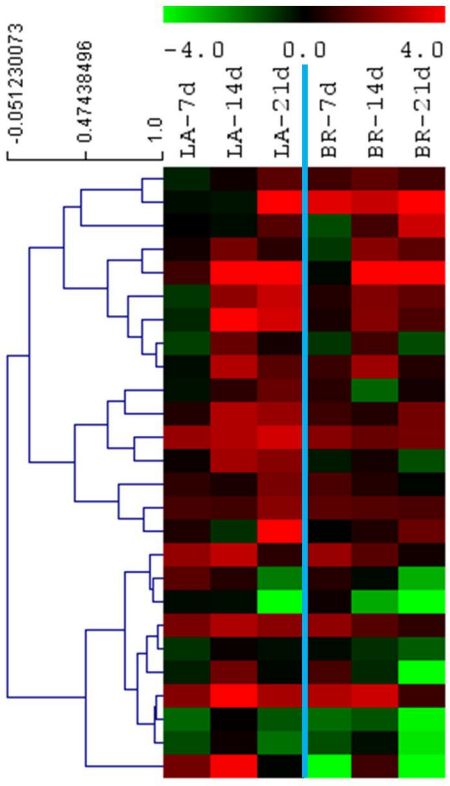
MES9 is activated to turn MeSA (transport for SAR) into SA (Dempsey et al., 2011).

NIMIN1 interacts with NPR1 to repress PR1 (Weigel et al., 2005).

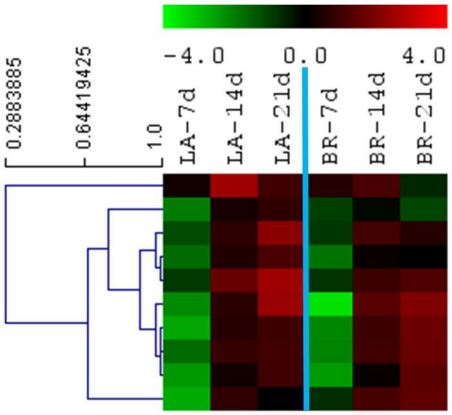
Heatmaps built with MeV using log2-fold changes from FPKM values. Hierarchical clustering was done with Pearson correlation.


A working model of SA mediated immunity
Ca2+
P
SA
NPR1
NIMIN1
NPR1
NPR3&4
SA
?
Ca2+CaM
CBP60g
SARD1
Ca2+CaM
CBP60a
Ca2+CPK5/11
WRKY28
P
Ca2+CaM
CAMTA3
WRKY
PR1
EDS1
ICS1
x
PAD4
EDS1
ICS1
Chorismate
Isochorismate
IPL?
Salicylic acid
SA
SA
EDS5
SA
MES9
MeSA
Seyfferth & Tsuda 2014

Similarities to responses to nematodes.

