Transcriptome changes in *Brassica napus* cultivars upon interaction with *Plasmodiophora brassicae* pathotype 5X.

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Cultivars Laurentian (R) and Brutor (S) present

divergent responses against pathotype 5x

Clubroot infestations

- *P. Brassicae* has spread rapidly for a soilborne pathogen.
- Currenty there are over 2700 cases across the province.
- Resistance was broken by pathotype 5x



courtesy of: Stephen Strelkov







7dai

14dai

21dai

RNA-seq analysis of Laurentian vs Brutor

cultivar	harvest	up	down	total
	7 dai	2946	2592	5538
Laurentian (R)	14 dai	1237	1510	2747
	21 dai	1570	2779	4349
Brutor (S)	7 dai	1936	1898	3834
	14 dai	1696	1719	3415
	21 dai	1221	5349	6570

The regulated genes are significant with a *q* value of 0.05, which is given after correction for multiple testing (Benjamini-Hockberg).

What we see from these data:

- Thousands of significantly regulated genes.
- A large number of regulated genes for Laurentian at 7 dai and for Brutor at 21 dai.
- More downregulated genes than upregulated for two time points in both cultivars.



LA (R) vs BR (S) 7 dai - MapMan



At 7 dai (days after inoculation) we see a general increased number of significantly regulated genes in resistant cultivar Laurentian (LA).



LA (R) vs BR (S) 14 dai - MapMan



At 14 dai the relative amount of regulated genes does not seem to vary greatly between both cultivars.



LA (R) vs BR (S) 21 dai - MapMan



At 21 day there is a strong downregulation in most categories for both cultivars but more radically in Brutor.







	r	7 dai	14 dai	21dai	Trends in functional category regulation	
	response to biotic stress				Trenus in junctional category regulation	
	response to abiotic stress					
	response to nematodes					
	syncytium formation					
	systemic acquired resistance				SAR is only enriched on the resistant cultivar.	
response to stress	receptor signalling				Receptor kinases are only enriched on the resistant cultivar.	
	protein phosphorylation-signalling					
	negative regulation of signalling					
	glucosinolate biosynthesis					
	camalexin biosynthesis				Production of metabolites are important mechanisms of defense and were	
	response to ROS				regulated in both cultivars.	
	glutamine-glutamate metabolism					
cell wall	cell wall modification				Both cultivars modify their cell wall , but the resistant cultivar uses mechanism	
	glucan metabolism				of cell wall deposition of callose to potentially create papillae.	
	phenylpropanoid metabolism					
	auxin signalling					
hormones	JA signalling					
	ET signalling				Auxin and BR signalling are only enriched on the susceptible cultivar.	
	ABA signalling				Most hormones respond at 7 and 14 dai and are downregulated 21 dai.	
	gibberellin signalling					
	BR signalling					
cell growth and cell size	root development					
	cell size regulation				Cell size regulation is modulated throughout the time course in Brutor.	
protein production and modification	ribosome biogenesis					
	translation					
	amino acid metabolism					
	proteolysis					
metabolism	polysaccharide biosynthesis					
	nitrate assimilation				Changes in the primary metabolism of the susceptible cultivar point to a sink of	
	glycolysis				nutrients in the root for pathogen utilization	
	fatty acid oxidation					





LA-BR receptor kinases



Leucine-rich receptor-like protein kinase family protein Leucine-rich repeat (LRR) family protein Leucine-rich repeat protein kinase family protein Leucine-rich receptor-like protein kinase family protein Receptor-like protein kinase-related family protein cysteine-rich RLK (RECEPTOR-like protein kinase) 6 Leucine-rich repeat protein kinase family protein Leucine-rich receptor-like protein kinase family protein Leucine-rich repeat protein kinase family protein Leucine-rich repeat protein kinase family protein Leucine-rich repeat protein kinase family protein Leucine-rich receptor-like protein kinase family protein myb domain protein 16 cysteine-rich RLK (RECEPTOR-like protein kinase) & Leucine-rich repeat protein kinase family protein Leucine-rich repeat receptor-like protein kinase family protein Protein kinase superfamily protein Protein kinase superfamily protein Leucine-rich repeat transmembrane protein kinase Protein kinase superfamily protein Protein kinase superfamily protein Protein kinase superfamily protein Malectin/receptor-like protein kinase family protein Leucine-rich repeat protein kinase family protein Leucine-rich repeat protein kinase family protein Protein kinase superfamily protein Leucine-rich receptor-like protein kinase family protein Leucine-rich receptor-like protein kinase family protein Leucine-rich repeat (LRR) family protein S-locus lectin protein kinase family protein Leucine-rich receptor-like protein kinase family protein receptor like protein 40 Leucine-rich receptor-like protein kinase family protein Leucine-rich receptor-like protein kinase family protein Leucine-rich receptor-like protein kinase family protein Concanavalin A-like lectin protein kinase family protein Malectin/receptor-like protein kinase family protein cysteine-rich RLK (RECEPTOR-like protein kinase) 18 Leucine-rich receptor-like protein kinase family protein Protein kinase superfamily protein Leucine-rich repeat protein kinase family protein Leucine-rich receptor-like protein kinase family protein Concanavalin A-like lectin protein kinase family protein cysteine-rich RLK (RECEPTOR-like protein kinase) 21 receptor like protein 47 Protein kinase superfamily protein Leucine-rich repeat protein kinase family protein Leucine-rich receptor-like protein kinase family protein Leucine-rich repeat protein kinase family protein Leucine-rich repeat transmembrane protein kinase Protein kinase superfamily protein Leucine-rich receptor-like protein kinase family protein Malectin/receptor-like protein kinase family protein Leucine-rich repeat protein kinase family protein Leucine-rich receptor-like protein kinase family protein Leucine-rich repeat transmembrane protein kinase

