



TECHNISCHE
UNIVERSITÄT
DRESDEN

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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

Resistant plants

Breeding

Transgenics

Biological control

Endophytes



Why

The defense reaction of the host is not strongly upregulated

Early infection:

Normal cell size
Plasmodia of pathogen start forming
Start of cell divisions

Biotic Stress

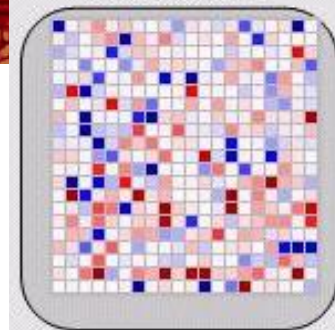


up
down

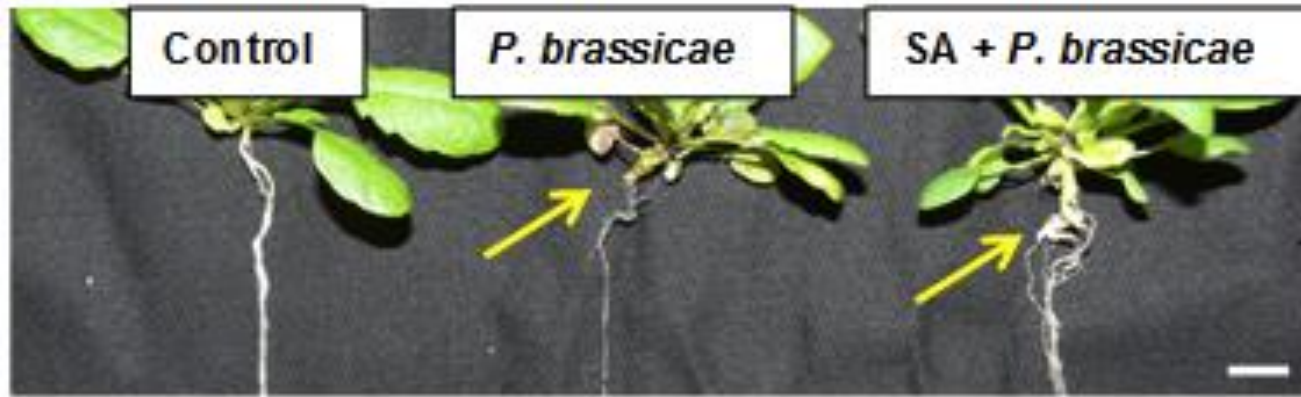
Late infection:

Hypertrophied cells
All developmental stages of the pathogen present
Cell divisions

Biotic Stress



Does it help to add salicylic acid?



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

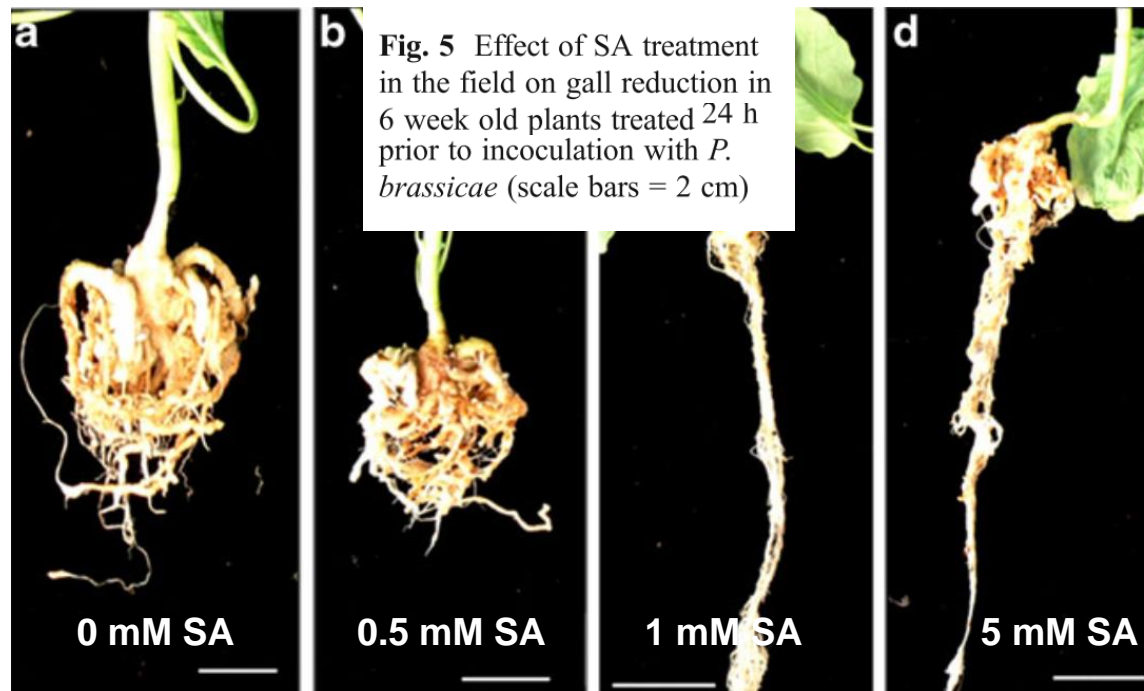
Lovelock et al, 2016, Mol. Plant Pathol.



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

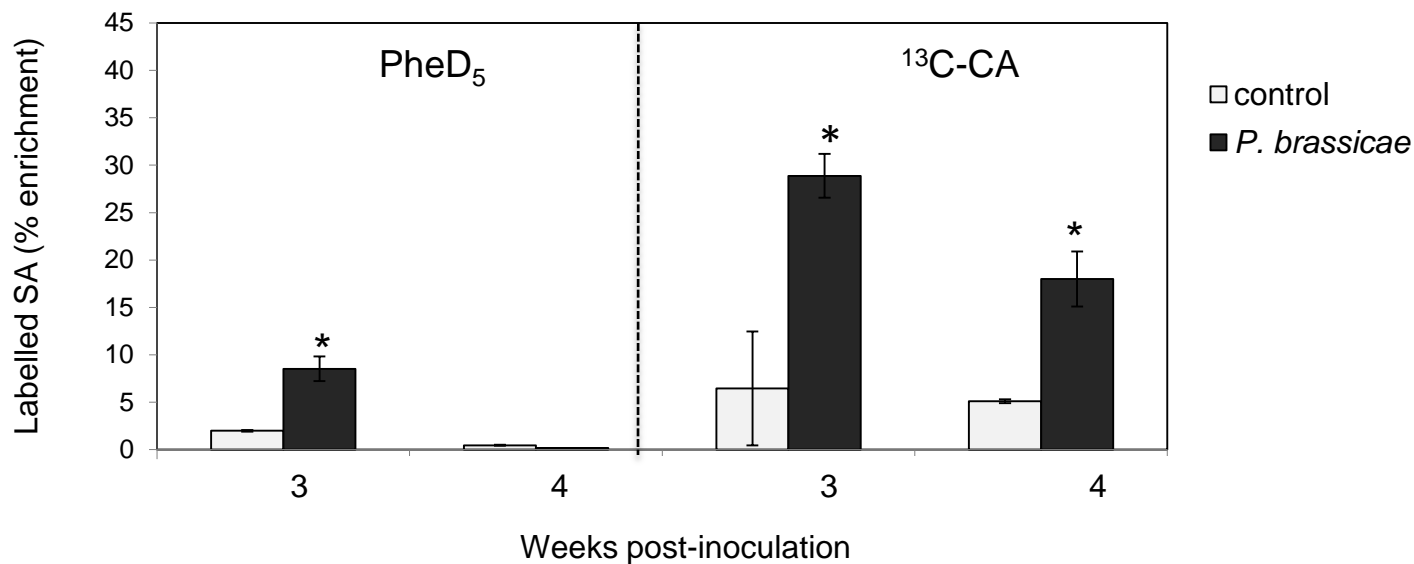
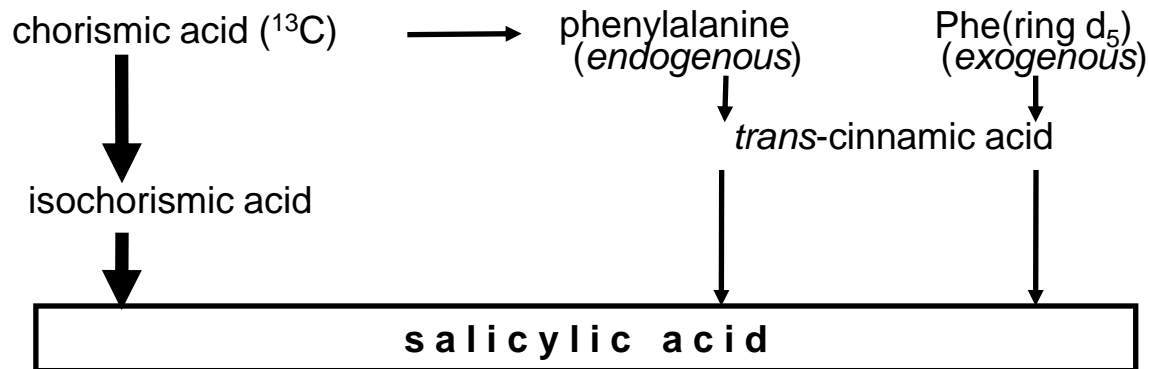
Agarwal et al., 2011, Funct. Plant Biol.

Does it help to add salicylic acid?



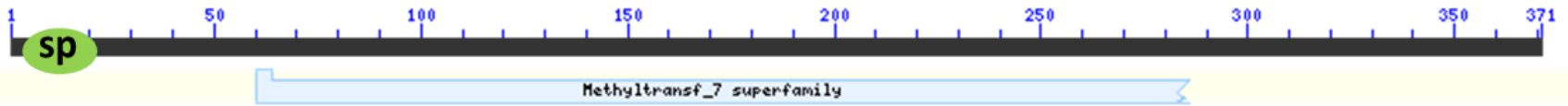
...but a pre-treatment with SA before inoculation can induce resistance

