



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

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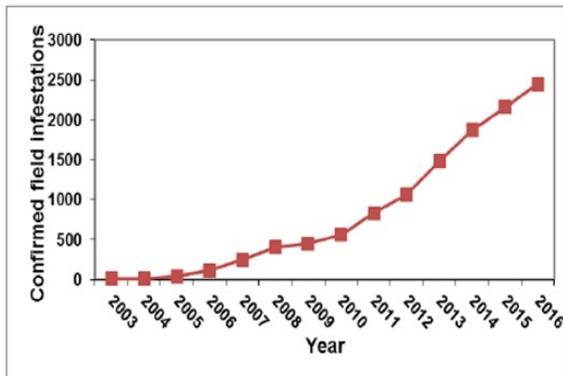
Cultivars Laurentian (R) and Brutor (S) present divergent responses against pathotype 5x

Clubroot infestations

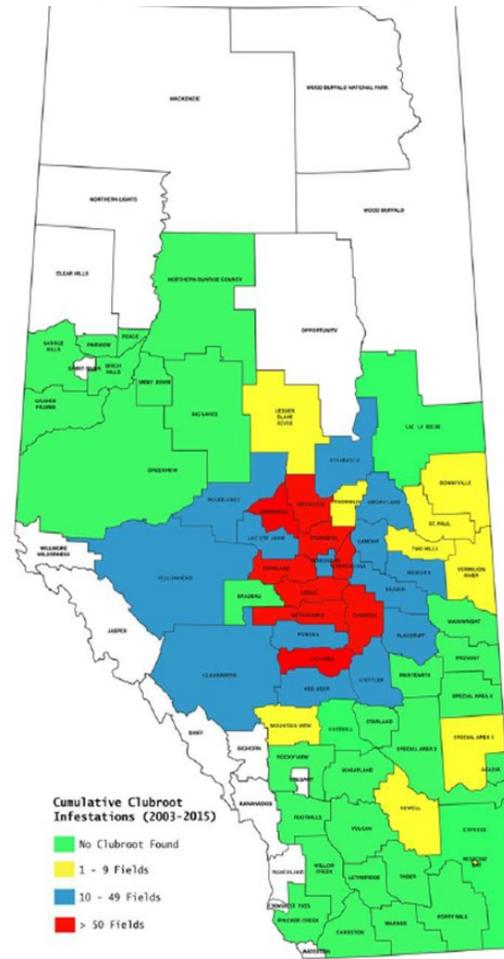
P. Brassicae has spread rapidly for a soilborne pathogen.

Currently there are over 2700 cases across the province.