LA-BR cell_wall

R





Pectin lyase-like superfamily protein Plant invertase/pectin methylesterase inhibitor superfamily Pectin lyase-like superfamily protein Glycosyl hydrolase superfamily protein Pectin lyase-like superfamily protein UDP-glucuronic acid decarboxylase 1 Plant invertase/pectin methylesterase inhibitor superfamily UDP-glucuronic acid decarboxylase 1 Leucine-rich repeat (LRR) family protein expansin Al galacturonosyltransferase δ Plant invertase/pectin methylesterase inhibitor superfamily NAD(P)-binding Rossmann-fold superfamily protein Pectin lyase-like superfamily protein Alpha-1,4-glucan-protein synthase family protein" COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored protein family expansin Al Pectin lyase-like superfamily protein expansin Al Pectin lyase-like superfamily protein Pectin lyase-like superfamily protein expansin Al plant natriuretic peptide A 🔶 polygalacturonase 2 Pectin lyase-like superfamily protein expansin Al Cellulose synthase family protein Pectin lvase-like superfamily protein Cupredoxin superfamily protein plant natriuretic peptide A 🚽 arabinogalactan protein 1 expansin Al Pectin lyase-like superfamily protein COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored protein family Pectin lyase-like superfamily protein UDP-glucuronic acid decarboxylase 1 xyloglucan endotransglucosylase/hydrolase 17 expansin Al cellulose synthase-like A3 Glycosyl hydrolase family protein Cellulose synthase family protein Pectin lyase-like superfamily protein Pectin lyase-like superfamily protein expansin Al Pectin lyase-like superfamily protein expansin-like A3 Pectin lyase-like superfamily protein plant natriuretic peptide A 🚄 Pectin lyase-like superfamily protein Pectin lyase-like superfamily protein xyloglucan endotransglucosylase/hydrolase 32 Pectin lyase-like superfamily protein Pectin lyase-like superfamily protein Pectin lyase-like superfamily protein Cellulose synthase family protein galacturonosyltransferase 3 galacturonosyltransferase 3 COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored protein family Cellulose synthase family protein expansin Al glycosyl hydrolase 9B8 plant natriuretic peptide A 🔫 expansin B3 Cupredoxin superfamily protein expansin Al Pectin lyase-like superfamily protein UDP-glucuronic acid decarboxylase 1