In Arabidopsis SA synthesis is increased in clubs



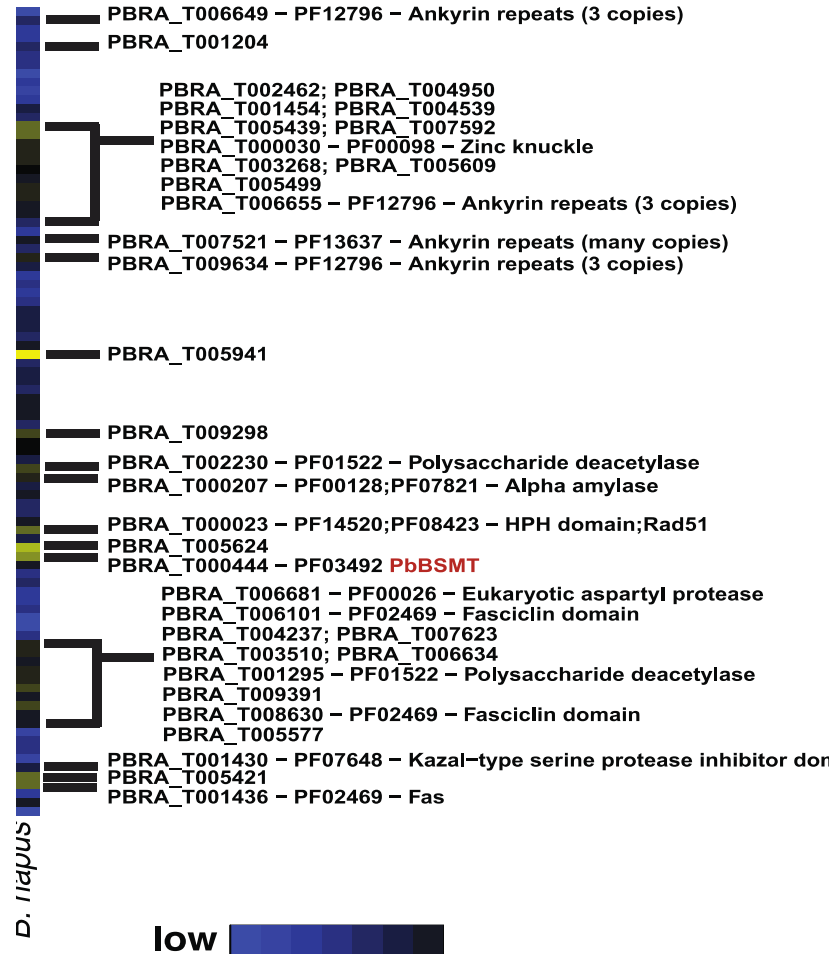
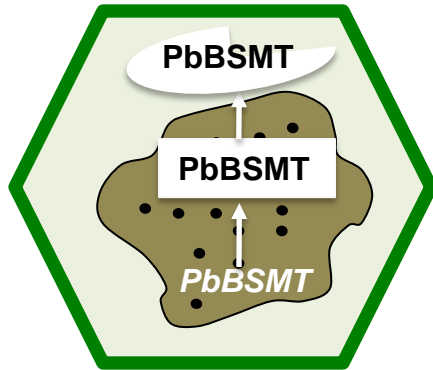
SA is a defense signal – why does it only partially work against *Plasmodiophora*?

Plasmodiophora has a protein with homology to plant SABATH methyltransferases

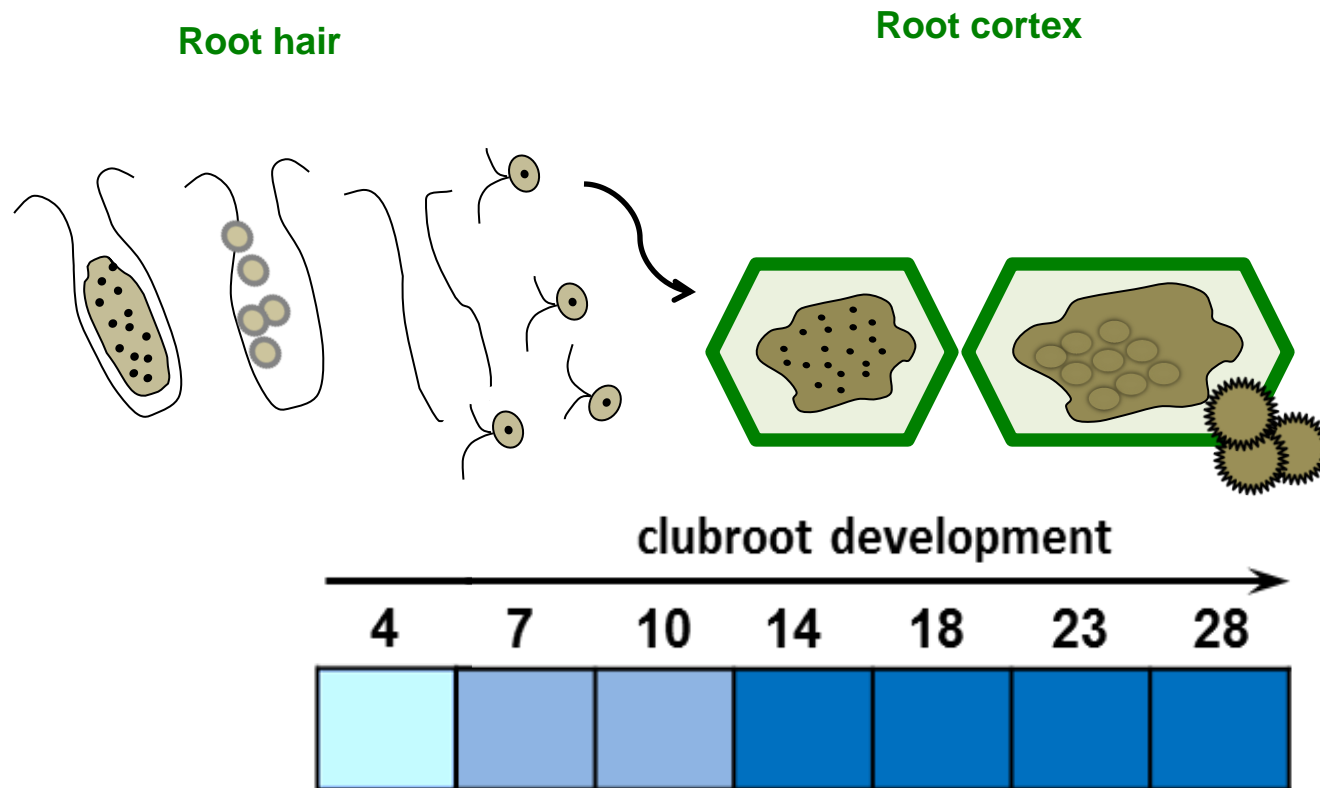


PbBSMT has a putative secretion signal

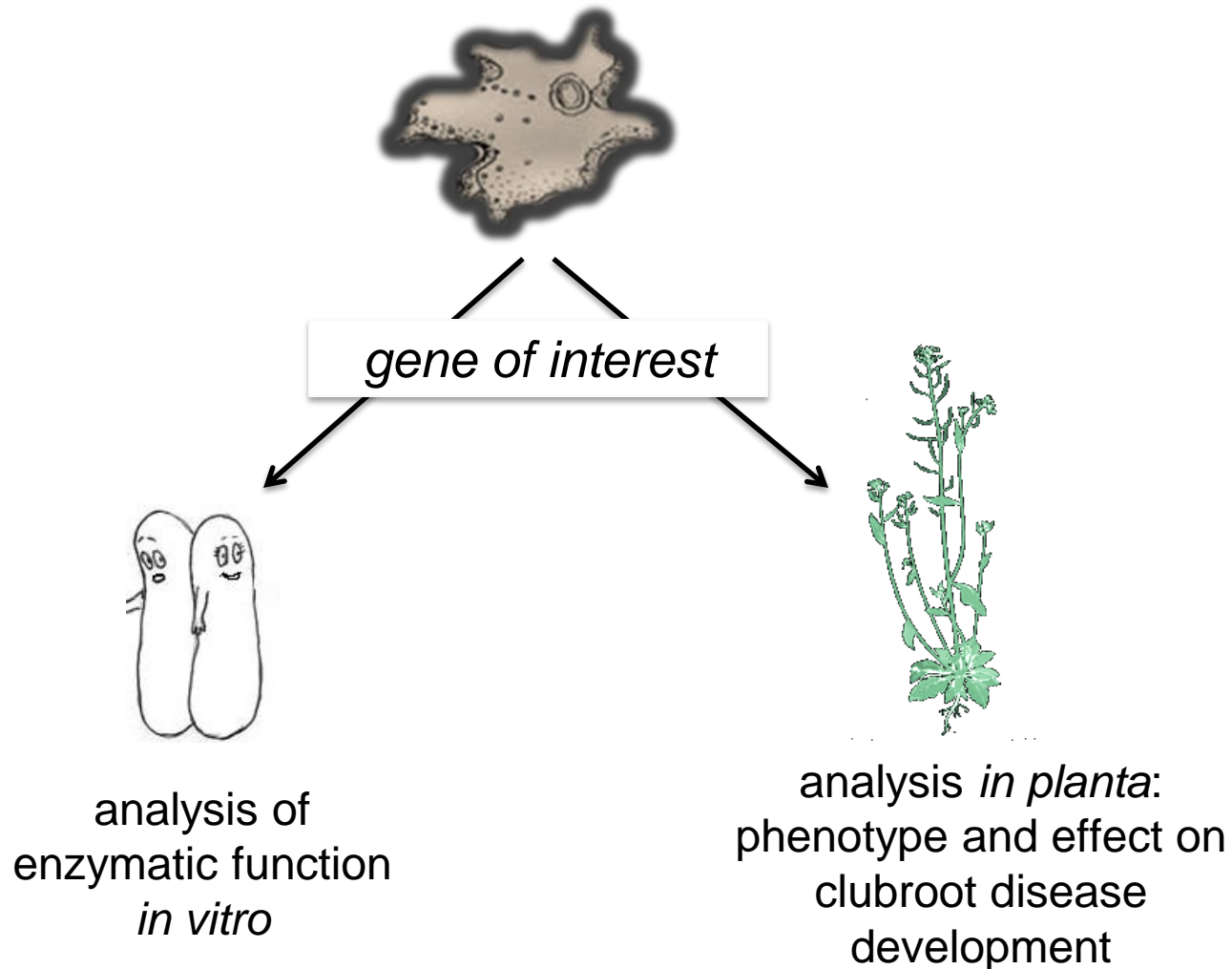
PbBSMT is among the highly expressed putative secreted effectors



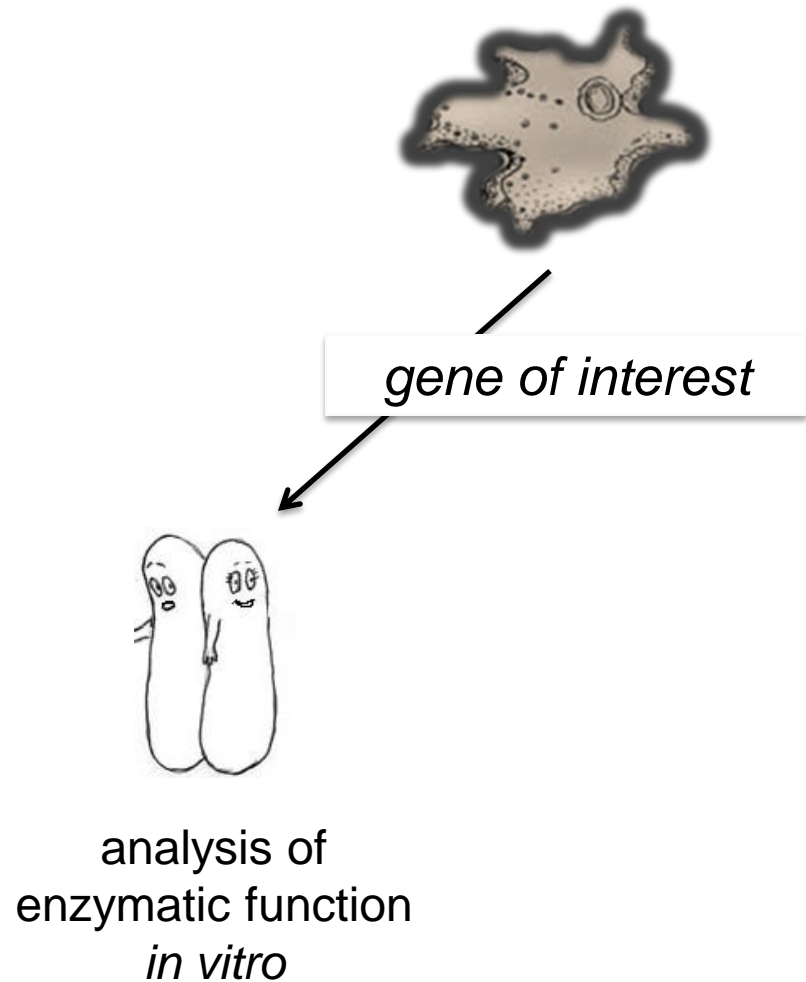
PbBSMT is highly expressed during the development of root galls *in planta*



