



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

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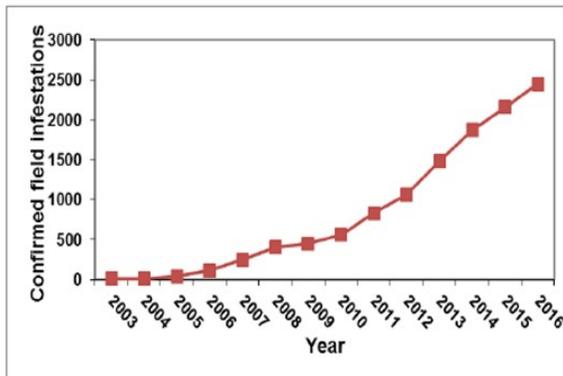
Cultivars Laurentian (R) and Brutor (S) present divergent responses against pathotype 5x

Clubroot infestations

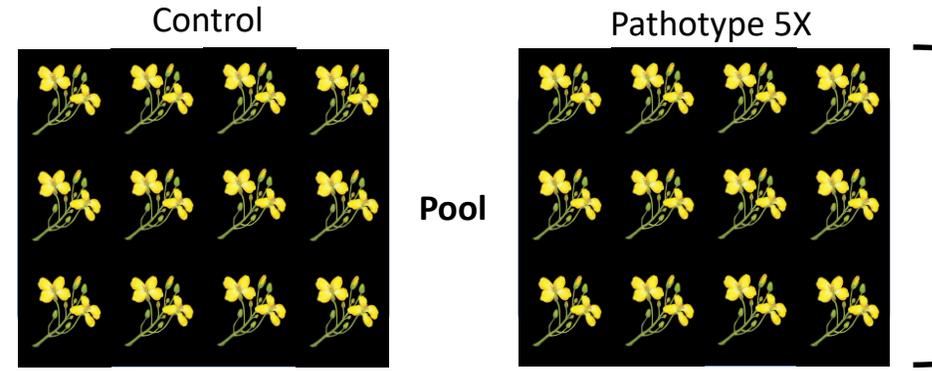
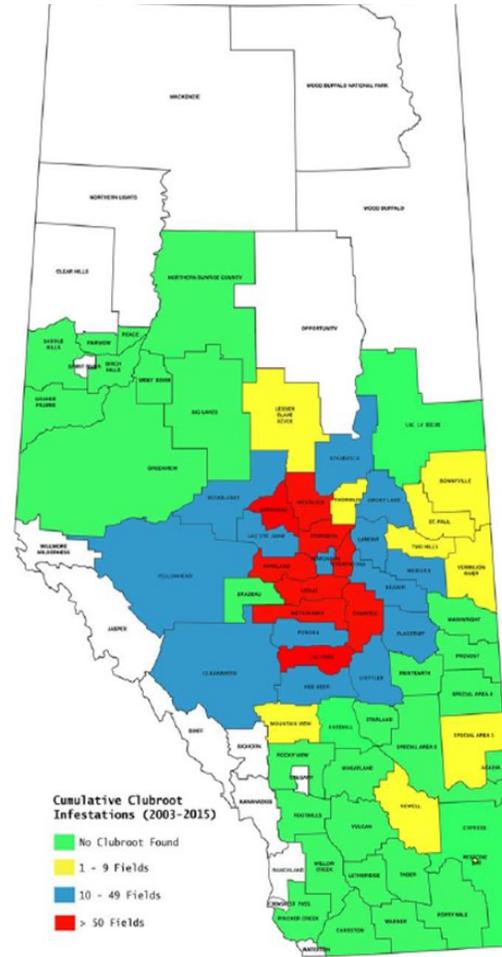
P. Brassicae has spread rapidly for a soilborne pathogen.

Currently there are over 2700 cases across the province.