LA-BR auxins

R



Auxin-responsive GH3 family protein Aluminium induced protein with YGL and LEDR motifs like COV 1 RWI-like superfamily protein Multi ise superiabily protein the state of the state of the state of the exhibitent induced calabodd in binding protein auch transport protein (BIG) Aluminium induced protein with YGL and LEDR motifs 0-fucosyltransferase family protein peptidase HCO/HES/HGU family protein peptiaks HE3/HE3/HE3 family protein baum-responsible family protein cyrothems P400, family TLI, subfamily A, polypeptide 1° -cyrothems P400, family TLI, subfamily A, polypeptide 1° -cyrothems P400, family TLI, subfamily A, polypeptide 1° indeiscartic acid-induced protein CM/IAA tamosriptional respirators family protein AM/IAA tamosriptional respirators family protein America sequences GDI family protein (b) COV Aus investmentive GiT family protein 1916 ibi 2016 ibi 2017 ibi 2018 ibi gluceside glucohydrolase 2 Amkin-responsive GH2 family protein Aux in responsive on a rawity protein Aux in responsive family protein SAUE-like aux in responsive protein family glucoside glucohydrolase 2 RAD (P)-likked oxidoreductase superfamily protein nisti) zeel BAD (P) linked oxidateshuttase superfamily protein intriliase) and experience line 1 denomy and and experience with YGL and LEDR works alluminum induced protein with YGL and LEDR meeting denomy associated protein with YGL and LEDR meeting gluesside glucohydrollase 2 glucoside glucohydrollase 3 glucoside glucohydrollase 5 (FCF family transcription factori Aluminum induced protein with YGL and LEDR meting denomy and the second second second second denomy and the second second second second second denomy and the second second second second Aluminum induced protein with YGL and LEDR meting denomy and the second second second second second aluminum induced second second second second denomy and the second second second second second denomination of the second second second second denomination second second second second second second second second denomination second second second second second second second denomination second s UDF-flycosyltcansfease superior family BDF-flycosyltcansfease superior family DDF-flycosyltcan inperton family DDF-flycosyltcan inperton New for flycosyltcan inperton Automatic family protein Automatic family protein Automatic family protein Automatic family protein Aluminum induced Flycosin with YGL and LEDE metifs Orthogottametic family protein Automatic family protein itrilase 1 nitzilase 1 Ausin-responsive GR3 family protein Ausin-responsive GR3 family protein Outer arms dynein light chain 1 protein Ausin-responsive GR3 family protein O-fucosyltransferase family protein ETI-like superfamily protein

S



NAD(P)-linked oxidoreductase superfamily protein nitrilase 1 < dormancy-associated protein-like 1 Aluminium induced protein with YGL and LRDR motifs Aluminium induced protein with YGL and LRDR motifs dormancy-associated protein-like 1 Aluminium induced protein with YGL and LRDR motifs glucoside glucohydrolase 2 0-fucosyltransferase family protein TCP family transcription factor Aluminium induced protein with YGL and LRDR motifs dormancy-associated protein-like 1 Aluminium induced protein with YGL and LRDR motifs UDP-Glycosyltransferase superfamily protein SAUR-like auxin-responsive protein family UDP-Glycosyltransferase superfamily protein Outer arm dynein light chain 1 protein Auxin efflux carrier family protein auxin-responsive GH3 family protein beta glucosidase 16 nitrilase 1 Auxin-responsive GH3 family protein dormancy-associated protein-like 1 0-fucosyltransferase family protein Aluminium induced protein with YGL and LRDR motifs 0-fucosyltransferase family protein Auxin-responsive GH3 family protein auxin-responsive family protein nitrilase l 🔫 Auxin-responsive GH3 family protein Auxin-responsive GH3 family protein

nitrilase 1 🔫 auxin transport protein (BIG) Transcriptional factor B3 family protein / auxin-re: SAUR-like auxin-responsive protein family Auxin-responsive family protein dormancy-associated protein-like 1 Auxin-responsive GH3 family protein auxin transport protein (BIG) Aluminium induced protein with YGL and LRDR motifs Aluminium induced protein with YGL and LRDR motifs glucoside glucohydrolase 2 Auxin-responsive GH3 family protein cullin-associated and neddylation dissociated Aluminium induced protein with YGL and LRDR motifs Auxin-responsive GH3 family protein Auxin-responsive GH3 family protein Aluminium induced protein with YGL and LRDR motifs glucoside glucohydrolase 2 RNI-like superfamily protein auxin-responsive family protein Auxin-responsive GH3 family protein Aluminium induced protein with YGL and LRDR motifs 0-fucosyltransferase family protein glucoside glucohydrolase 2 Auxin-responsive GH3 family protein Auxin-responsive GH3 family protein Outer arm dynein light chain 1 protein dormancy-associated protein-like 1 RNI-like superfamily protein 0-fucosyltransferase family protein glucoside glucohydrolase 2 nitrilase 1 🚽



Conclusions

- Host responses in resistant (or partially resistant) and susceptible cultivars against pathotype 5x show changes in defense mechanisms, protein modification and degradation, hormone regulation, cell growth and cell wall regulation, and adjustments in primary and secondary metabolism.
- The resistant cultivar shows a larger amount of genes earlier, and maintains regulation of defense mechanisms for a longer period of time when compared with the susceptible cultivar.
- Mechanisms of auxin and brassinosteroid regulation may be key in the compatible interaction, and the susceptible cultivar behaves as a sink of carbohydrates and nitrogen-derived compounds 21 dai.
- Genes which have been characterized for resistance in Arabidopsis, in wild relatives or different cultivar-pathotype interactions should be verified for a similar interaction in new associations, since pattern of expression and genome complexity may differ.
 - For candidate gene finding and mutagenesis: CR genes, negative regulators (S), positive regulators (R).



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