Heterologous expression of *PbBSMT* in *E. coli* and Arabidopsis to elucidate its function

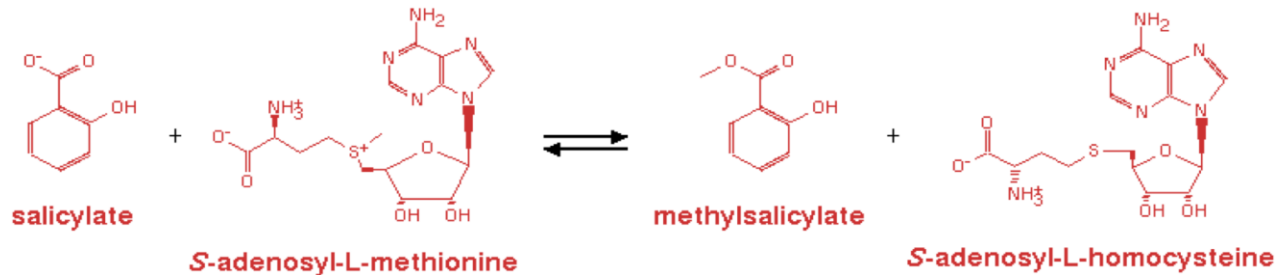
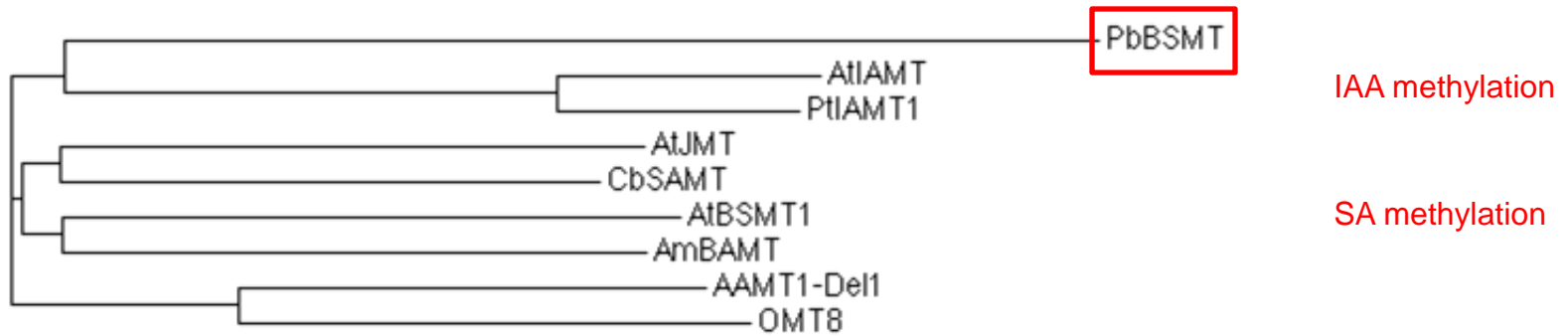


Heterologous expression of *PbBSMT* in *E. coli* and Arabidopsis to elucidate its function

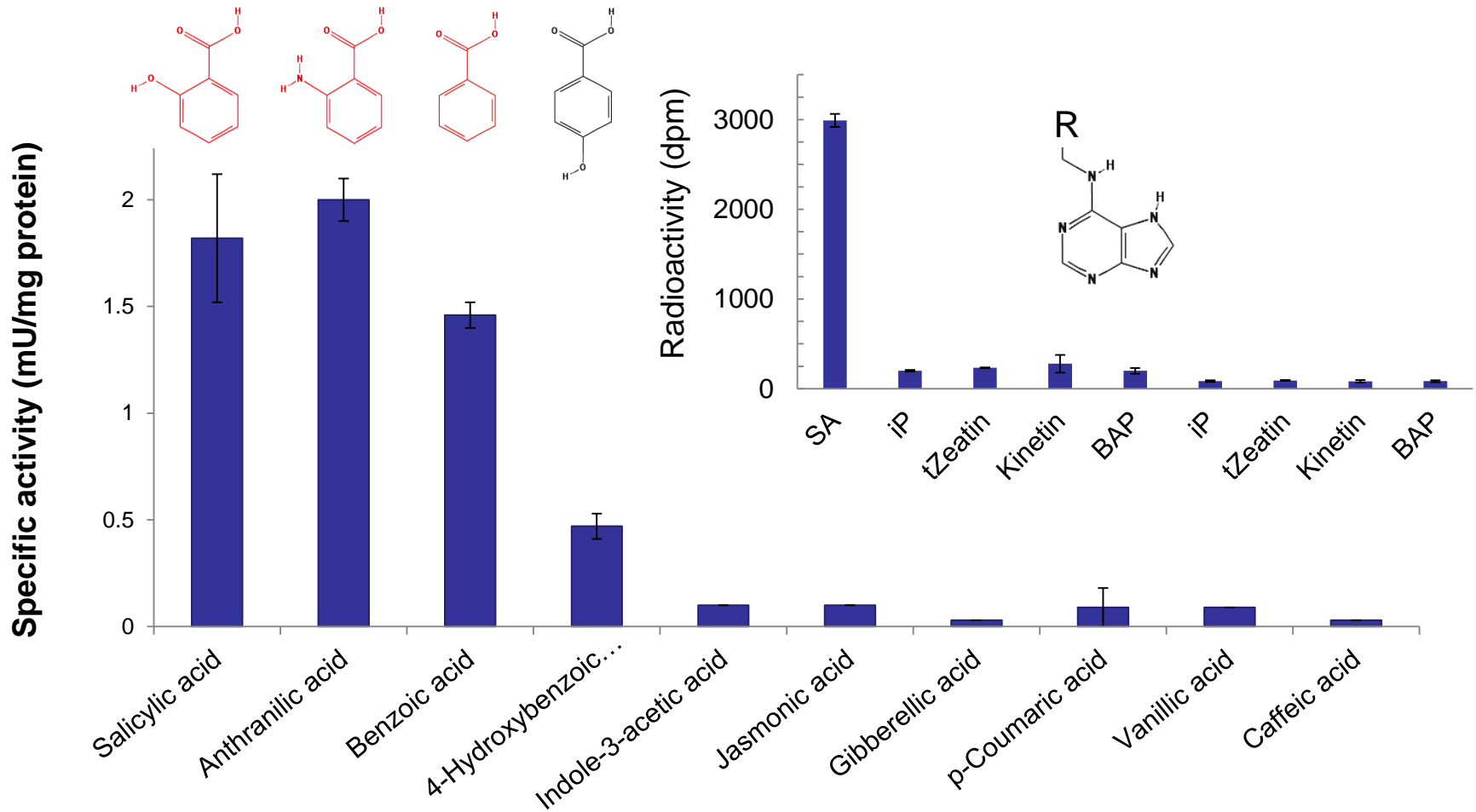


SA is a defense signal – why does it only partially work against *Plasmodiophora*?

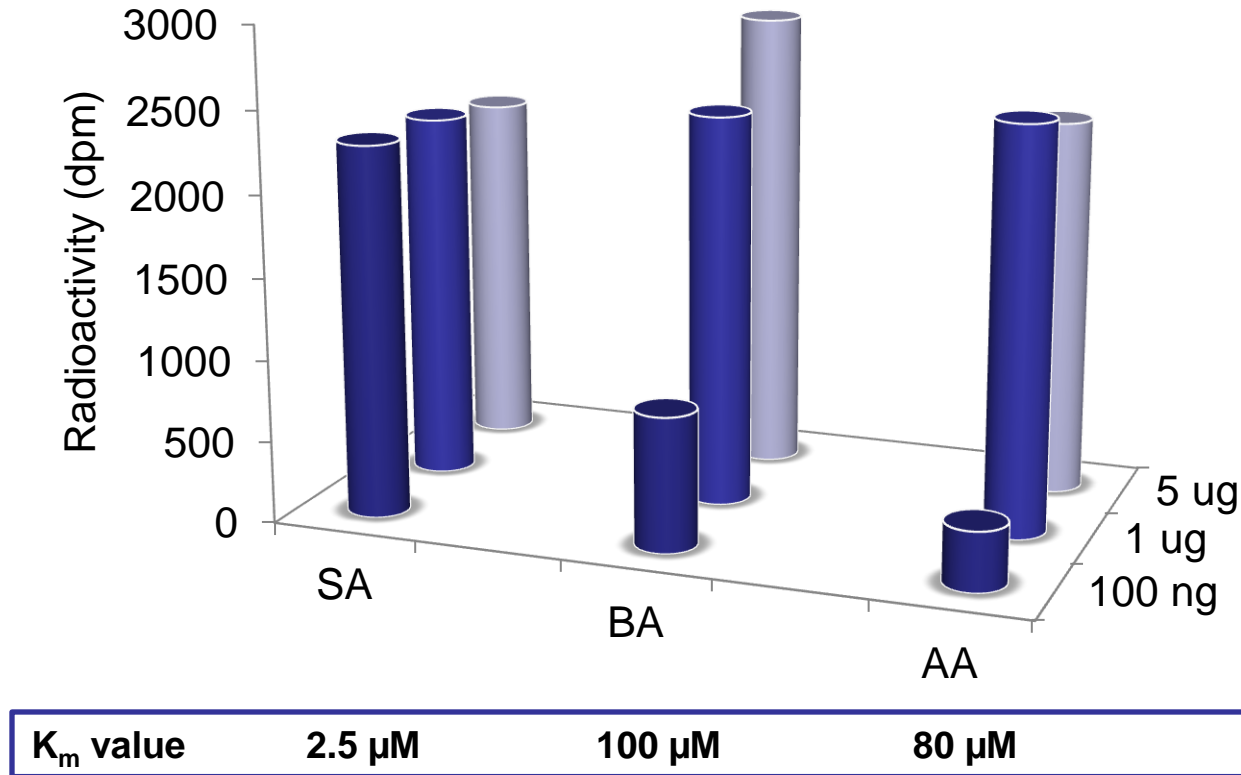
Plasmodiophora has a protein with homology to plant SABATH methyltransferases