Resistance was broken by pathotype 5x



courtesy of: Stephen Strelkov



RNA-seq analysis of Laurentian vs Brutor

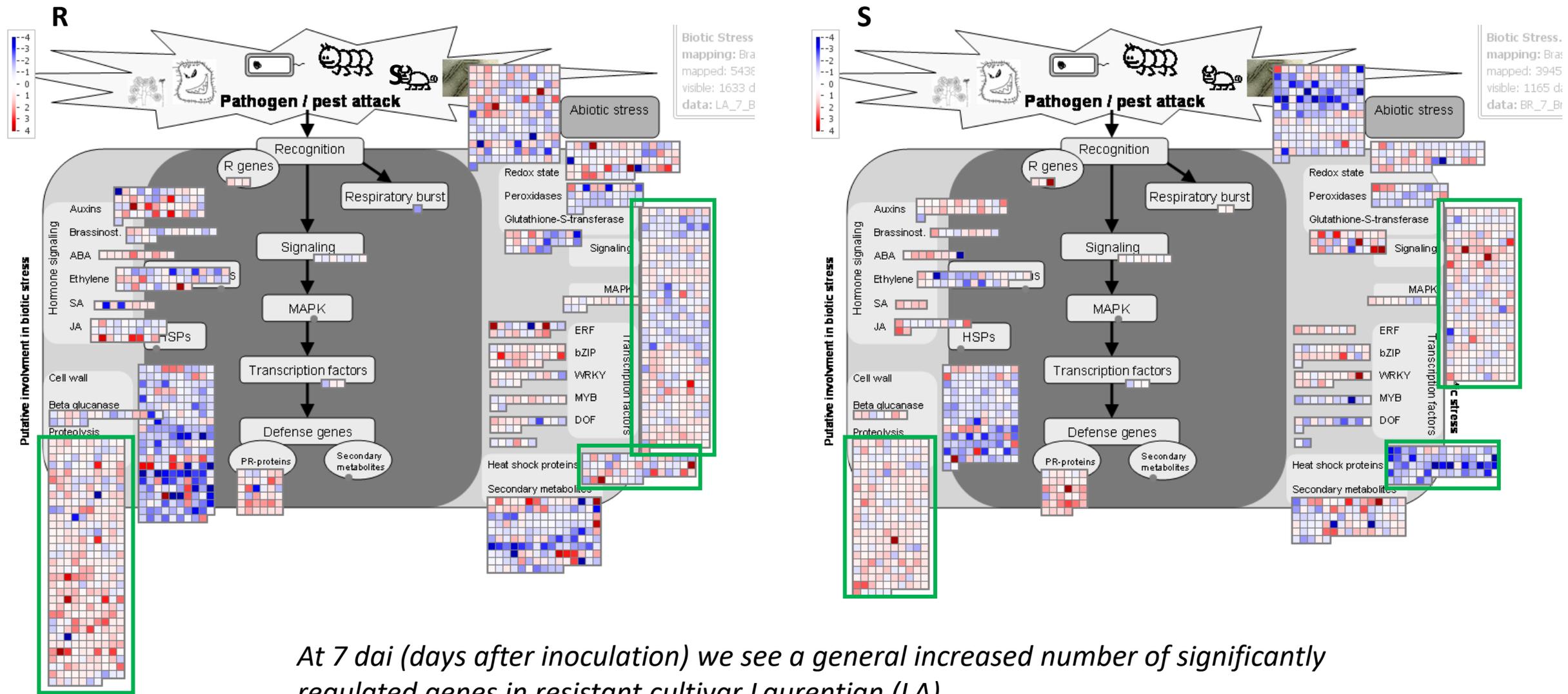
cultivar	harvest	up	down	total
Laurentian (R)	<i>7 dai</i>	2946	2592	5538
	<i>14 dai</i>	1237	1510	2747
	<i>21 dai</i>	1570	2779	4349
Brutor (S)	<i>7 dai</i>	1936	1898	3834
	<i>14 dai</i>	1696	1719	3415
	<i>21 dai</i>	1221	5349	6570

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:

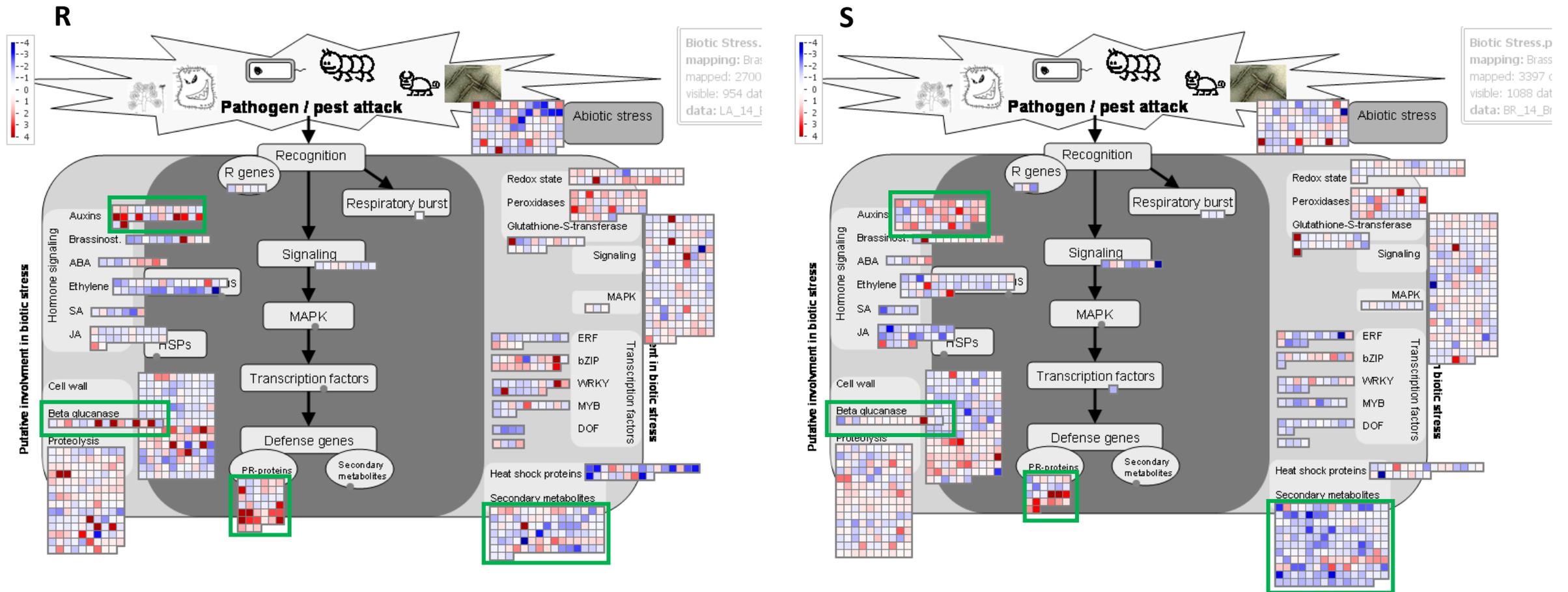
- ❖ Thousands of significantly regulated genes.
- ❖ A large number of regulated genes for Laurentian at 7 dai and for Brutor at 21 dai.
- ❖ More downregulated genes than upregulated for two time points in both cultivars.