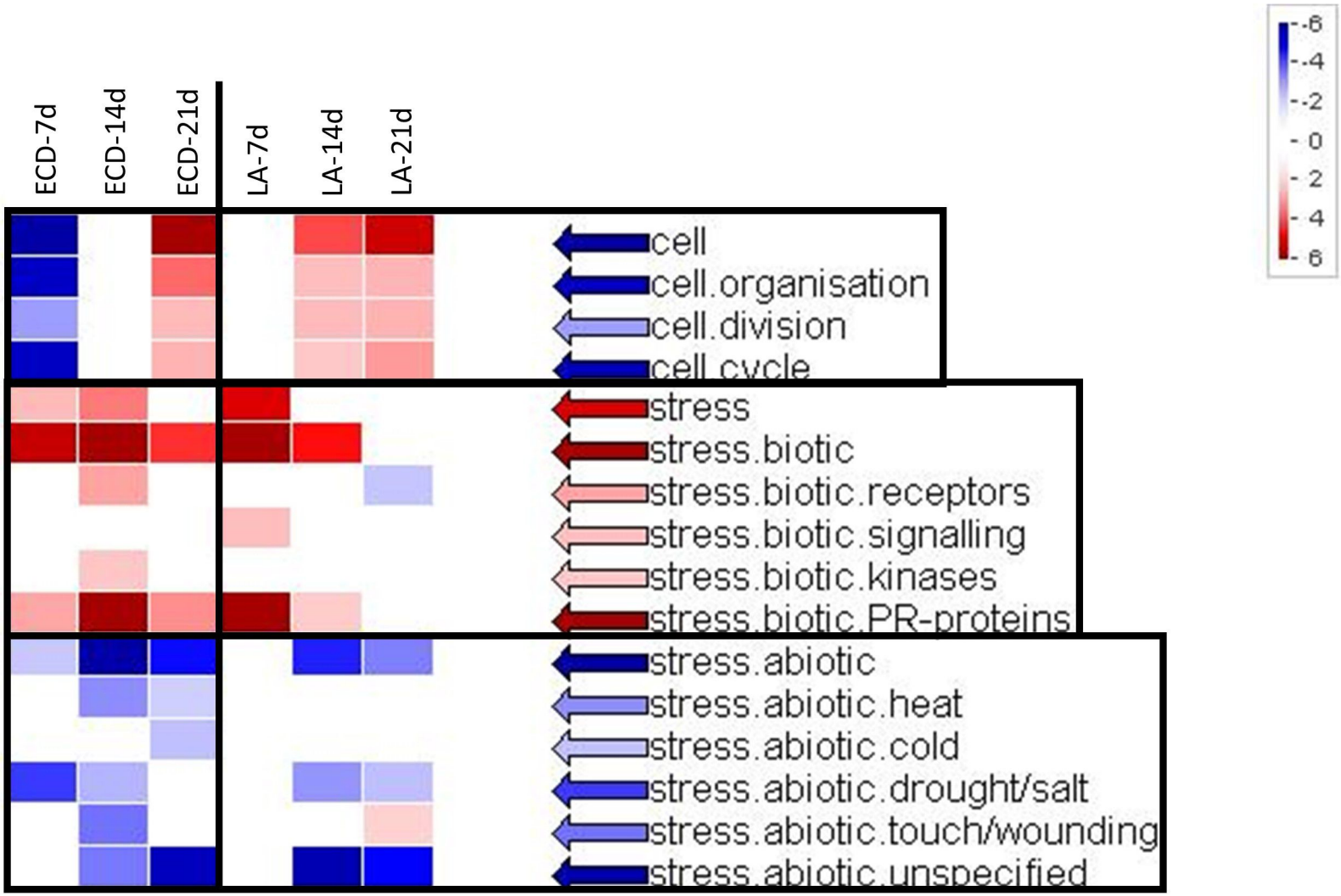


Linked to polar auxin transport (Prat et al., 2018) .
Knockouts in At result in resistance to nematodes (Grunewald et al., 2008) .



Expansins enriched in the category syncytium formation may be triggered to favor pathogen development through cell enlargement.

A second experiment comparing two more cultivars against pathotype 3A, which is widespread in Alberta, shows a clear picture of cell development and confirms patterns of defense – (M.Sc. Qinqin Zhou).



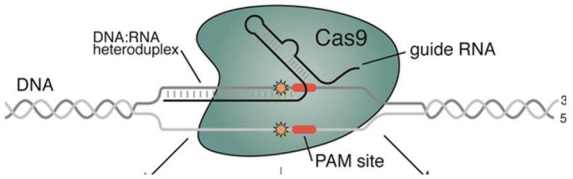
Heatmaps built with MapMan/PageMan.

Candidate genes for resistance or susceptibility can be used to validate function or increase cultivar resistance via gene editing (CRISPR-Cas technology).