PbBSMT can methylate salicylic acid, benzoic acid and anthranilic acid



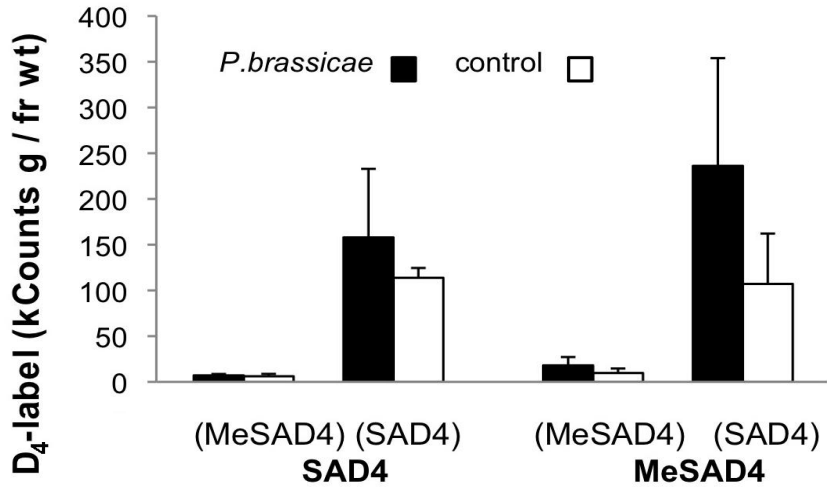
Salicylic acid is the only substrate methylated efficiently



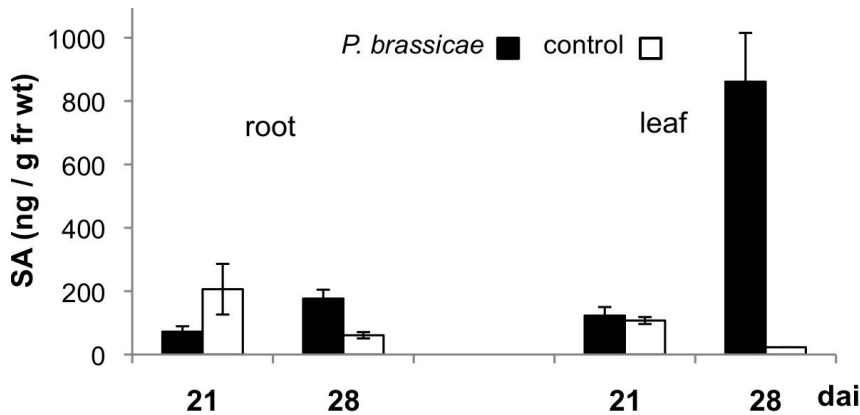
SA is most likely the natural substrate

Where does Methyl-SA go in the plants?

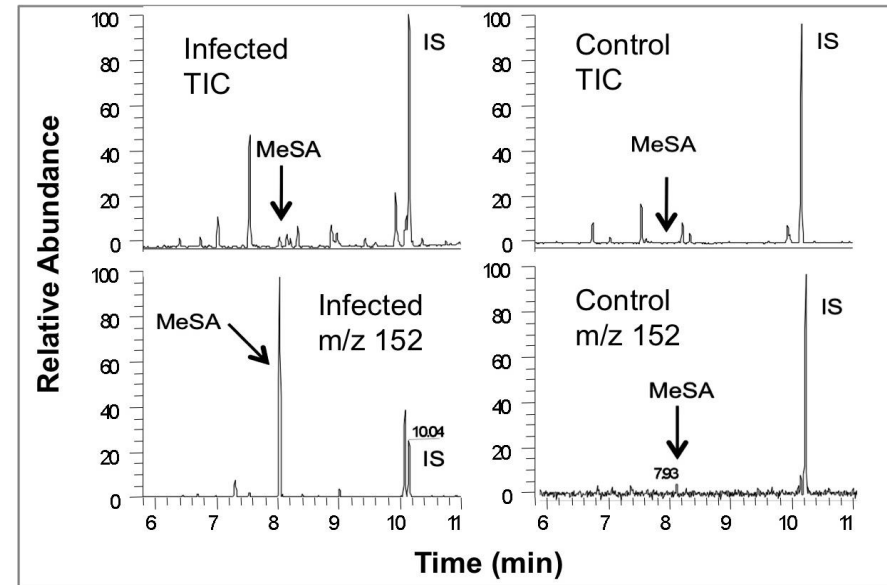
Me-SA is better transported from infected roots to leaves than SA



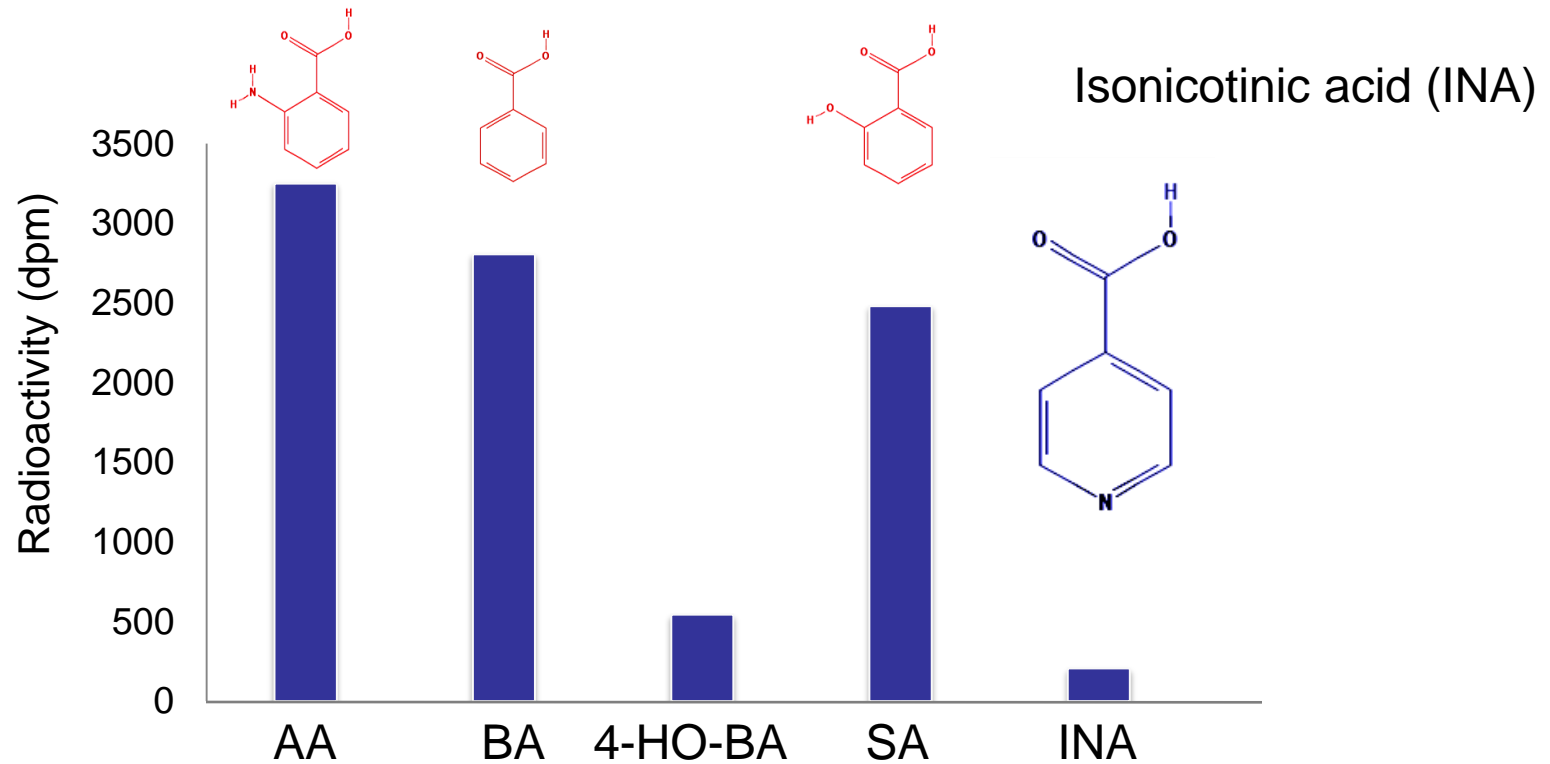
-> more SA in leaves compared to roots



Leaves of infected Arabidopsis plants emit Me-SA



SA analogs as inducers of defense?



Treatment with isonicotinic acid enhances the vigour of plants but does not reduce clubroot symptoms

