

CENTRE FOR CROP AND DISEASE MANAGEMENT



School of Molecular and Life Sciences, Curtin University


Towards effector-assisted breeding for Sclerotinia resistance



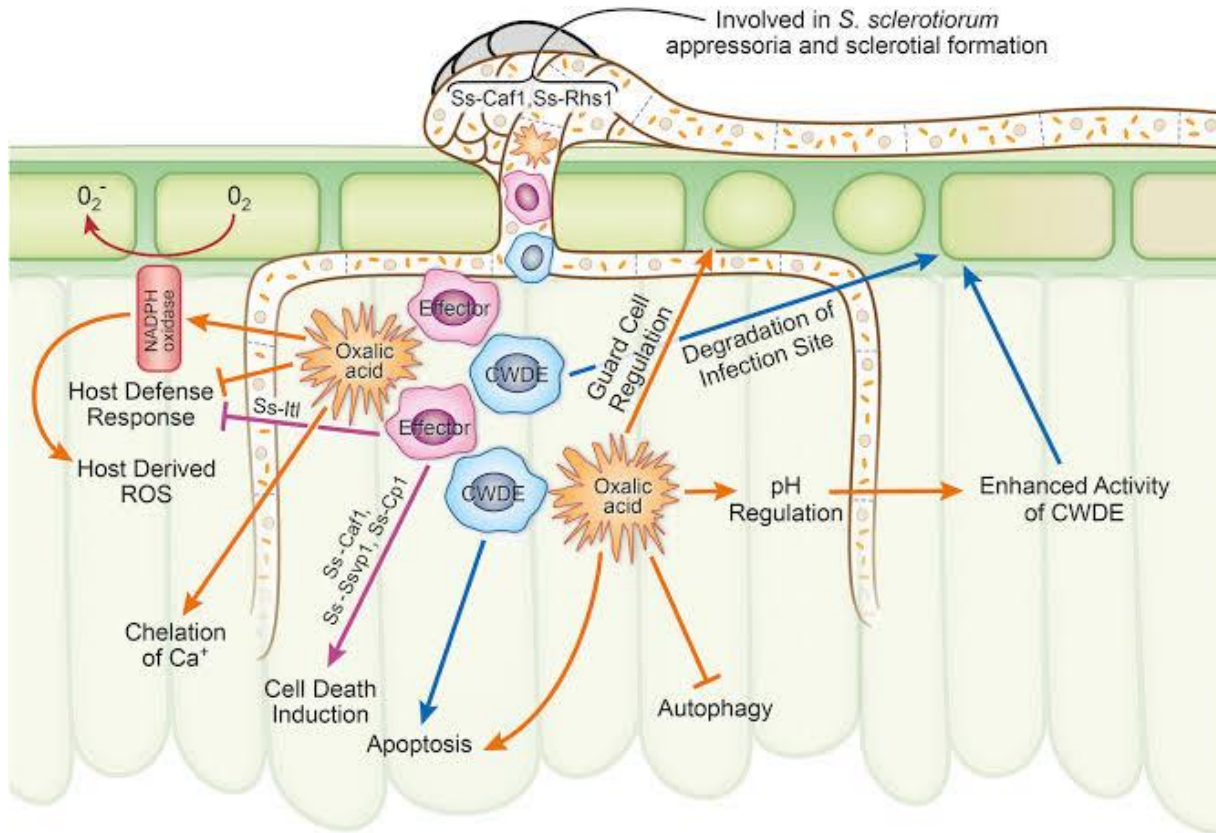
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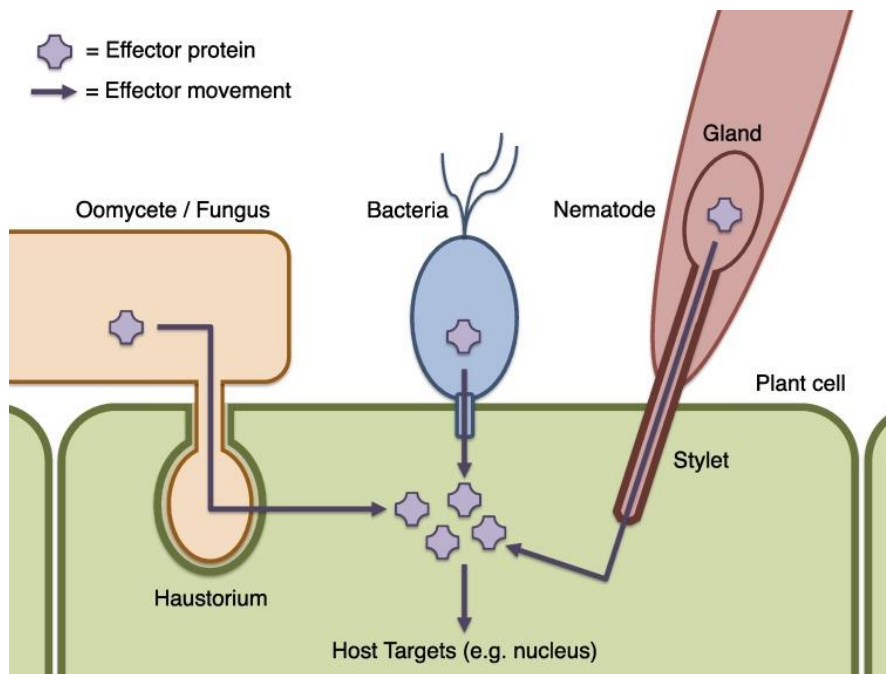
Sclerotinia sclerotiorum arsenal