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



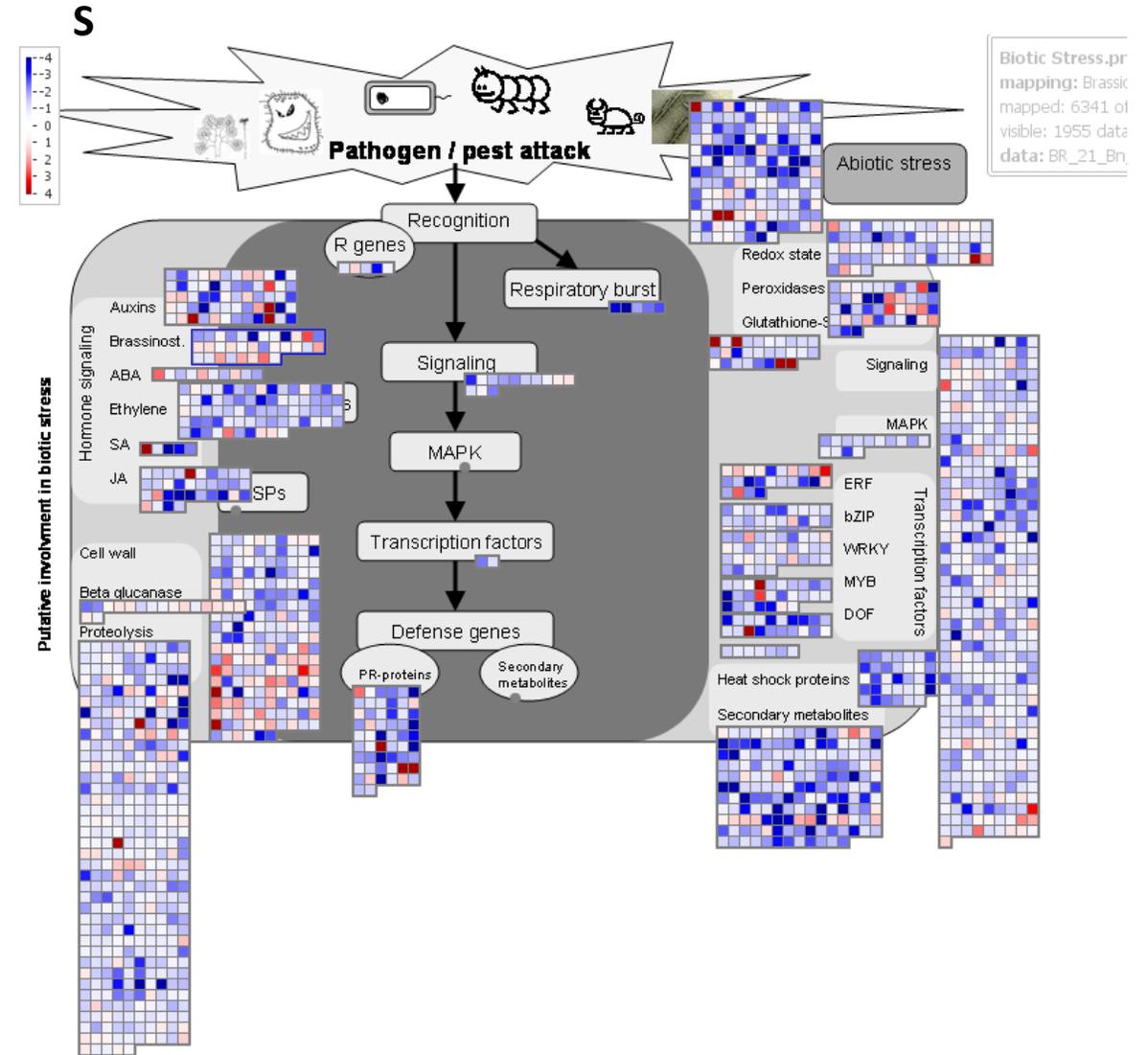
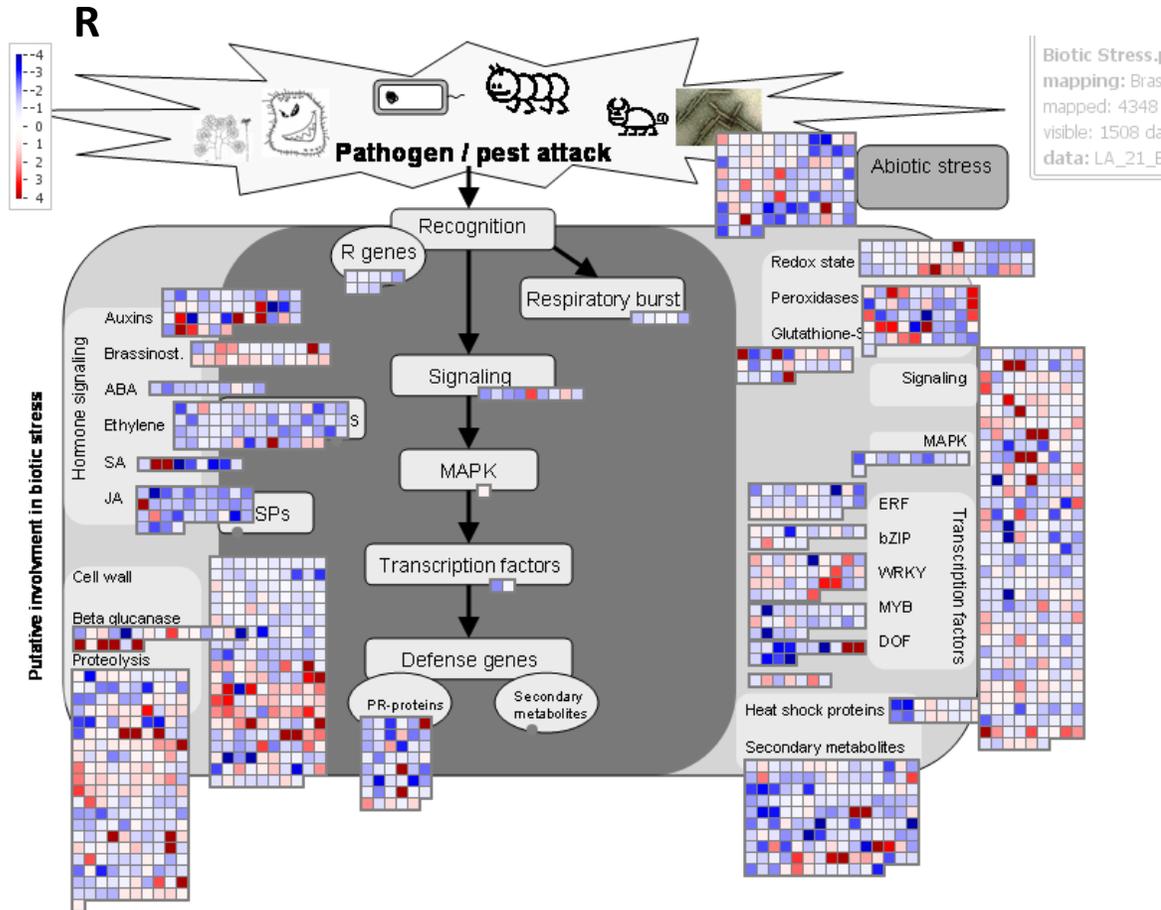
At 7 dai (days after inoculation) we see a general increased number of significantly regulated genes in resistant cultivar Laurentian (LA).