Resistance was broken by pathotype 5x



courtesy of: Stephen Strelkov



Control

Pathotype 5X

Pool

8

reps

per

cage

➔

7dai

14dai

21dai



RNA-seq analysis of Laurentian vs Brutor

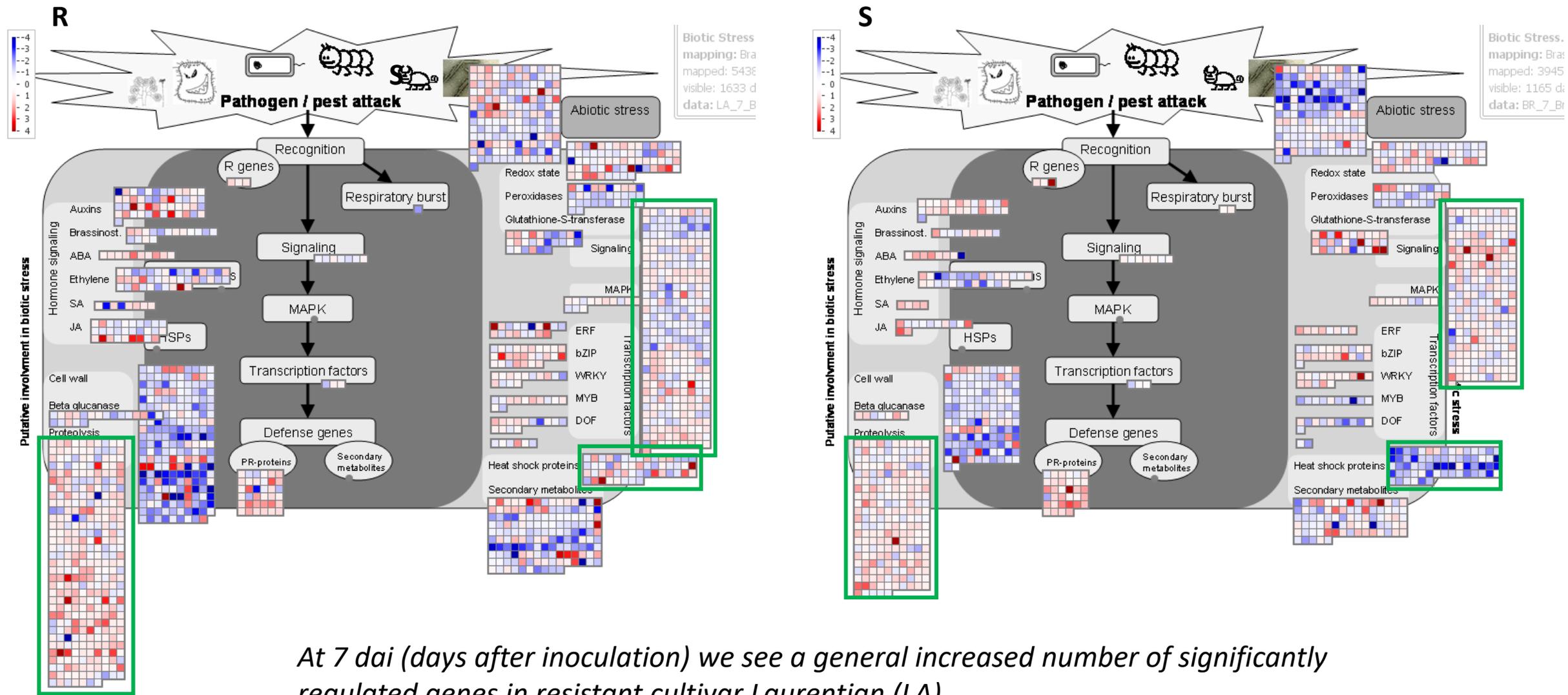
cultivar	harvest	up	down	total
Laurentian (R)	<i>7 dai</i>	2946	2592	5538
	<i>14 dai</i>	1237	1510	2747
	<i>21 dai</i>	1570	2779	4349
Brutor (S)	<i>7 dai</i>	1936	1898	3834
	<i>14 dai</i>	1696	1719	3415
	<i>21 dai</i>	1221	5349	6570

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:

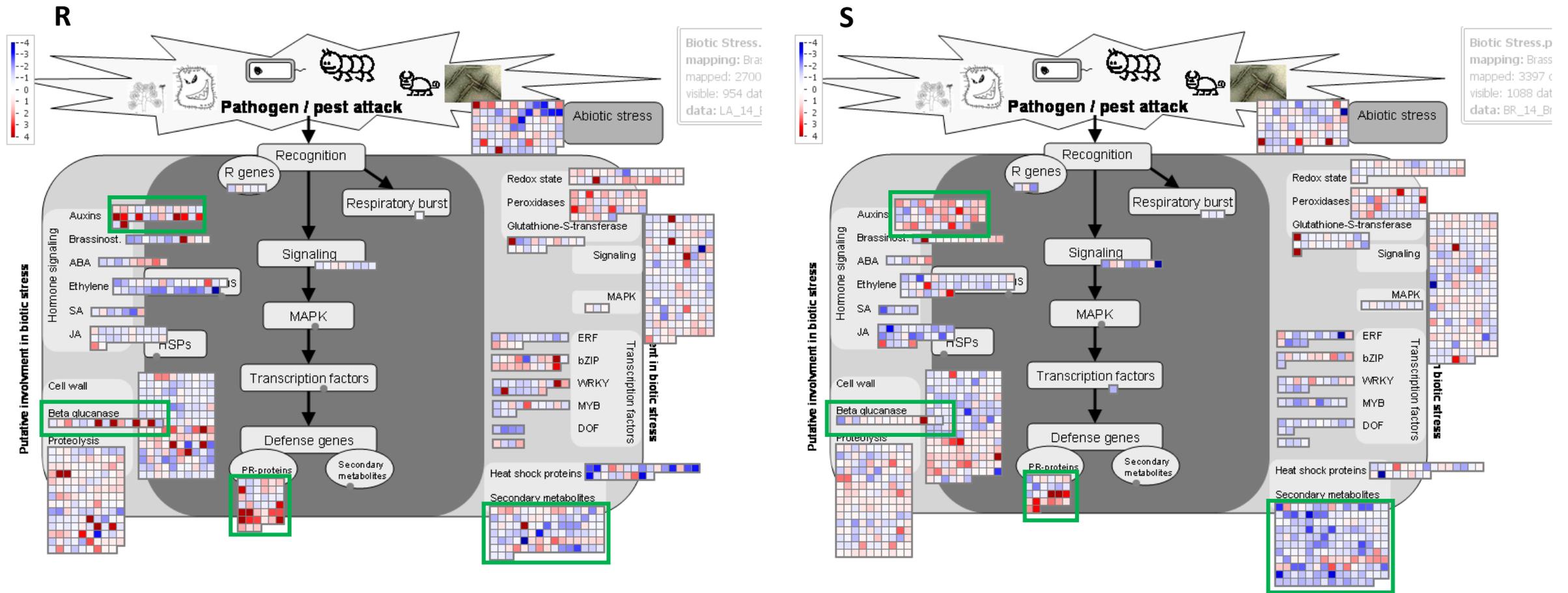
- ❖ Thousands of significantly regulated genes.
- ❖ A large number of regulated genes for Laurentian at 7 dai and for Brutor at 21 dai.
- ❖ More downregulated genes than upregulated for two time points in both cultivars.