Candidate gene selection

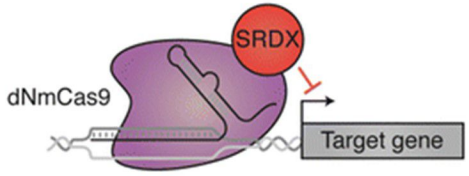
Defense/resistance gene
(WRKY, PNP, CHIT, SA-related, R-genes)

Susceptibility gene
(WRKY, EXP, LTPs)



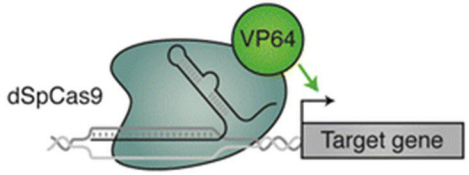
Functional validation

Modulation



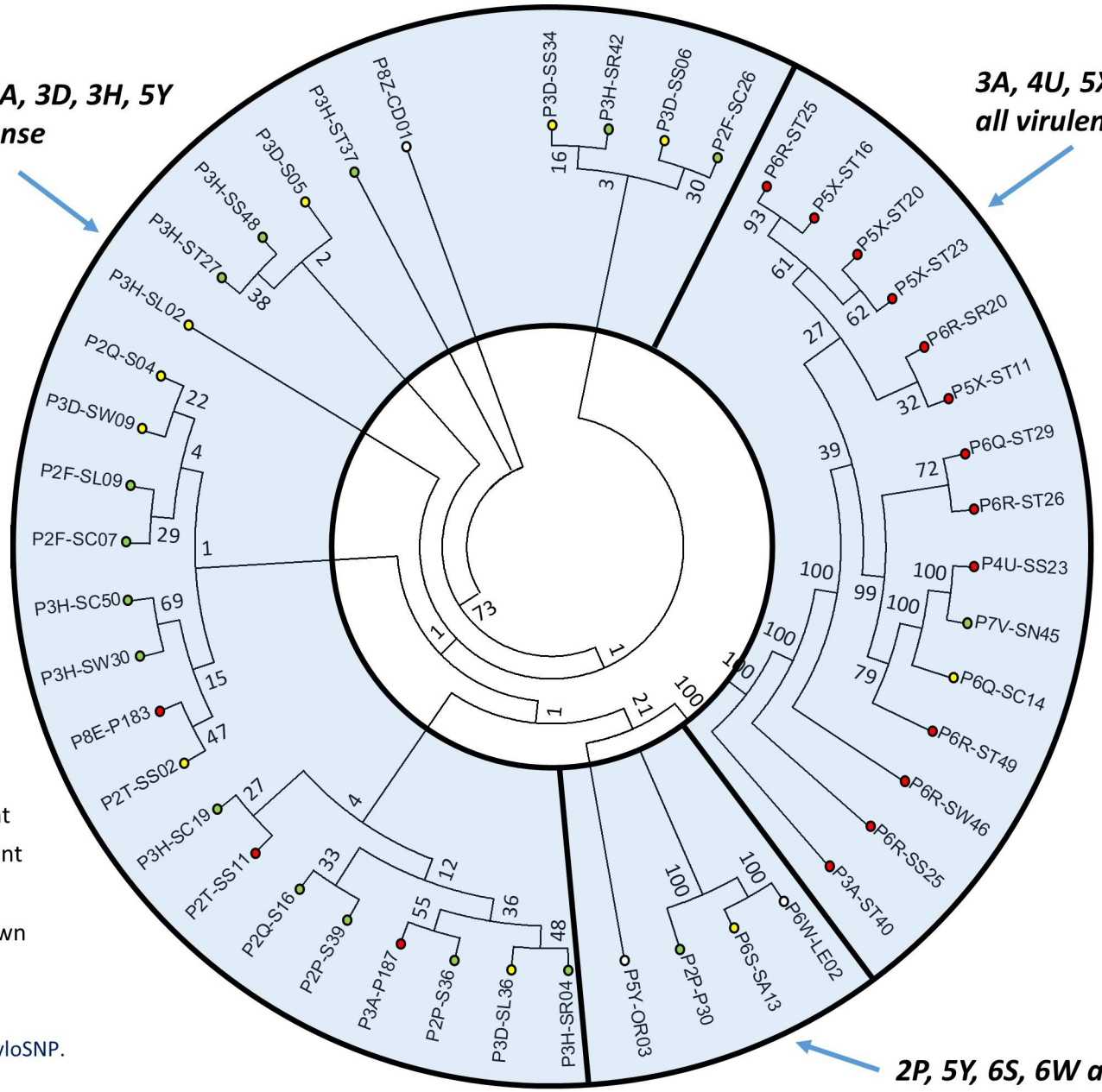
Partial repression to find a development/defense balance