no treatment



Infection rate

100%

76

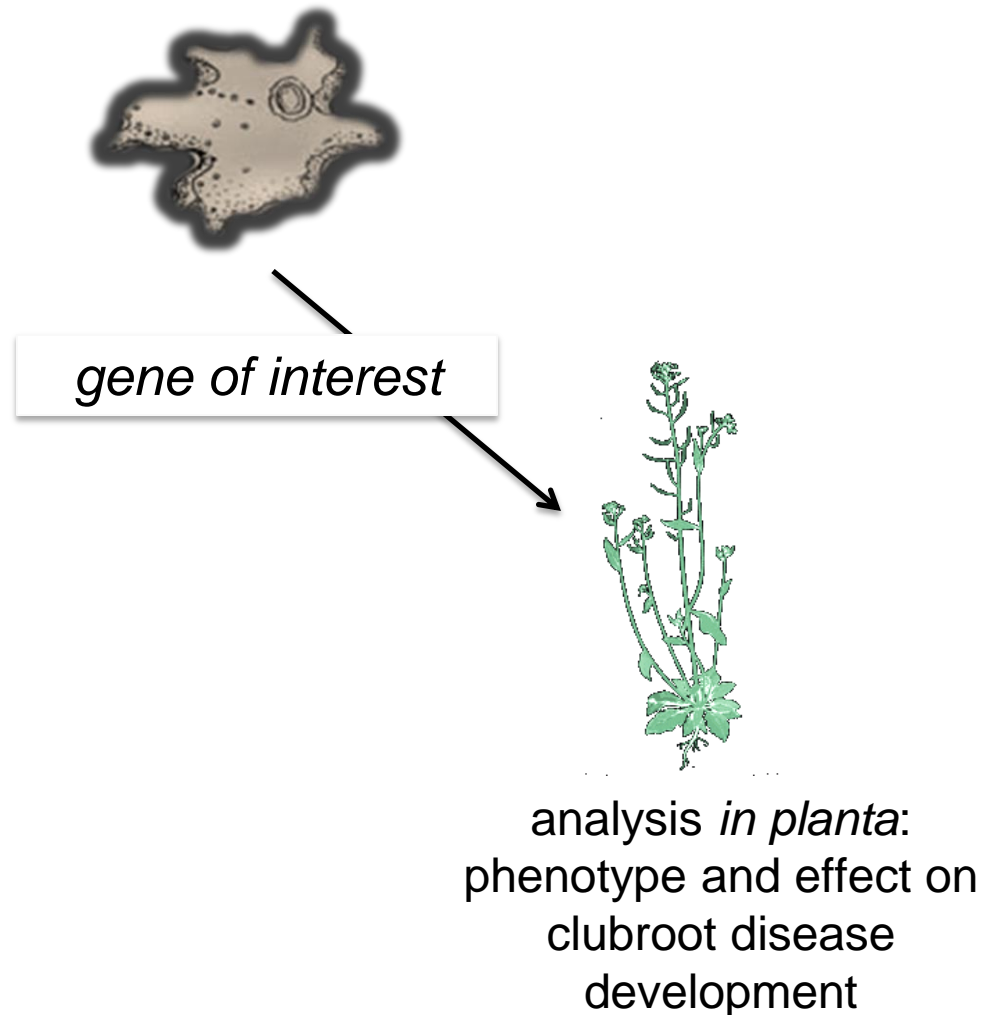


100%

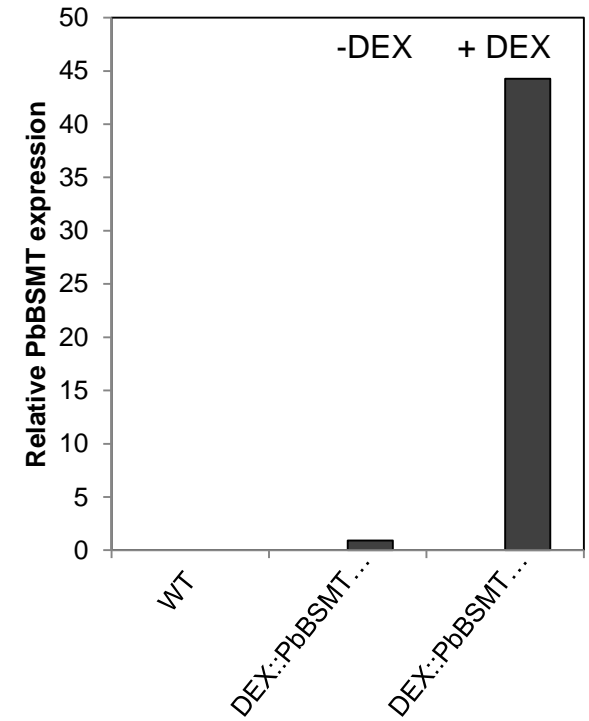
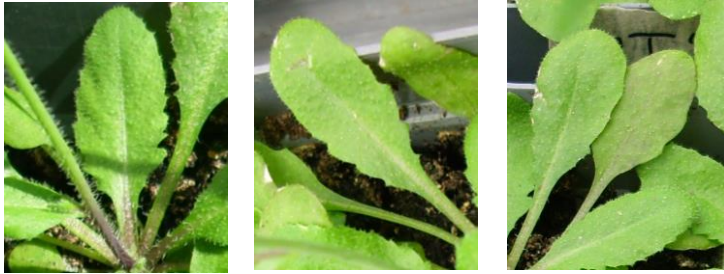
79

isonicotinic acid

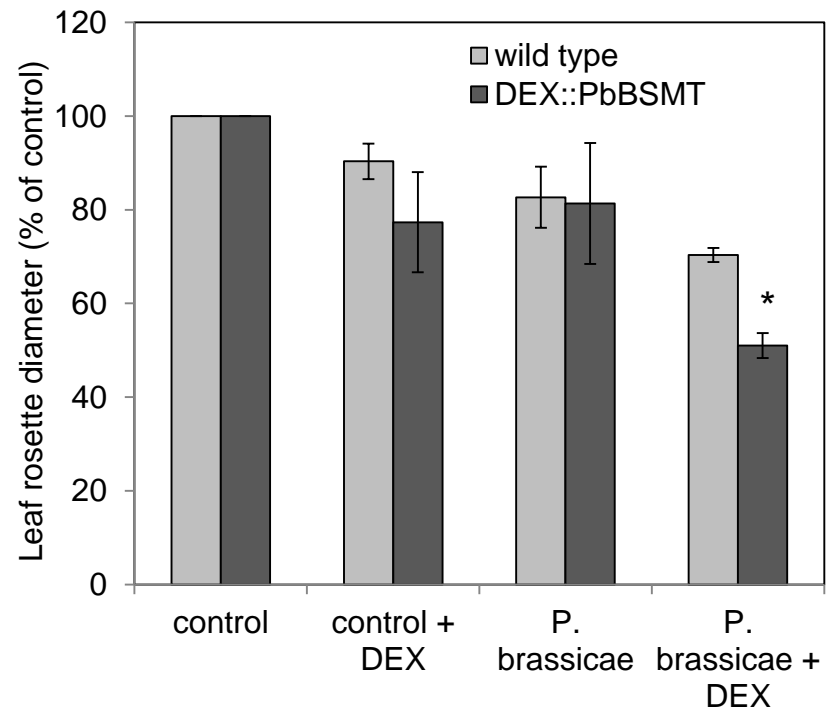
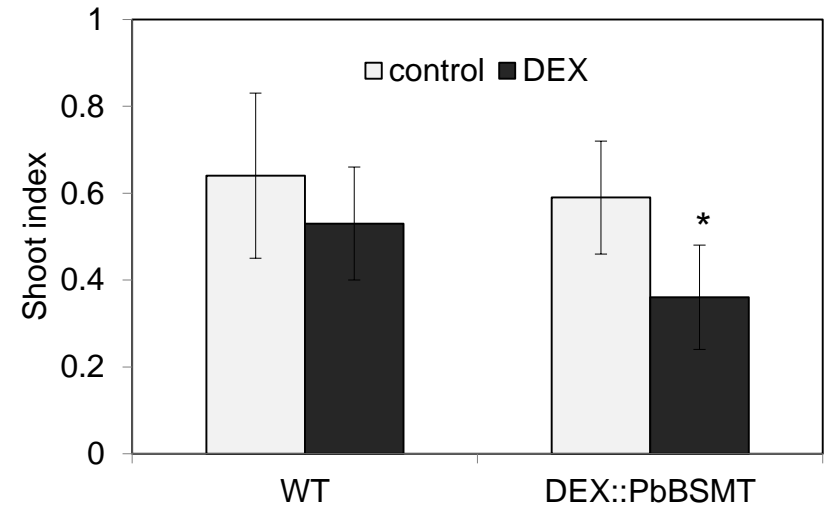
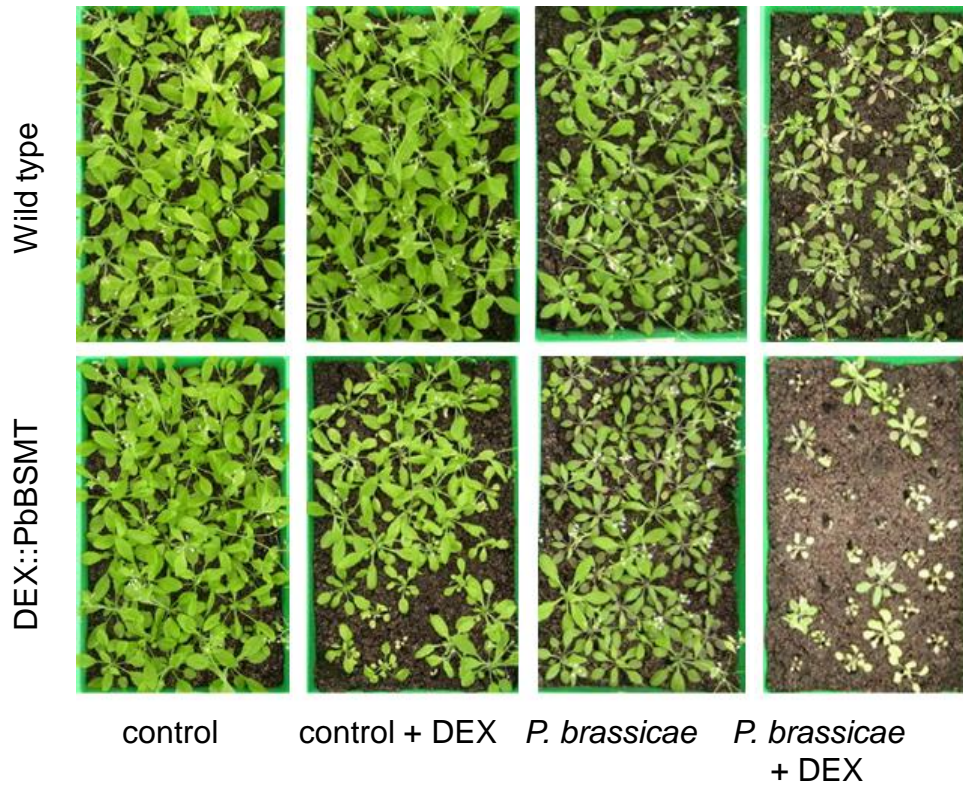
Heterologous expression of *PbBSMT* in *E. coli* and Arabidopsis to elucidate its function



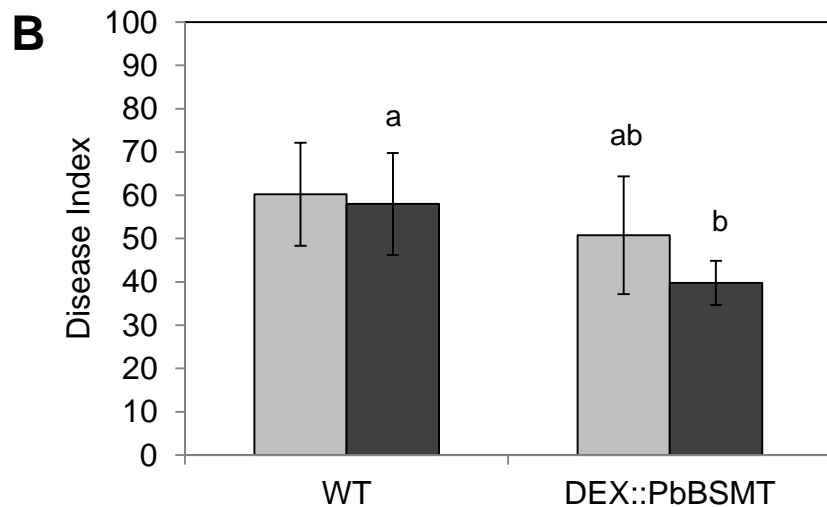
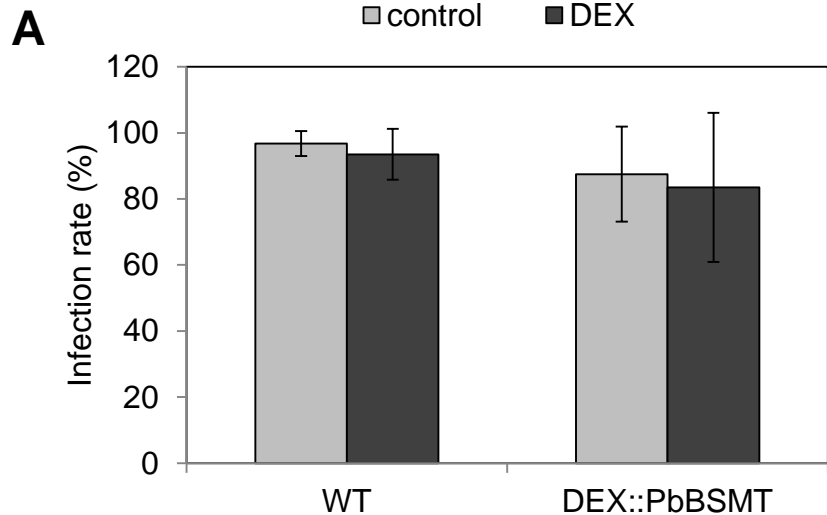
Overexpression of *PbBSMT* severely compromises Arabidopsis performance



