Using knowledge on plant hormone metabolism by *Plasmodiophora brassicae* - a possibility to control the clubroot pathogen
Clubroot disease control is difficult

- Chemical control
- Calcium (liming)
- pH
- Drainage
- Crop rotation
- Catch crops
  - Breeding
    - Resistant plants
      - Transgenics
        - Biological control
          - Endophytes
The defense reaction of the host is not strongly upregulated.

**Early infection:**
- Normal cell size
- Plasmodia of pathogen start forming
- Start of cell divisions

**Late infection:**
- Hypertrophied cells
- All developmental stages of the pathogen present
- Cell divisions

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Siemens et al. (2006) MPMI
Does it help to add salicylic acid?

Treatment with SA during disease development does not reduce disease symptoms...


...but a pre-treatment with SA before inoculation can induce resistance

Does it help to add salicylic acid?

...but a pre-treatment with SA before inoculation can induce resistance

In Arabidopsis SA synthesis is increased in clubs

chorismic acid ($^{13}$C) → phenylalanine (endogenous) → Phe(ring $d_5$) (exogenous) → trans-cinnamic acid → salicylic acid

SA is a defense signal – why does it only partially work against *Plasmodiophora*?

*Plasmodiophora* has a protein with homology to plant SABATH methyltransferases

PbBSMT has a putative secretion signal
*PbBSMT* is among the highly expressed putative secreted effectors.
*PbBSMT* is highly expressed during the development of root galls *in planta*.

Schwelm et al., 2016, EJPP
Heterologous expression of PbBSMT in *E. coli* and Arabidopsis to elucidate its function

- analysis of enzymatic function *in vitro*
- analysis *in planta*: phenotype and effect on clubroot disease development
Heterologous expression of *PbBSMT* in *E. coli* and Arabidopsis to elucidate its function

gene of interest

analysis of enzymatic function

*in vitro*
SA is a defense signal – why does it only partially work against *Plasmodiophora*?

*Plasmodiophora* has a protein with homology to plant SABATH methyltransferases.
PbBSMT can methylate salicylic acid, benzoic acid and anthranilic acid

Specific activity (mU/mg protein)

Radioactivity (dpm)

Sabine Jülke
Salicylic acid is the only substrate methylated efficiently. 

Radioactivity (dpm)

<table>
<thead>
<tr>
<th></th>
<th>SA</th>
<th>BA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ug</td>
<td>1 ug</td>
<td>100 ng</td>
<td>1000</td>
</tr>
</tbody>
</table>

$K_m$ value

<table>
<thead>
<tr>
<th></th>
<th>2.5 µM</th>
<th>100 µM</th>
<th>80 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2500</td>
<td>2500</td>
<td>1500</td>
</tr>
</tbody>
</table>

SA is most likely the natural substrate.
Where does Methyl-SA go in the plants?

Me-SA is better transported from infected roots to leaves than SA

Leaves of infected Arabidopsis plants emit Me-SA

-> more SA in leaves compared to roots

SA analogs as inducers of defense?

Radioactivity (dpm)

<table>
<thead>
<tr>
<th>Compound</th>
<th>AA</th>
<th>BA</th>
<th>4-HO-BA</th>
<th>SA</th>
<th>INA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3500</td>
<td>3000</td>
<td>1500</td>
<td>2500</td>
<td>50</td>
</tr>
</tbody>
</table>

Isonicotinic acid (INA)

Sabine Jülke
Treatment with isonicotinonic acid enhances the vigour of plants but does not reduce clubroot symptoms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Infection Rate</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>no treatment</td>
<td>100%</td>
<td>76</td>
</tr>
<tr>
<td>isonicotinonic acid</td>
<td>100%</td>
<td>79</td>
</tr>
</tbody>
</table>
Heterologous expression of *PbBSMT* in *E. coli* and Arabidopsis to elucidate its function

*gene of interest*

analysis *in planta*: phenotype and effect on clubroot disease development
Overexpression of *PbBSMT* severely compromises Arabidopsis performance

Bulman et al., Plant Biol. 2018
DEX::PbBSMT plants are more susceptible to clubroot

Bulman et al., Plant Biol. 2018
Clubroot symptoms of DEX::PbBSMT plants

**A**

Infection rate (%)

- Control
- DEX

**B**

Disease Index

- WT
- DEX::PbBSMT

**C**

Images showing clubroot symptoms.