Increase expression to improve resistance



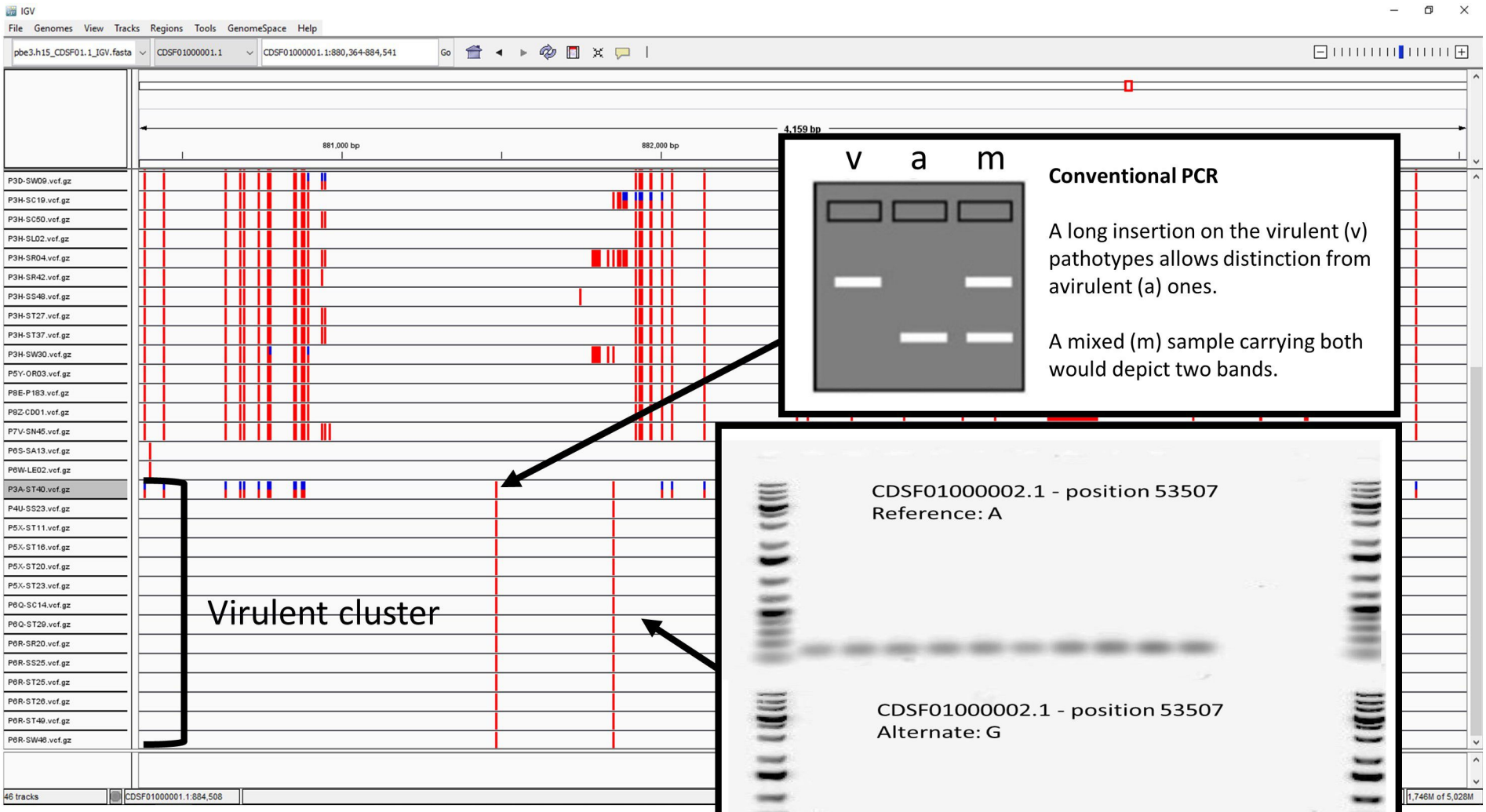
Paul and Yiping (2016)

We are using whole genome sequencing of P. brassicae isolates to find evolutionary relationships and create pathotype-specific diagnostic tools – (Ph.D. Homa Askarian).