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



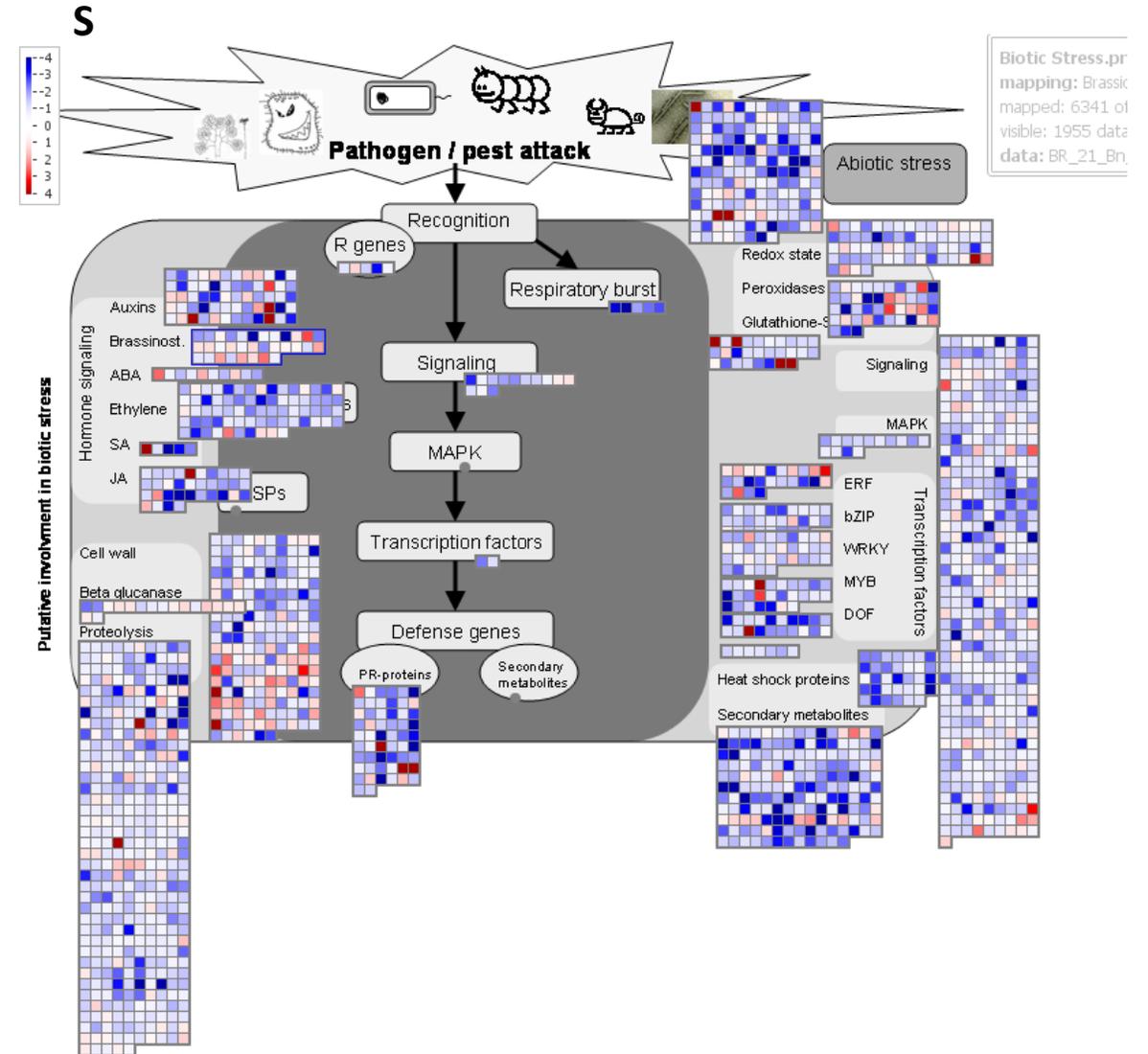
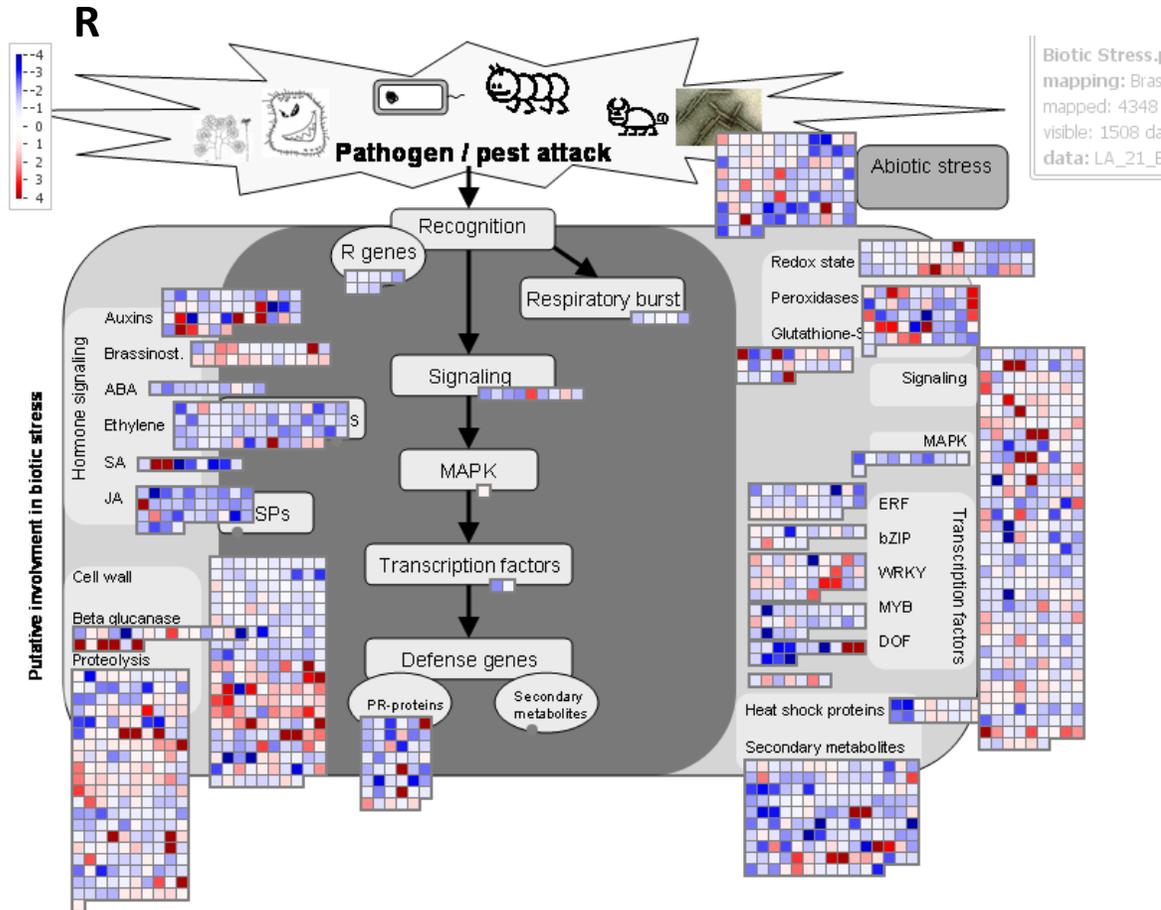
At 7 dai (days after inoculation) we see a general increased number of significantly regulated genes in resistant cultivar Laurentian (LA).