DEX::PbBSMT plants are more susceptible to clubroot



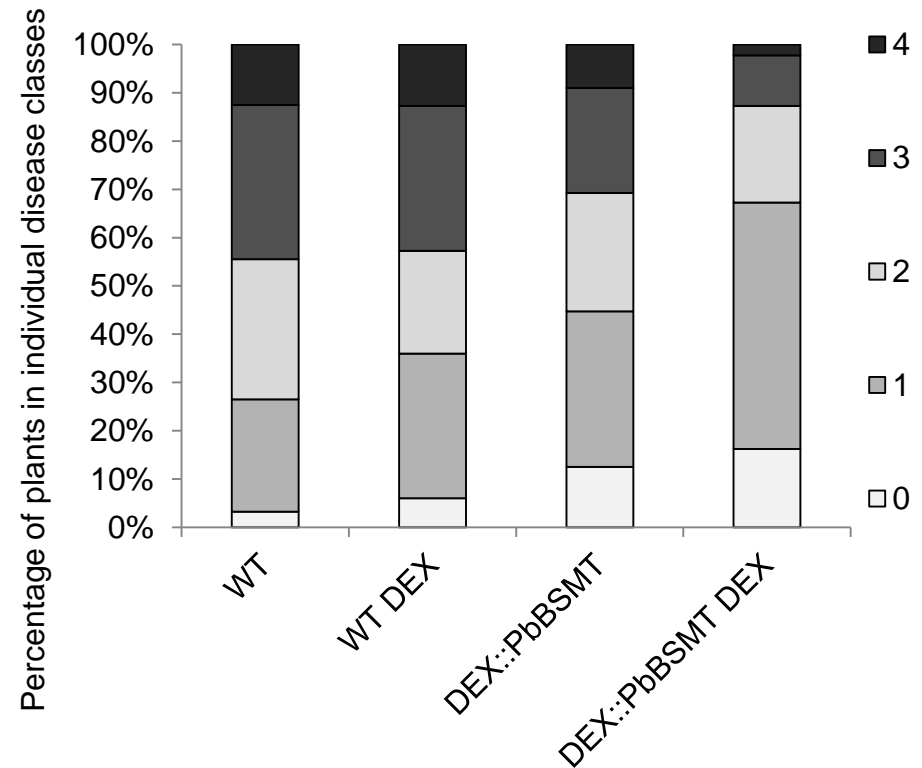
Clubroot symptoms of DEX::PbBSMT plants



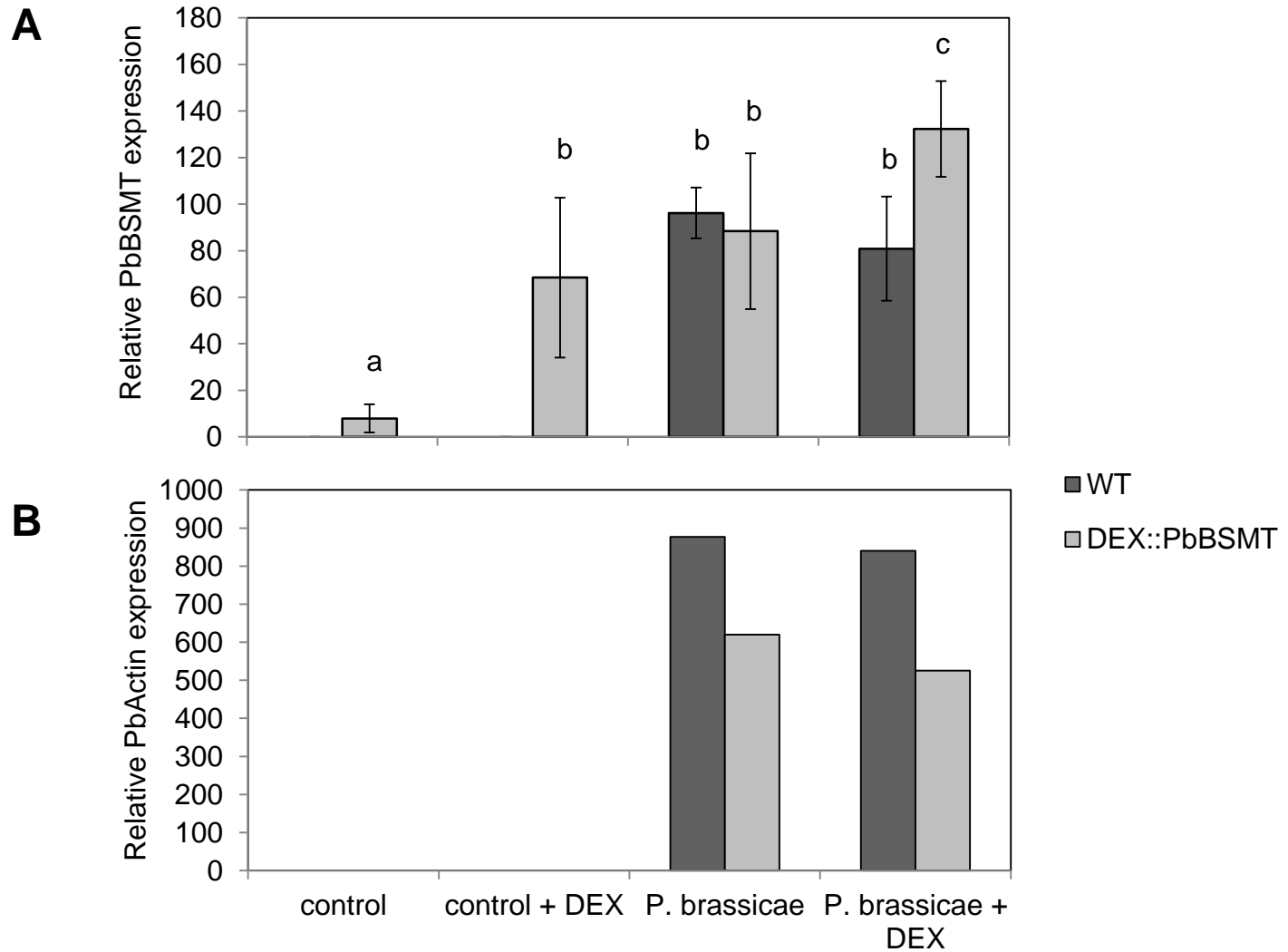
C



D

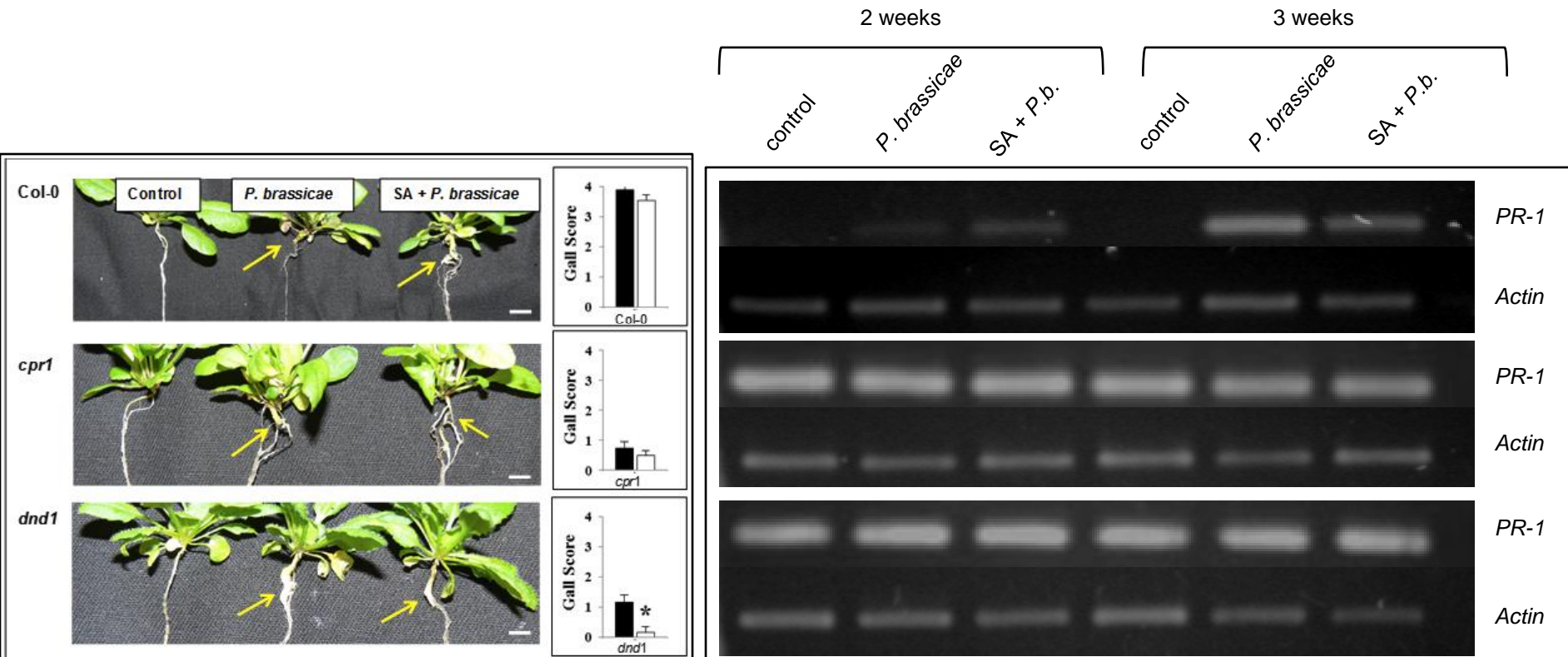


Relative *PbBSMT1* expression in DEX::*PbBSMT* clubroots

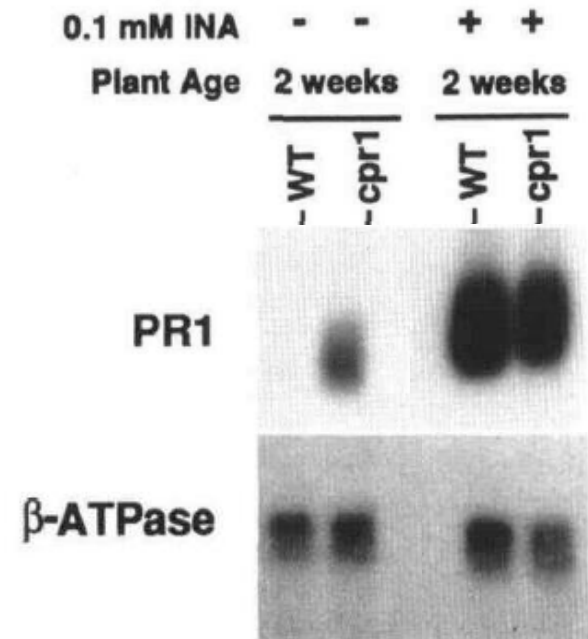
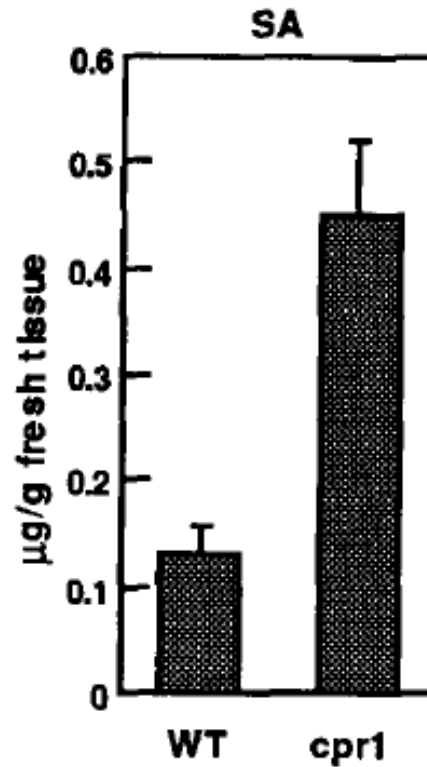
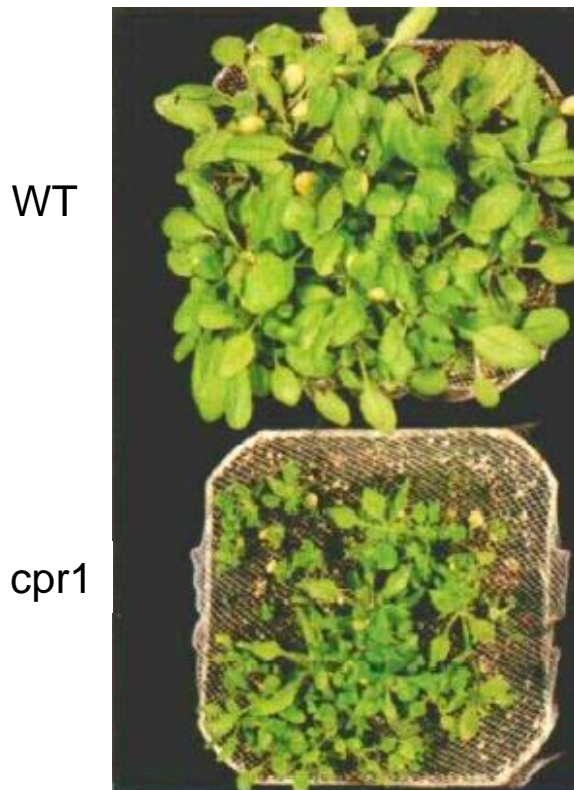


Can we use plants with constitutive defense response or elevated SA levels to increase resistance to clubroot?