Analysis performed using PhyloSNP.
100 boostrap replicates.

Polymorphism can be exploited to create cluster/pathotype/isolate-specific detection assays – (M.Sc. Heather Tso).



Currently we have identified 128 INDEL + SNPs by comparing 45 full genomes, to use for our diagnostic assays.

Conclusions

- *B. napus immunity to P. brassicae is mainly activated through SA-mediated signalling and sustained for longer in resistant cultivars.*

- *Many genes indicate common responses to nematode infestations. The effects of increased cell size and developmental alterations of the host may account for these common features.*

- *Genes found through RNA-seq studies constitute an abundant source for functional validation and improving resistance through gene-editing approaches.*

- *Diversity among pathotypes indicates common sources of virulence and is useful for pathotype-specific diagnostic assays.*

Acknowledgments

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- *Heather Tso (M.Sc. student) – rh-PCR standardization.*
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References

- Hewezi, T., Piya, S., Qi, M., Balasubramaniam, M., Rice, J. H., and Baum, T. J. (2016). *Arabidopsis* miR827 mediates post-transcriptional gene silencing of its ubiquitin E3 ligase target gene in the syncytium of the cyst nematode *Heterodera schachtii* to enhance susceptibility. Plant J. 88, 179–192. doi:10.1111/tpj.13238.
- Dempsey, DM.A., Vlot, A.C., Wildermuth, M.C., Klessig, D.F. (2011). Salycilic acid synthesis and metabolism. The Arabidopsis Book. E0156. doi: 10.1199/tab.0156.
- Lee, M. W., Lu, H., Jung, H. W., and Greenberg, J. T. (2007). A Key Role for the *Arabidopsis* WIN3 Protein in Disease Resistance Triggered by *Pseudomonas syringae* That Secrete AvrRpt2. Mol. Plant-Microbe Interact. 20, 1192–1200.
- Paul, J.W., Yiping, I.I.I., 2016. CRISPR / Cas9 for plant genome editing : accomplishments , problems and prospects. Plant Cell Rep. 35, 1417–1427. doi:10.1007/s00299-016-1985-z
- Seyfferth, C., Tsuda, K. (2014). Salicylic acid signal transduction: the initiation of biosynthesis, perception and transcriptional reprogramming. Front. Plant. Sci. 5, 697. doi: 1003389/fpls.2014.00697.
- Wang, L., Tsuda, K., Truman, W., Sato, M., Nguyen, L. V, Katagiri, F., et al. (2011). CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling. Plant J. 3000, 1029–1041. doi:10.1111/j.1365-313X.2011.04655.x.
- Wang, S., Yu, F., Zhang, W., Tang, J., Li, J., Yu, L., et al. (2019). Comparative transcriptomic analysis reveals gene expression changes during early stages of *Plasmodiophora brassicae* cab infection in cabbage (*Brassica oleracea* var . capitata L .). Can. J. Plant Pathol. 41, 188–199. doi:10.1080/07060661.2019.1567592.
- Weigel, R.R., Pfitznee, U.M., Gatz, C. (2005). Interaction of NIMIN1 with NPR1 modulates PR gene expression in *Arabidopsis*. Plant Cell. 14, 1279-1291.
- Yaeno, T., and Iba, K. (2008). BAH1 / NLA , a RING-Type Ubiquitin E3 Ligase , regulates the accumulation of salicylic acid and immune responses to *Pseudomonas syringae* DC3000 1 [W][OA]. Plant Physiol. 148, 1032–1041. doi:10.1104/pp.108.124529.