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

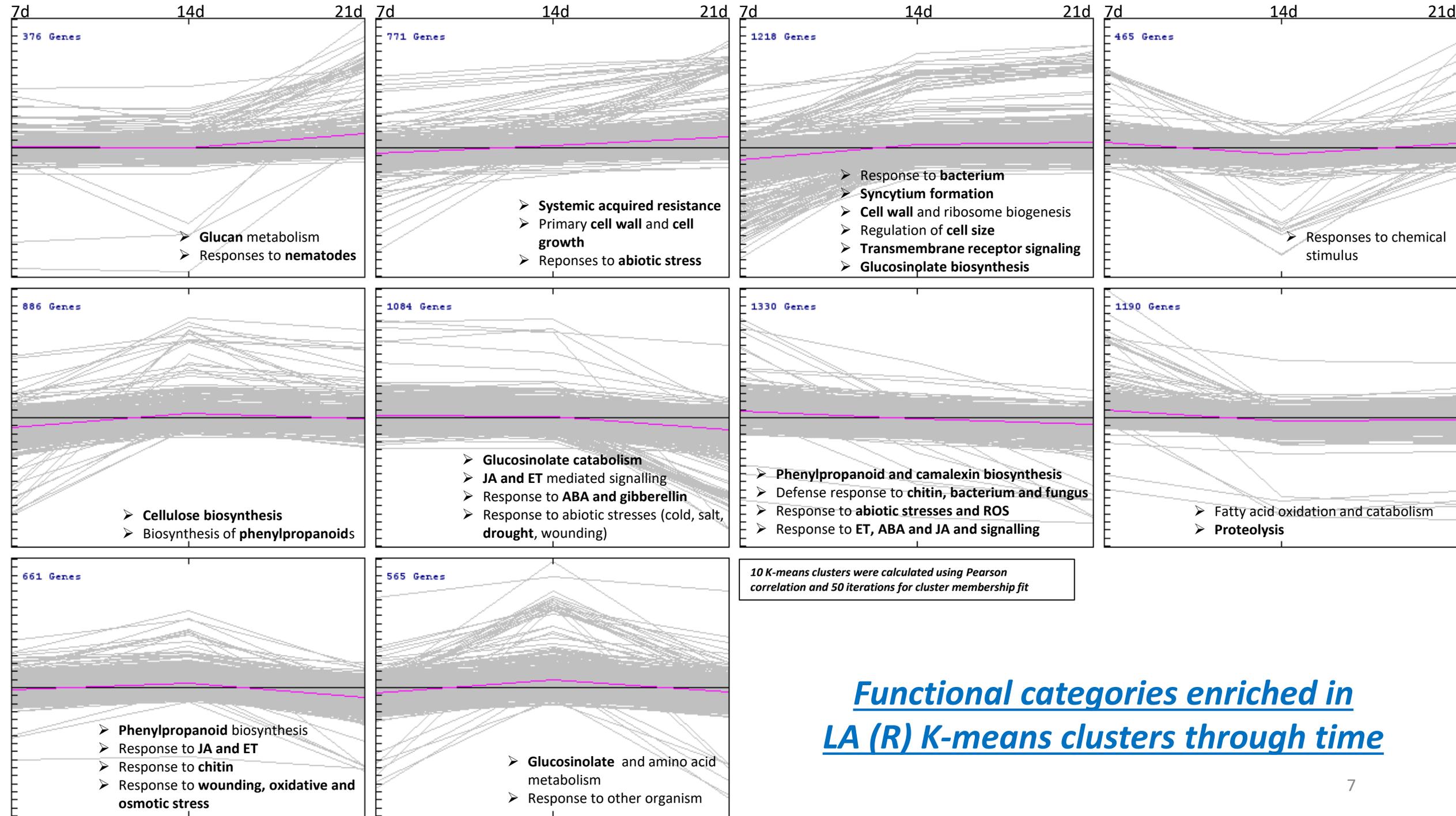


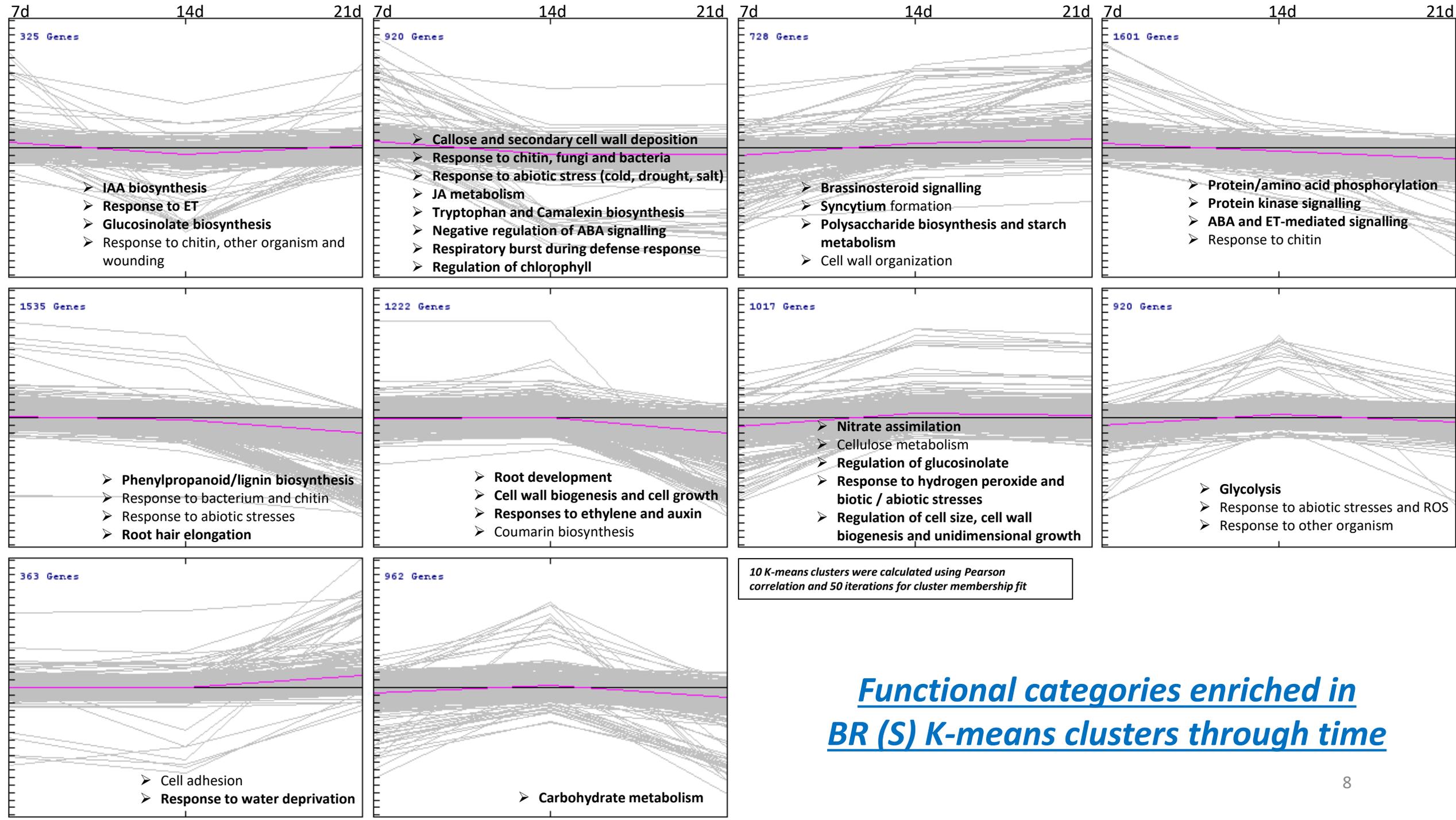
At 14 dai the relative amount of regulated genes does not seem to vary greatly between both cultivars.

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



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





Trends in functional category regulation

		7 dai	14 dai	21dai
response to stress	response to biotic stress	Red	Blue	Blue
	response to abiotic stress	Red	Blue	Blue
	response to nematodes	White	Red	Red
	syncytium formation	White	Red	Red
	systemic acquired resistance	White	Red	Red
	receptor signalling	White	Red	Red
	protein phosphorylation-signalling	Blue	Blue	White
	negative regulation of signalling	Blue	White	White
	glucosinolate biosynthesis	Red	Red	Blue
	camalexin biosynthesis	Blue	White	White
	response to ROS	Blue	Blue	Blue
	glutamine-glutamate metabolism	Blue	White	White
cell wall	cell wall modification	Blue	Red	Red
	glucan metabolism	White	Red	Red
	phenylpropanoid metabolism	Blue	Blue	White
hormones	auxin signalling	Blue	Blue	Blue
	JA signalling	Red	Red	White
	ET signalling	Blue	Red	Blue
	ABA signalling	Blue	Red	White
	gibberellin signalling	Red	Red	White
cell growth and cell size	BR signalling	White	Blue	Blue
	root development	Blue	Blue	White
protein production and modification	cell size regulation	Blue	Red	Red
	ribosome biogenesis	White	Red	Blue
	translation	White	Red	Red
	amino acid metabolism	Red	Red	Blue
metabolism	proteolysis	Red	White	White
	polysaccharide biosynthesis	White	Blue	Blue
	nitrate assimilation	White	Blue	Blue
	glycolysis	White	Blue	White
	fatty acid oxidation	Red	Blue	White

SAR is only enriched on the resistant cultivar.
 Receptor kinases are only enriched on the resistant cultivar.

Production of **metabolites** are important mechanisms of defense and were regulated in both cultivars.