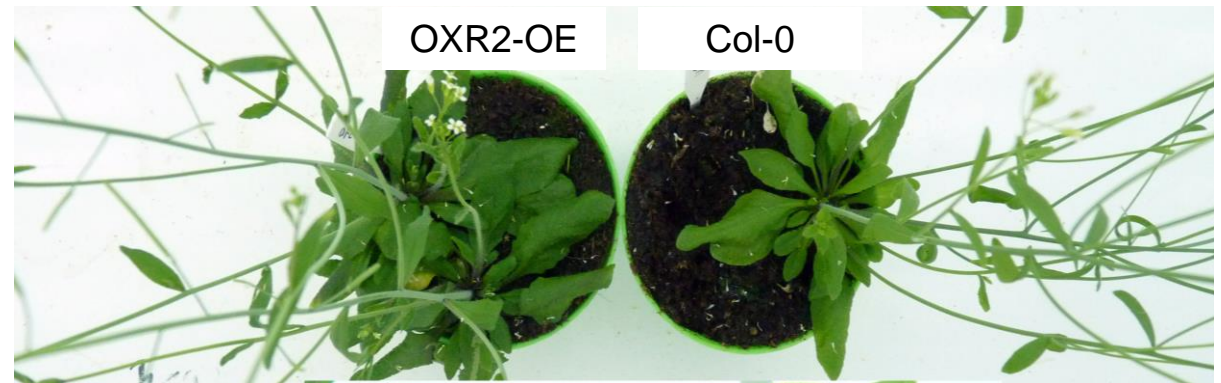
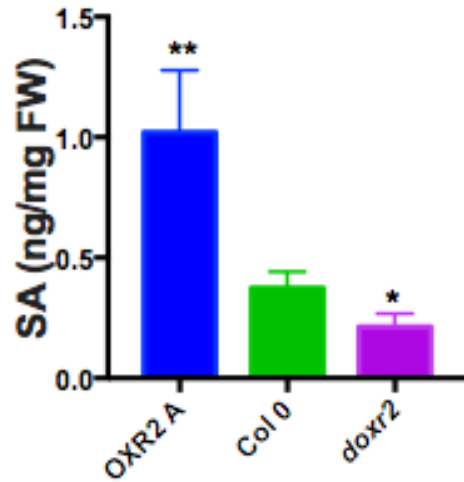
Mutants with constitutive SA signaling are more tolerant to clubroot infection



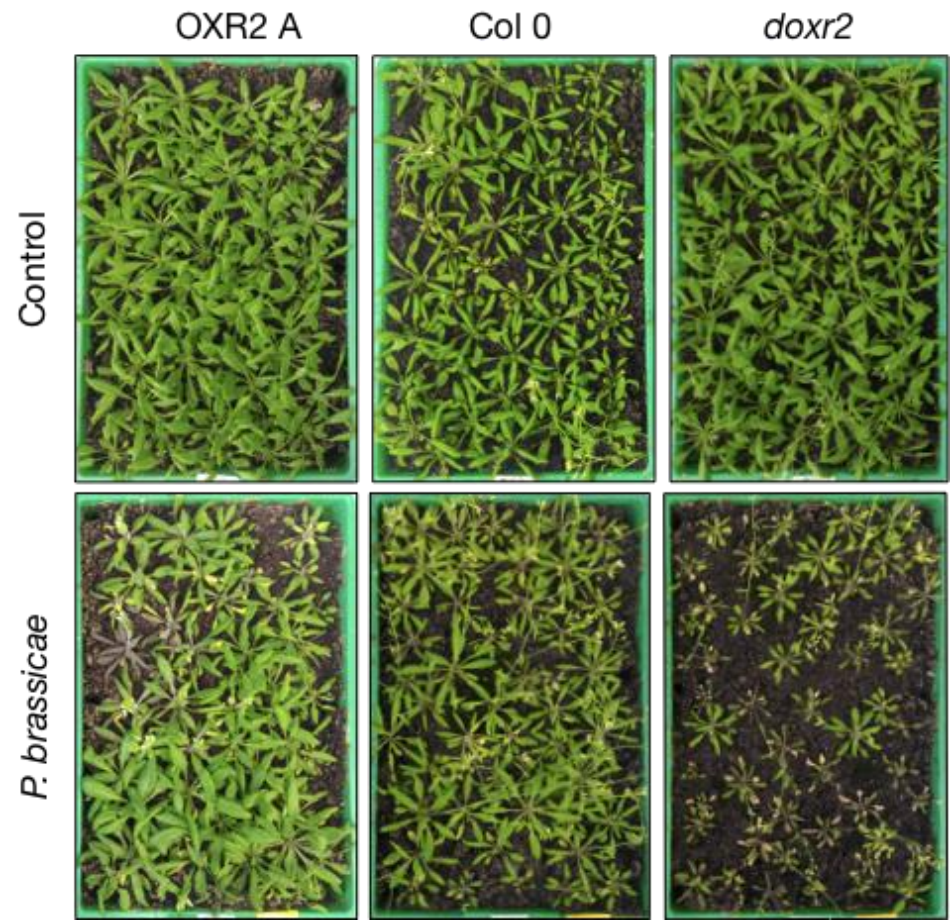
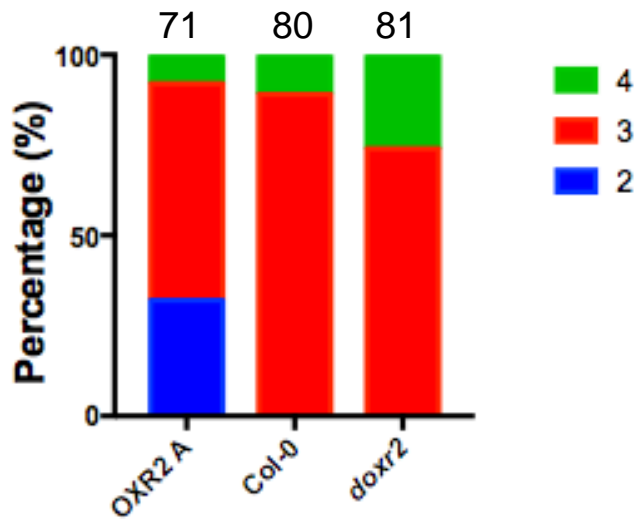
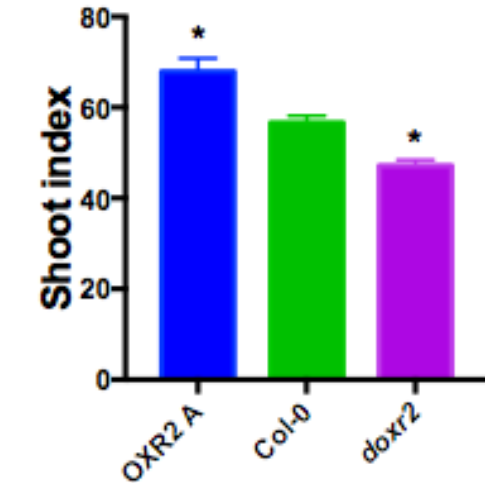
Arabidopsis mutants with constitutive SA response are dwarfs



Novel Arabidopsis mutants with elevated SA levels which are not dwarfs



Novel Arabidopsis mutants with elevated SA levels show tolerance to clubroot



Different biocontrol agents induce different host resistance responses

Heteroconium chaetospira Induces Resistance to Clubroot via Upregulation of Host Genes Involved in Jasmonic Acid, Ethylene, and Auxin Biosynthesis

Rachid Lahlali¹, Linda McGregor¹, Tao Song¹, Bruce D. Gossen¹, Kazuhiko Narisawa², Gary Peng^{1*}

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).

Gene family ^x	Metabolic pathway	Transcript levels relative to control (fold-change) ^y	
		Pb only	BC2HB1 + Pb
<i>BnSAM3</i>	Ethylene	0.66±0.15	1.14±0.12
<i>BnACO</i>	Ethylene	0.50±0.20	23.97±3.80**z
<i>BnOPR2</i>	Jasmonic acid	5.75±0.60**z	18.09±3.7**
<i>BnAAO1</i>	Auxin	12.77±2.5*	38.53±4.75**
<i>BnPR-1</i>	PR-1 protein	6.70±1.21*	5.32±1.20*
<i>BnPR-2</i>	PR-2 protein	0.11±0.01	6.9±1.08**
<i>BnPR-5</i>	PR-5 protein	1.02±0.25	1.02±0.32
<i>BnCCR</i>	Phenylpropanoid	1.20±0.30	2.05±0.22*
<i>BnOPCL</i>	Phenylpropanoid	1.30±0.25	1.78±0.40*

