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

**Control**  
**Pathotype 5X**

**Pool**

8 reps per cage

7dai  
14dai  
21dai

courtesy of: Stephen Streikov
**RNA-seq analysis of Laurentian vs Brutor**

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

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

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.
At 7 dai (days after inoculation) we see a general increased number of significantly regulated genes in resistant cultivar Laurentian (LA).
At 14 dai the relative amount of regulated genes does not seem to vary greatly between both cultivars.
At 21 dai there is a strong downregulation in most categories for both cultivars but more radically in Brutor.
Functional categories enriched in LA (R) K-mean clusters through time

- Glucan metabolism
- Responses to nematodes
- Cellulose biosynthesis
- Biosynthesis of phenylpropanoids
- Glucosinolate catabolism
- JA and ET mediated signalling
- Response to ABA and gibberellin
- Response to abiotic stresses (cold, salt, drought, wounding)
- Glucosinolate and amino acid metabolism
- Response to other organism

- Systemic acquired resistance
- Primary cell wall and cell growth
- Responses to abiotic stress
- Glucosinolate biosynthesis
- Cellulose biosynthesis
- Biosynthesis of phenylpropanoids
- Glucosinolate catabolism
- JA and ET mediated signalling
- Response to ABA and gibberellin
- Response to abiotic stresses (cold, salt, drought, wounding)
- Glucosinolate and amino acid metabolism
- Response to other organism

- Response to bacterium
- Syncytium formation
- Cell wall and ribosome biogenesis
- Regulation of cell size
- Transmembrane receptor signaling
- Glucosinolate biosynthesis
- Fatty acid oxidation and catabolism
- Proteolysis

- Response to abiotic stresses (cold, salt, drought, wounding)
- Glucosinolate catabolism
- JA and ET mediated signalling
- Response to ABA and gibberellin
- Response to abiotic stresses (cold, salt, drought, wounding)
- Glucosinolate and amino acid metabolism
- Response to other organism

10 K-means clusters were calculated using Pearson correlation and 50 iterations for cluster membership fit.
Callose and secondary cell wall deposition
- Response to chitin, fungi and bacteria
- Response to abiotic stress (cold, drought, salt)
- JA metabolism
- Tryptophan and Camalexin biosynthesis
- Negative regulation of ABA signalling
- Respiratory burst during defense response
- Regulation of chlorophyll

Phenylpropanoid/lignin biosynthesis
- Response to bacterium and chitin
- Response to abiotic stresses
- Root hair elongation

IAA biosynthesis
- Response to ET
- Glucosinolate biosynthesis
- Response to chitin, other organism and wounding

IAA biosynthesis
- Response to ET
- Glucosinolate biosynthesis
- Response to chitin, other organism and wounding

Nitrate assimilation
- Cellulose metabolism
- Regulation of glucosinolate
- Response to hydrogen peroxide and biotic / abiotic stresses
- Regulation of cell size, cell wall biogenesis and unidimensional growth

Carbohydrate metabolism
- Cell adhesion
- Response to water deprivation
- Carbohydrate metabolism

Protein/amino acid phosphorylation
- Protein kinase signalling
- ABA and ET-mediated signalling
- Response to chitin

Brassinosteroid signalling
- Syncytium formation
- Polysaccharide biosynthesis and starch metabolism
- Cell wall organization

Glycolysis
- Response to abiotic stresses and ROS
- Response to other organism

Functional categories enriched in BR (S) K-means clusters through time
# Trends in functional category regulation

<table>
<thead>
<tr>
<th>Response to Stress</th>
<th>7 dai</th>
<th>14 dai</th>
<th>21 dai</th>
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</thead>
<tbody>
<tr>
<td>Response to biotic stress</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
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<tr>
<td>Response to abiotic stress</td>
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<td>✔️</td>
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<tr>
<td>Response to nematodes</td>
<td>✔️</td>
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<tr>
<td>Syncytium formation</td>
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<tr>
<td>Systemic acquired resistance</td>
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<tr>
<td>Receptor signalling</td>
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<tr>
<td>Negative regulation of signalling</td>
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<tr>
<td>Glucosinolate biosynthesis</td>
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<td>Camalexin biosynthesis</td>
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<td>Response to ROS</td>
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<td>Glutamine-glutamate metabolism</td>
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**Cell Wall**

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<tr>
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<th>7 dai</th>
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<th>21 dai</th>
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<tbody>
<tr>
<td>Cell wall modification</td>
<td>✔️</td>
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<tr>
<td>Glucan metabolism</td>
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<tr>
<td>Phenylpropanoid metabolism</td>
<td>✔️</td>
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</table>

**Hormones**

<table>
<thead>
<tr>
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<th>7 dai</th>
<th>14 dai</th>
<th>21 dai</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auxin signalling</td>
<td>✔️</td>
<td>✔️</td>
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</tr>
<tr>
<td>JA signalling</td>
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<tr>
<td>ET signalling</td>
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<tr>
<td>ABA signalling</td>
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<tr>
<td>Gibberellin signalling</td>
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<tr>
<td>BR signalling</td>
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**Cell Growth and Cell Size**

<table>
<thead>
<tr>
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<th>21 dai</th>
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</thead>
<tbody>
<tr>
<td>Root development</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
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<tr>
<td>Cell size regulation</td>
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</table>

**Protein Production and Modification**

<table>
<thead>
<tr>
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<th>7 dai</th>
<th>14 dai</th>
<th>21 dai</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribosome biogenesis</td>
<td>✔️</td>
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<tr>
<td>Translation</td>
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<tr>
<td>Amino acid metabolism</td>
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<td>✔️</td>
<td>✔️</td>
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<tr>
<td>Proteolysis</td>
<td>✔️</td>
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</tbody>
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**Metabolism**

<table>
<thead>
<tr>
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<th>21 dai</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharide biosynthesis</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
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<tr>
<td>Nitrate assimilation</td>
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<td>✔️</td>
<td>✔️</td>
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<tr>
<td>Glycolysis</td>
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<tr>
<td>Fatty acid oxidation</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

**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
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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