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

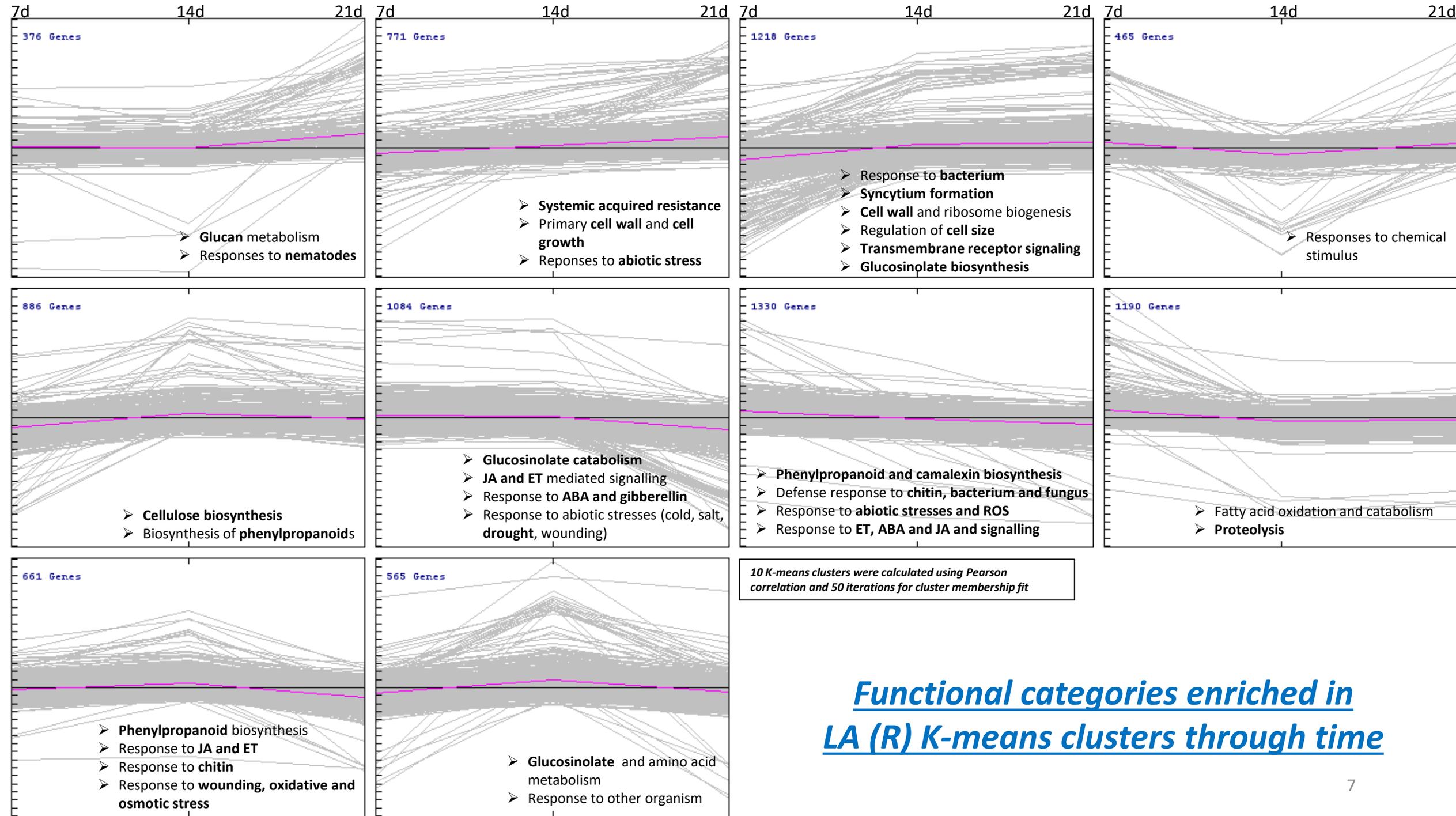


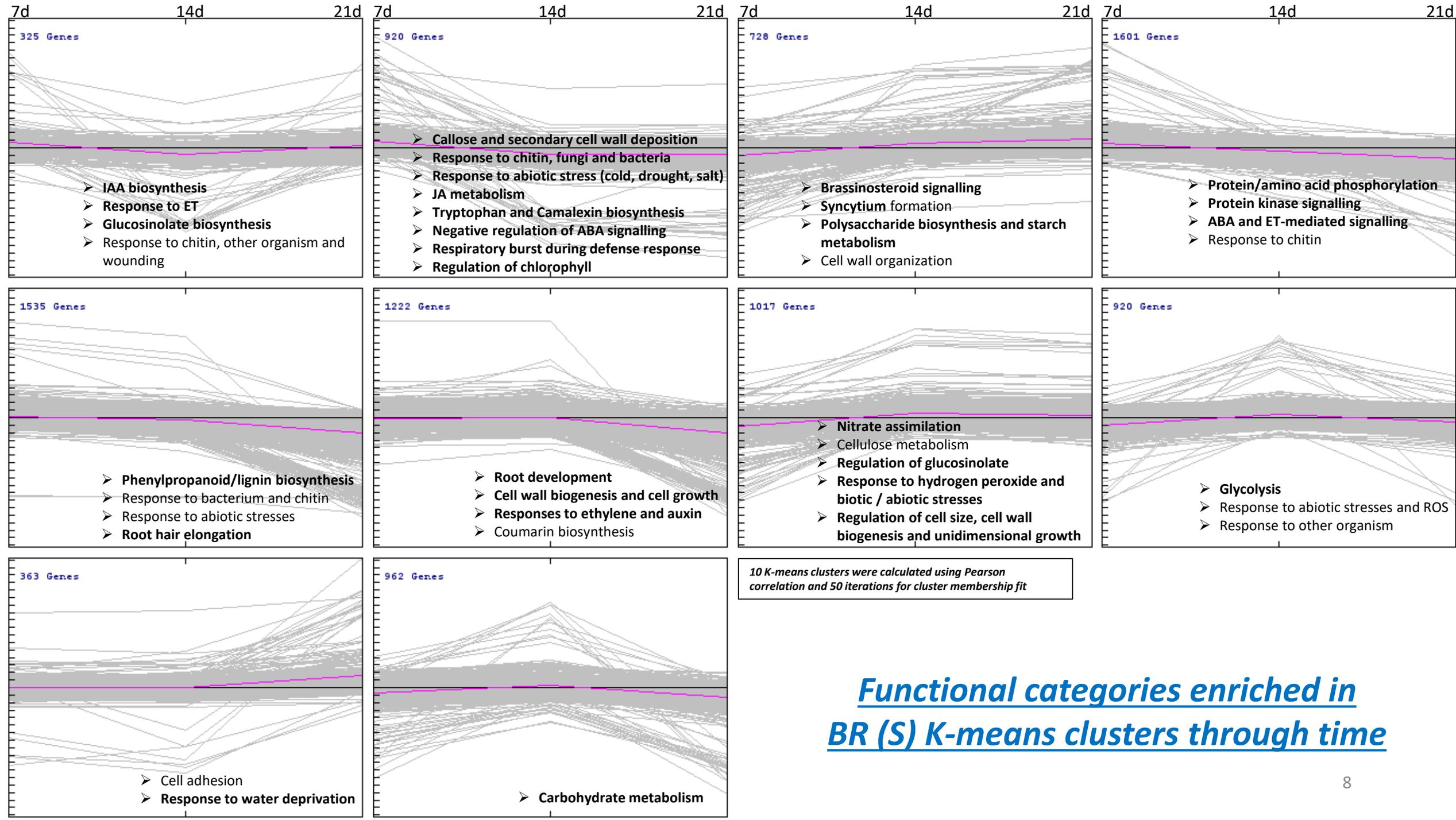
At 14 dai the relative amount of regulated genes does not seem to vary greatly between both cultivars.