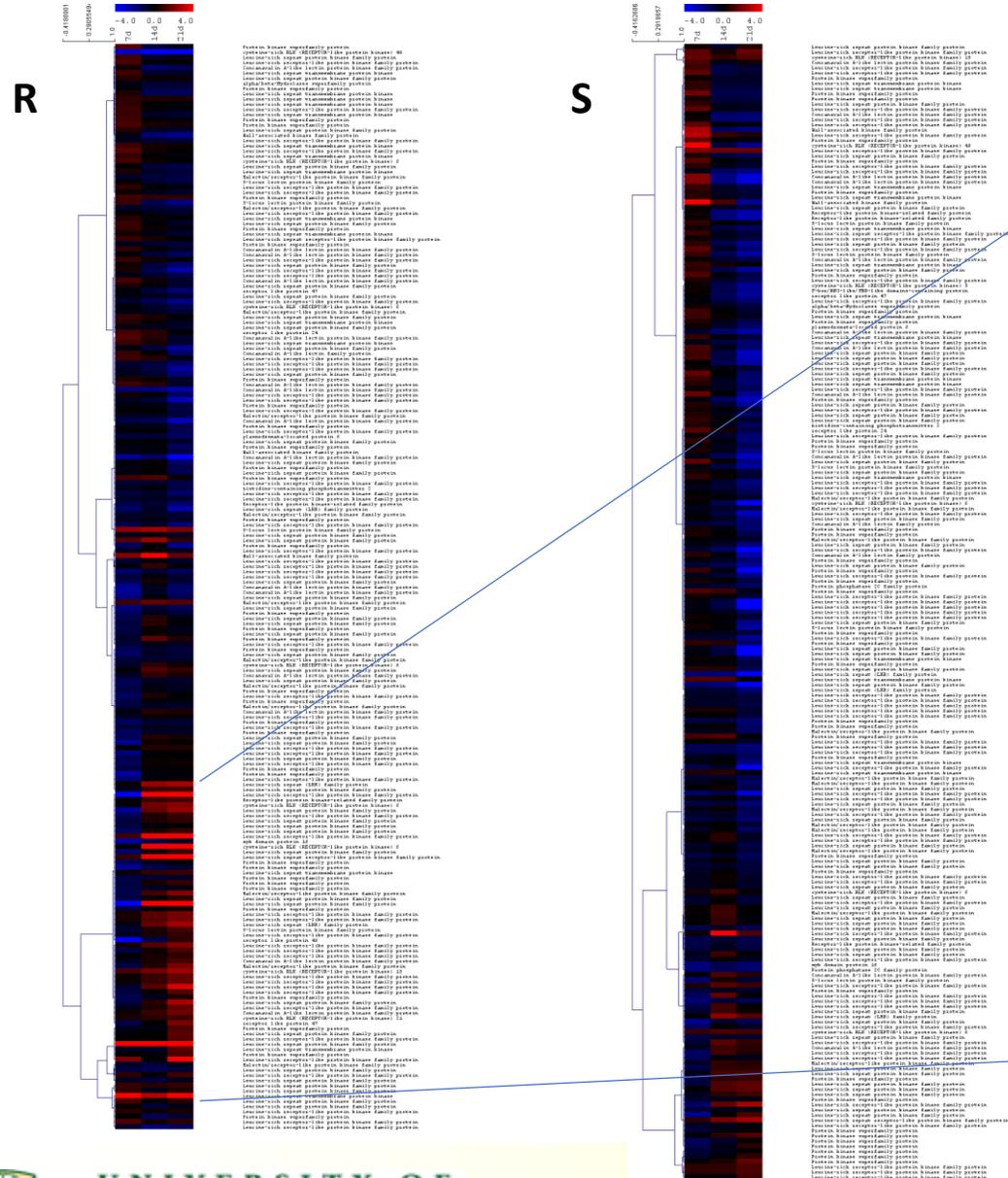
Both cultivars **modify their cell wall**, but the resistant cultivar uses mechanism of **cell wall deposition of callose** to potentially create papillae.

Auxin and BR signalling are only enriched on the susceptible cultivar.
 Most hormones respond at 7 and 14 dai and are downregulated 21 dai.

Cell size regulation is modulated throughout the time course in Brutor.

Changes in the **primary metabolism** of the susceptible cultivar point to a sink of nutrients in the root for pathogen utilization