**D**

Percentage of plants in individual disease classes

- WT
- WT DEX
- DEX::PbBSMT
- DEX::PbBSMT DEX

Bulman et al., Plant Biol. 2018
Relative *PbBSMT1* expression in DEX::PbBSMT clubroots

A

![Bar graph showing relative PbBSMT expression](image)

B

![Bar graph showing relative PbActin expression](image)

Bulman et al., Plant Biol. 2018
Can we use plants with constitutive defense response or elevated SA levels to increase resistance to clubroot?
Mutants with constitutive SA signaling are more tolerant to clubroot infection

Arabidopsis mutants with constitutive SA response are dwarfs

WT

cpr1

Bowling et al. (1994) Plant Cell
Novel Arabidopsis mutants with elevated SA levels which are not dwarfs

Colombatti, Mencia, Welchen 2018, submitted
Novel Arabidopsis mutants with elevated SA levels show tolerance to clubroot

Regina Mencia
Elina Welchen
Different biocontrol agents induce different host resistance responses

**Table 4.** The expression (transcript levels) of nine genes potentially related to defence responses in canola at 14 days after a treatment with *Heteroconium chaetospira* BC2HB1 plus *Plasmodiophora brassica* (Pb) or Pb alone, relative to the control (n = 8).

<table>
<thead>
<tr>
<th>Gene family</th>
<th>Metabolic pathway</th>
<th>Transcript levels relative to control (fold-change)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pb only</td>
</tr>
<tr>
<td><strong>BnSAM3</strong></td>
<td>Ethylene</td>
<td>0.66±0.15</td>
</tr>
<tr>
<td><strong>BnACO</strong></td>
<td>Ethylene</td>
<td>0.50±0.20</td>
</tr>
<tr>
<td><strong>BnOPR2</strong></td>
<td>Jasmonic acid</td>
<td>5.75±0.60**</td>
</tr>
<tr>
<td><strong>BnAAO1</strong></td>
<td>Auxin</td>
<td>12.77±2.5*</td>
</tr>
<tr>
<td><strong>BnPR-1</strong></td>
<td>PR-1 protein</td>
<td>6.70±1.21*</td>
</tr>
<tr>
<td><strong>BnPR-2</strong></td>
<td>PR-2 protein</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td><strong>BnPR-5</strong></td>
<td>PR-5 protein</td>
<td>1.02±0.25</td>
</tr>
<tr>
<td><strong>BnCCR</strong></td>
<td>Phenylpropanoid</td>
<td>1.20±0.30</td>
</tr>
<tr>
<td><strong>BnOPCL</strong></td>
<td>Phenylpropanoid</td>
<td>1.30±0.25</td>
</tr>
</tbody>
</table>
Defense can be induced by the fungal endophyte *Acremonium alternatum*

Pb only  Pb + *Acremonium*

Chinese cabbage - Doan et al. (2010) Acta Hort


The live fungus is not necessary to induce tolerance

Susann Auer
Defense can be induced in Arabidopsis by *Acremonium alternatum* via the salicylic acid pathway up three days after inoculation.
Summary

Mutants with elevated SA levels are more tolerant to clubroot.

Salicylic acid is methylated by the protist and that could result in downregulation of defense.

Overexpression of PbBSMT in Arabidopsis causes a more susceptible phenotype.

Early addition of SA reduces clubroot symptoms, while late treatments do not.

Tolerance can be induced by Acremonium alternatum via induction of the SA-dependent plant defense pathway.
Thanks to:

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Susann Auer
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Regina Mencia
Diana Seidler
Sabine Jülke

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(New Zealand)
Arne Schwelm
(Uppsala, Innsbruck)
Elina Welchen
(Argentina)