Influence of nitrogen constraint on quantitative resistance to clubroot in *Brassica napus*

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Use of quantitative resistance (QR) to construct resistant varieties

- Quantitative variation is more abundant in nature
- QR is more difficult to overcome by pathogen populations
- Diversity of (cellular and physiological) mechanisms underlying QTL

However QR is difficult to use:

- Many genetic factors having weak effect
- Expression of QR depending on biotic and abiotic environments

How biotic (microbiota) and abiotic factors (water availability, temperature, nutritional-nitrogen constraints) can modulate the effect / expression of clubroot quantitative resistance?
Pathosystem Clubroot – *Brassicaceae*

**Disease distribution in France**

- Absent
- Low frequent
- Medium-frequent
- Common
- Very common

**Genetics of quantitative resistance to clubroot**

- Different sources of clubroot QR
- Complex genetic architecture of QR (QTL and epiQTL)
- Weak to strong QTL effects according to both host genotype and *P. brassicae* isolate

*Brassica napus*, *Brassica oleracea*, *Arabidopsis thaliana*
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**Environmental factors favouring clubroot development**

- Soil pH
- Soil calcium content
- Temperature
- Soil moisture
- Fertilization (nitrogen fertilization)
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Impact of Nitrogen on disease development

- Nitrogen deficiency or over fertilization not only influences plant growth and development, but also disease development

- Low / high-nitrogen supply can boost... or repress plant diseases

- Many ways in which nitrogen can positively or negatively influence plant diseases
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What about the *Brassica napus / P. brassicae* pathosystem?

- Few studies have suggested that a high-nitrogen supply tends to reduce the damage caused by *P. brassicae* infection

- Winter oilseed rape is usually considered to have a high requirement for nitrogen

- ...But increasing demands for adaptation to low-input agricultural practices (especially low nitrogen input)
Diversity, genetics and molecular mechanisms involved in N x clubroot quantitative resistance responses
Experimental design

92 winter oilseed rape inbred lines
   Diversity set

108 doubled haploids
   ('Darmor-bzh' x 'Yudal')
   Segregating population

2 isolates (Europe)
   eH
   K92-16

5 isolates (Canada)
   SACAN-ss3
   SACAN-ss1
   ORCA-ss4
   AbotJE-ss1
   L-G1 + L-G2 + L-G3

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   L-G1 + L-G2 + L-G3

Experimental setup:
- Sowing
- Inoculation
- Differential fertilization
- DI measure
  - 7 days post sowing (dps)
  - 0 day post inoculation (dpi)
  - 56 dps
  - 49 dpi
Fully developed clubs and important symptoms are observed under both low and high-nitrogen supply...

<table>
<thead>
<tr>
<th>Clubroot class symptoms</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>2+</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>under N8 supply</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
</tr>
<tr>
<td>under N1 supply</td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
</tr>
</tbody>
</table>

Laperche et al. 2017
Genotype-dependent modulation of the clubroot response triggered by nitrogen

Diversity of clubroot responses among 92 oilseed rape genotypes against infection by eH isolate under high and low-nitrogen supply
The influence of nitrogen supply on host clubroot response depends on both plant and pathogen genotypes.

Canadian pathotypes (William’s classification)

Y Aigu in collaboration with S. Strelkov (Univ. Alberta)

Aigu et al. in prep
Genetic architecture of N-dependent clubroot resistance

108 DH progeny from the cross Darmor-$bzh$ x Yudal / isolate eH of $P. brassicae$

QTL architecture is similar under N1 & N8

The magnitude of the QTL effect is dependent on the fertilization level

Laperche et al. 2017
Variation of nitrogen supply exerts a switch on the effects of the two QTL controlling resting spore content.
*PbBn_C02* is the main genetic factor implied in the N1-driven resistance to isolate eH
Molecular mechanisms involved in N-dependent clubroot resistance

Choice of contrasted genotypes to be compared...

‘Yudal’  HD018

Comparing dynamics of cellular responses to clubroot infection...

Sowing  Inoculation  Root sampling  Root sampling  Root sampling
Differential fertilization  7 days post sowing (dps)  21 dps  34 dps  49 dps
0 day post inoculation (dpi)  14 dpi  27 dpi  42 dpi

Metabolomic analyses
Transcriptomic analyses
Both genotypes display similar metabolic and transcriptomic responses to nitrogen deficiency in non-inoculated conditions.

<table>
<thead>
<tr>
<th>Genes regulated in response to long-term nitrogen deficiency</th>
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<tbody>
<tr>
<td>Down regulated</td>
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<tr>
<td>Up regulated</td>
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</table>

<table>
<thead>
<tr>
<th>Amino acids</th>
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</thead>
<tbody>
<tr>
<td>Glutamine</td>
</tr>
<tr>
<td>SABA</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Alpha-Alanine</td>
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<tr>
<td>Glutamic acid</td>
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<td>Aspartic acid</td>
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<tr>
<td>Asparagine</td>
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<td>Valine</td>
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<tr>
<td>Tyrosine</td>
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<tr>
<td>Alpha-Alanine</td>
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<tr>
<td>Methionine</td>
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</table>

Aigu et al. (in prep)
In inoculated roots, SA-responses are the major features in both genotypes and in both nitrogen conditions. SA-responses to infection are not sufficient to explain the low-nitrogen clubroot resistance in Yudal.
Very few transcriptomic regulations are specific to ‘Yudal x N1’

The expression of 80 genes is specifically induced in infected roots of Yudal under low-nitrogen condition

NRT genes and auxin responsive genes
Summary

✓ Oilseed rape response to clubroot can be modulated by nitrogen supply

✓ Modulation of clubroot response triggered by nitrogen depends on both plant genotype and pathogen isolate

✓ QTL PbBn-CO2 controls partial resistance under low nitrogen supply

✓ Resistance harbored by Yudal in low-nitrogen conditions
  
  • Does not involve massive transcriptional or metabolome reprogramming
  • Is not associated to SA-related responses

  ➢ Current work to clone QTL PbBn-CO2
Summary

Similar results were obtained in Arabidopsis:

✓ Modulation of the effect of clubroot resistance QTL by flooding (water availability during the secondary phase of the *P. brassicae* life-cycle) (Gravot et al, 2016)

✓ Modulation of the effect of clubroot resistance epigenetic QTL by temperature (Liégard et al, under revision)
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Importance of linking physiological and genetic analysis for the study of abiotic-biotic stress interactions and predict the modulation of resistance in various environments
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