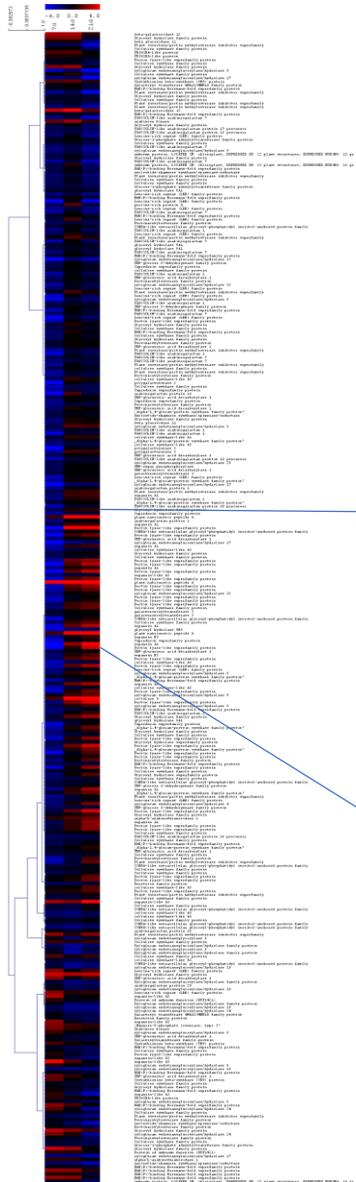
LA-BR receptor kinases



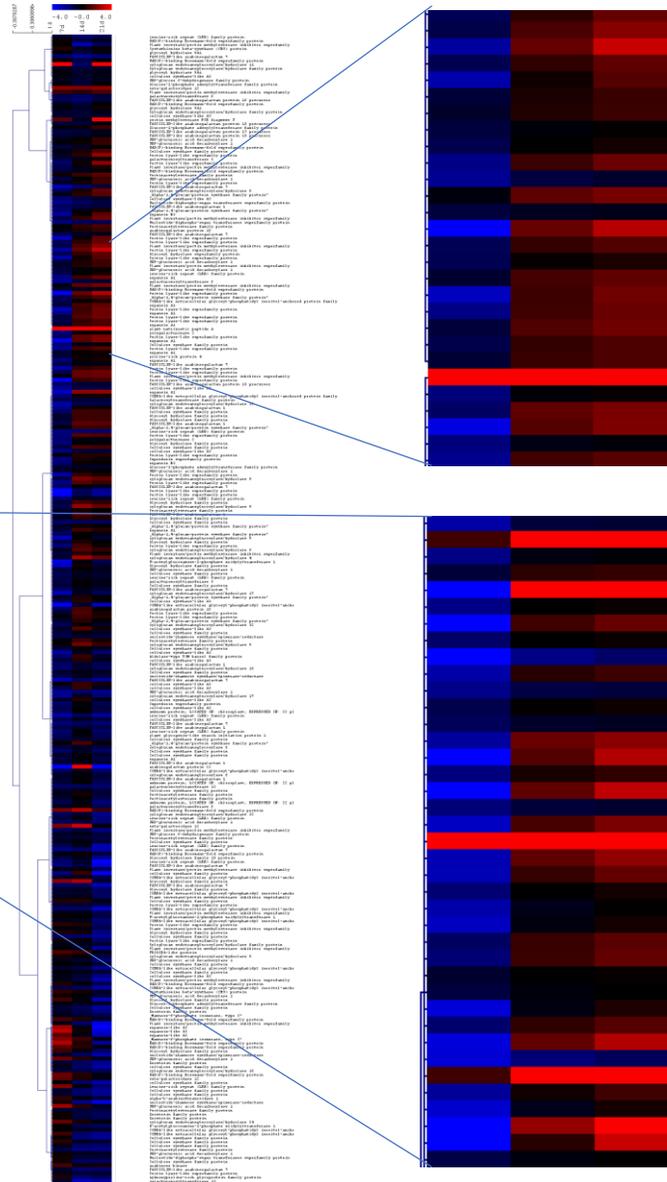
- Leucine-rich receptor-like protein kinase family protein
- Leucine-rich repeat (LRR) family protein
- Leucine-rich repeat protein kinase family protein
- Leucine-rich receptor-like protein kinase family protein
- Receptor-like protein kinase-related family protein
- cysteine-rich RLK (RECEPTOR-like 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 receptor-like 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) 6
- 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 transmembrane protein kinase
- Protein kinase superfamily protein
- Leucine-rich receptor-like protein kinase family 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 receptor-like protein kinase family protein
- Leucine-rich repeat transmembrane protein kinase

LA-BR cell wall

R



S



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 A1
 galacturonosyltransferase 6
 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 A1
 Pectin lyase-like superfamily protein
 expansin A1
 Pectin lyase-like superfamily protein
 Pectin lyase-like superfamily protein
 expansin A1
 plant natriuretic peptide A ←
 polygalacturonase 2
 Pectin lyase-like superfamily protein
 expansin A1
 Cellulose synthase family protein
 Pectin lyase-like superfamily protein

Cupredoxin superfamily protein
 plant natriuretic peptide A ←
 arabinogalactan protein 1
 expansin A1
 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 A1
 cellulose synthase-like A3
 Glycosyl hydrolase family protein
 Cellulose synthase family protein
 Pectin lyase-like superfamily protein
 Pectin lyase-like superfamily protein
 expansin A1
 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
 Cellulose synthase family protein
 galacturonosyltransferase 3
 galacturonosyltransferase 3
 COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored protein family
 Cellulose synthase family protein
 expansin A1
 glycosyl hydrolase 9B8
 plant natriuretic peptide A ←
 expansin B3
 Cupredoxin superfamily protein
 expansin A1
 Pectin lyase-like superfamily protein
 UDP-glucuronic acid decarboxylase 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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