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

→ 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.

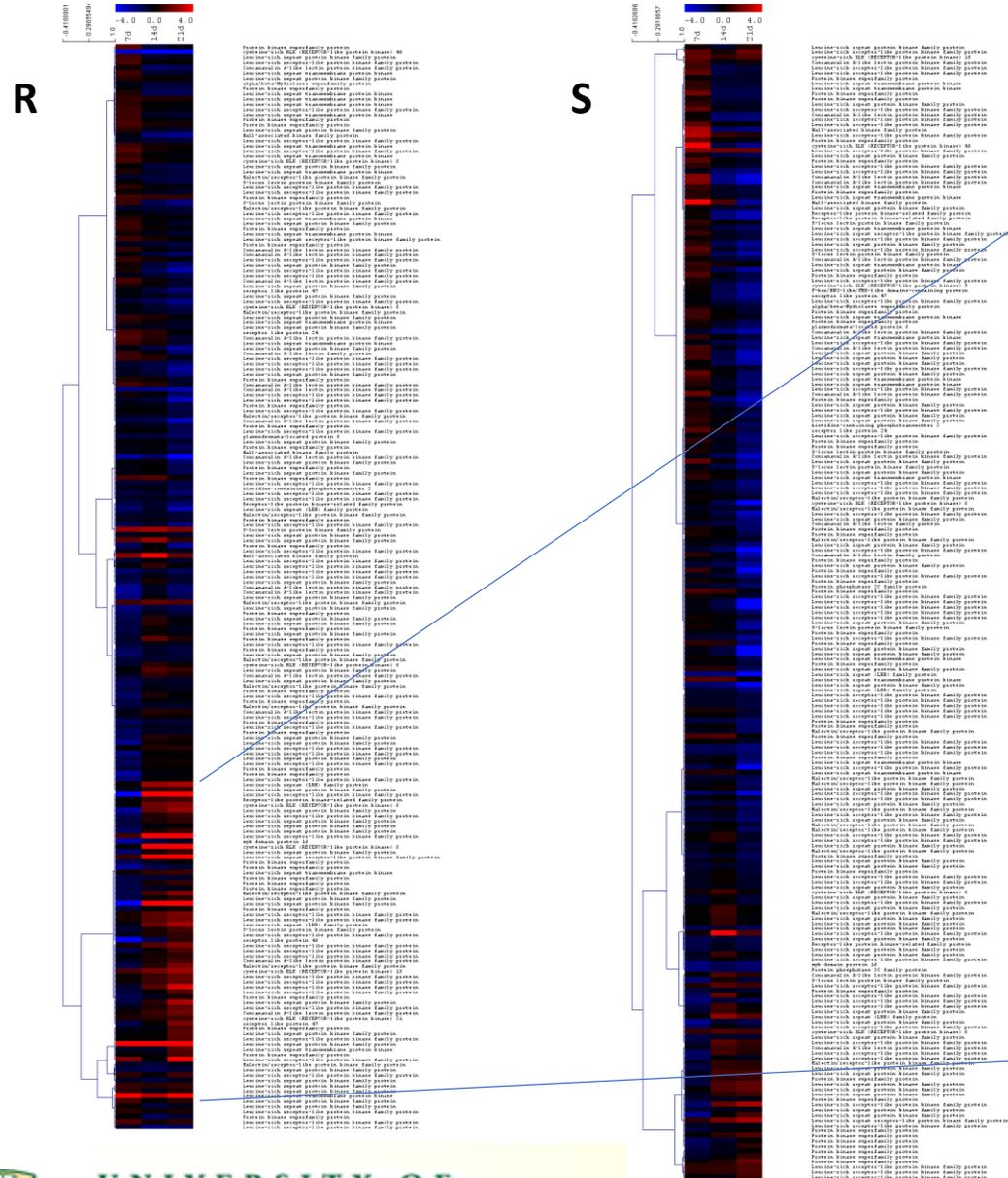
Auxin and BR signalling are only enriched on the susceptible cultivar.
 Most hormones respond at 7 and 14 dai and are downregulated 21 dai.