Defense can be induced by the fungal endophyte *Acremonium alternatum*

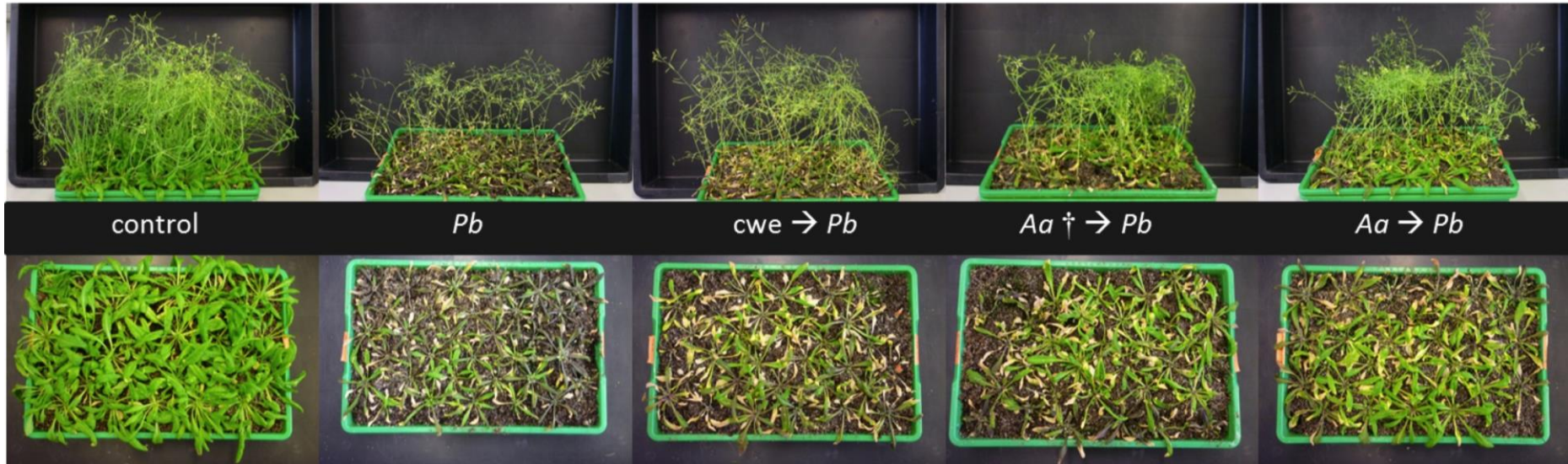
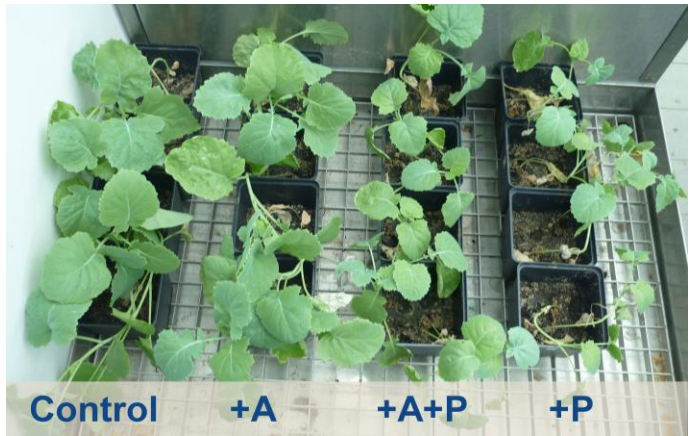
Pb only

Pb + *Acremonium*



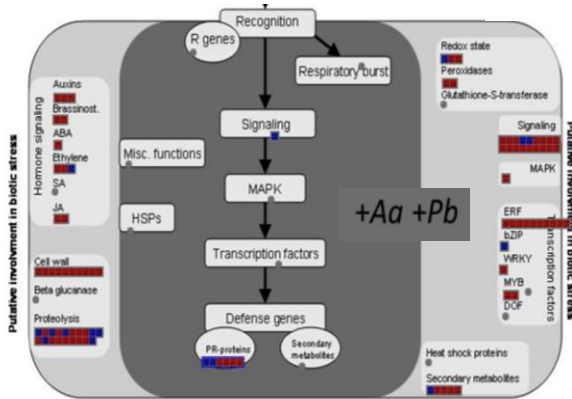
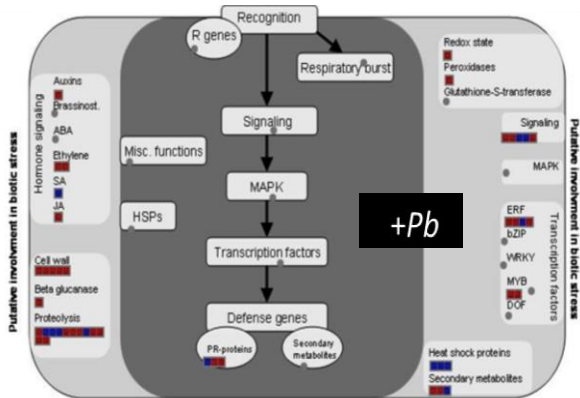
Chinese cabbage -
Doan et al. (2010)
Acta Hort

Oilseed rape - Auer and
Ludwig-Müller (2014)
Albanian J Agriculture



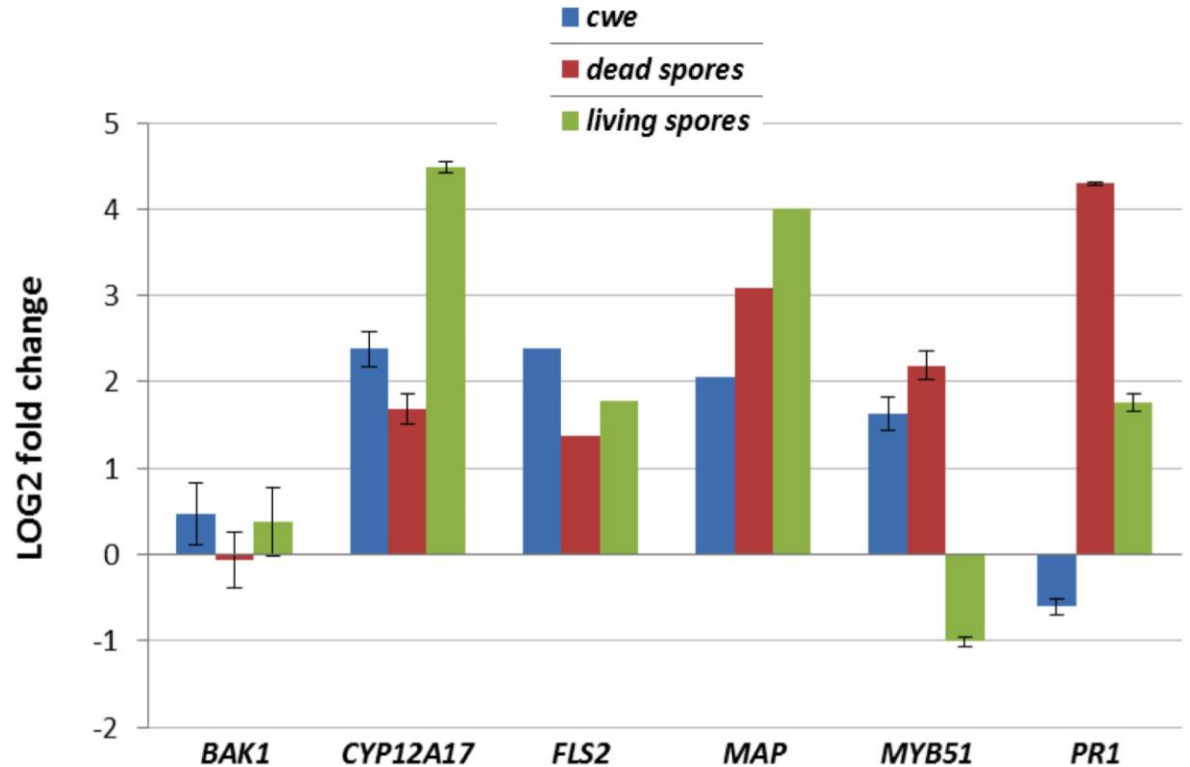
The live fungus is not necessary to induce tolerance

Defense can be induced in Arabidopsis by *Acremonium alternatum* via the salicylic acid pathway

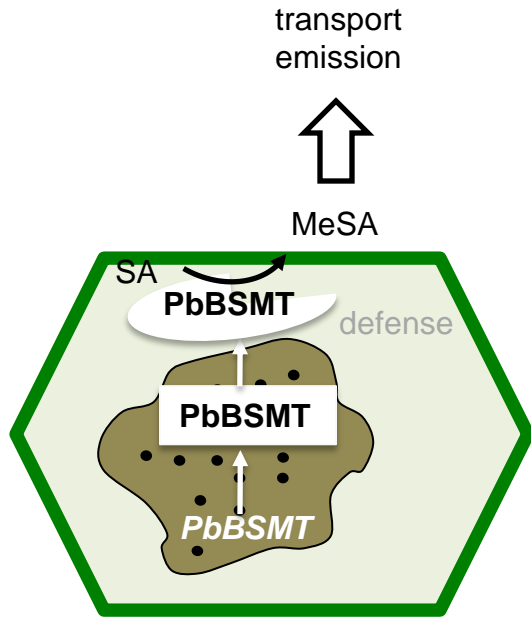


up down

three days after inoculation



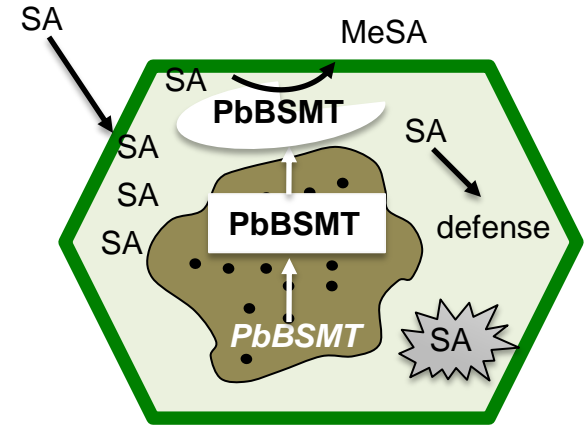
Summary



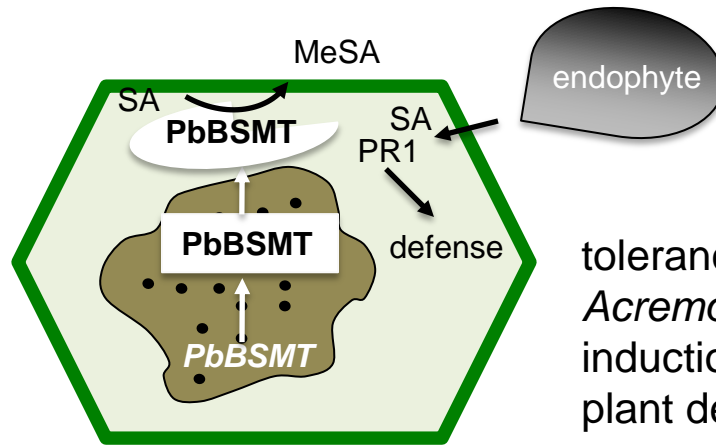
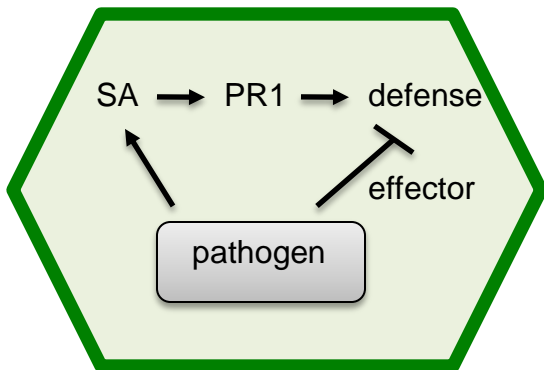
overexpression of PbBSMT in Arabidopsis causes a more susceptible phenotype

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

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



mutants with elevated SA levels are more tolerant to clubroot



tolerance can be induced by *Acremonium alternatum* via induction of the SA-dependent plant defense pathway

Thanks to:



the group - especially:

- Susann Auer
- Freia Benade
- Regina Mencia
- Diana Seidler
- Sabine Jülke →



and all former members who contributed

collaborations:

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

Funding:



Bundesministerium
für Bildung
und Forschung



Bundesministerium
für Ernährung
und Landwirtschaft