→ 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

Laurentian (resistant) ■
 Brutor (susceptible) ■

LA-BR receptor kinases

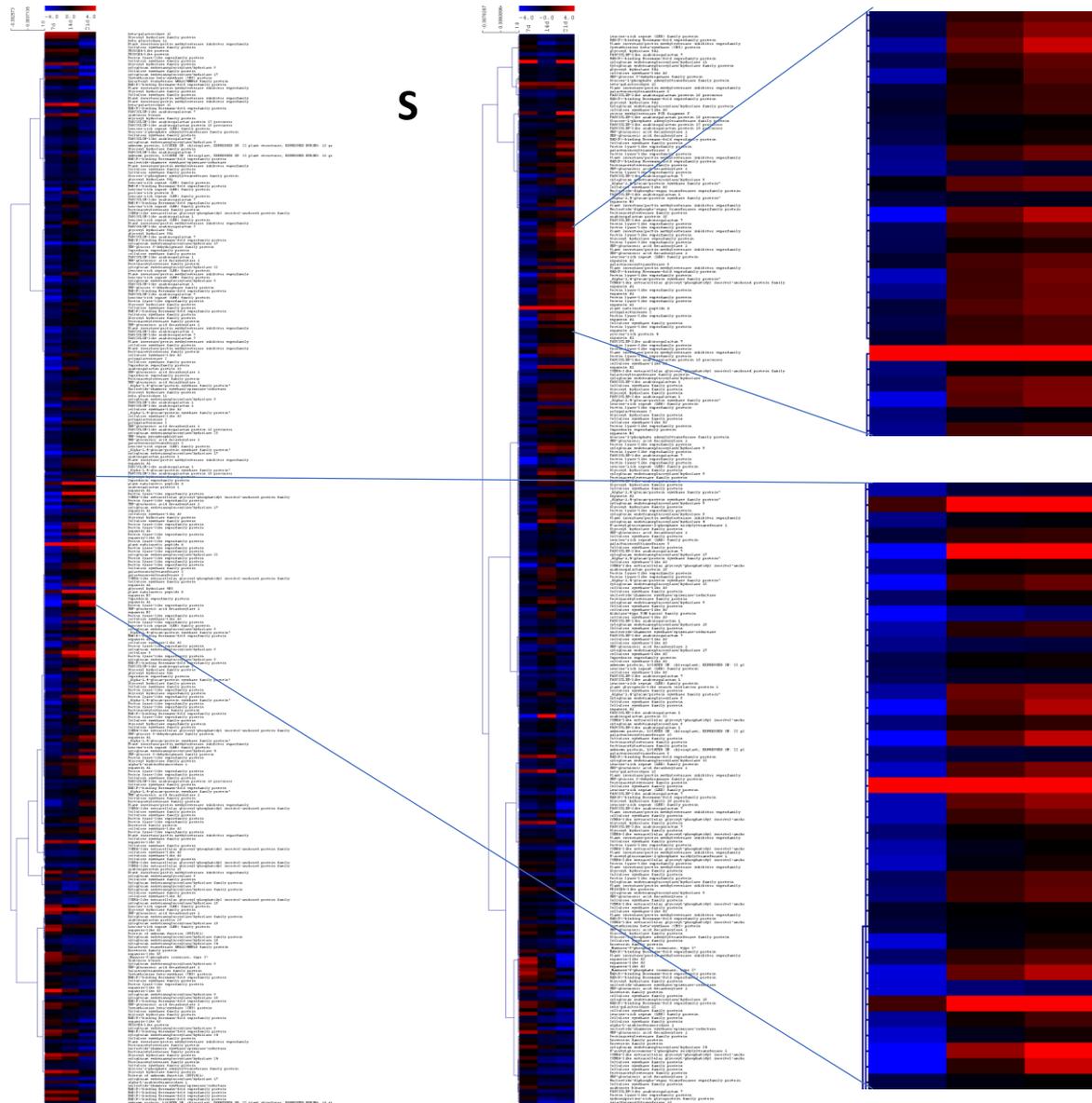


Leucine-rich receptor-like protein kinase family protein
 Leucine-rich repeat (LRR) family protein
 Leucine-rich repeat protein kinase family protein
 Leucine-rich receptor-like protein kinase family protein
 Receptor-like protein kinase-related family protein
 cysteine-rich RLK (RECEPTOR-like protein 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 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
 3-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 protein kinase family protein
 Leucine-rich repeat 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
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 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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