McCaghey et al., 2019

- Oxalic acid
- Cell wall degrading enzymes (CWDEs)
- Effectors

Effector = a molecule that promotes infection



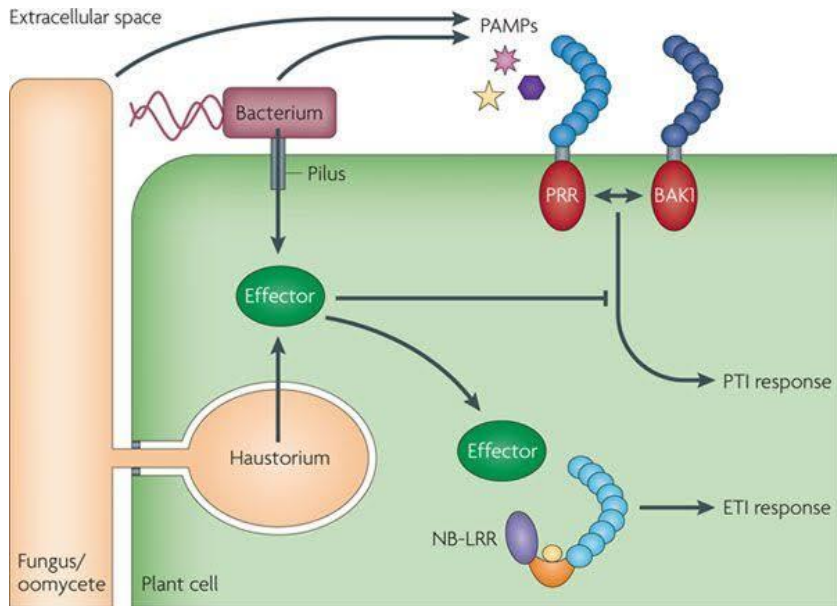
Cock et al., 2013

- Typically small secreted proteins

Effector functions:

1. Induce cell death
2. Suppress defence
3. Evade defence
4. Enable pathogen growth

Necrotrophic effectors hijack host immune system



Nature Reviews | Genetics

- Effector-triggered immunity (ETI) is a rapid, robust defence response to biotrophic pathogens.
- Can be hijacked by necrotrophic pathogens to induce cell death and cause susceptibility.

NLR = NB(S)-LRR = Nucleotide binding-site and leucine-rich repeat immune receptor

Dodds & Rathjen, 2010

Characterized *S. sclerotiorum* effectors

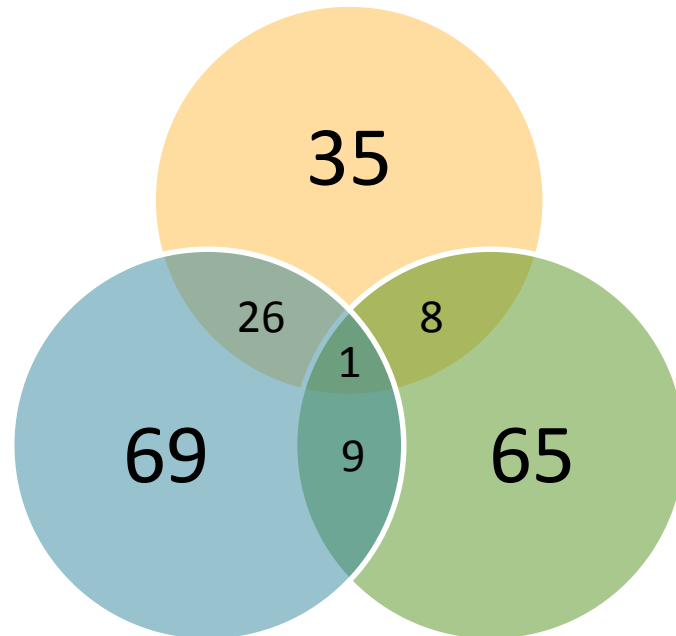
Gene name	Protein	Function	Localisation
Ssv263	Hypothetical secreted protein	Virulence	
SsCVNH	Secreted protein	Virulence, sclerotial development	
Ss-Caf1	Secreted protein with a putative Ca ²⁺ -binding EF-hand motif	Appressorium formation, sclerotial development, induction of host cell death	Inside host cells
SsSSVP1	Secreted protein	Virulence, induction of host cell death	Inside host cells (mitochondria)
SsCP1/SsSm1	Cerato-platanin protein	Virulence, sclerotial formation, induction of host cell death	Apoplast
Ss-Rhs1	Rearrangement hot spot repeat-containing protein	Virulence, sclerotial formation, appressorium formation	
SsNEP1	Necrosis-and ethylene-inducing peptide	Induction of host cell death	Apoplast
SsNEP2	Necrosis-and ethylene-inducing peptide	Induction of host cell death	Apoplast
SsITL	Integrin-like protein	Virulence, defence suppression	Inside host cells

Induce cell death

Suppress defence

S. sclerotiorum effector candidates

Derbyshire et al. (2017)



Hegedus & Seifbarghi
(unpublished)

Guyon et al. (2014)

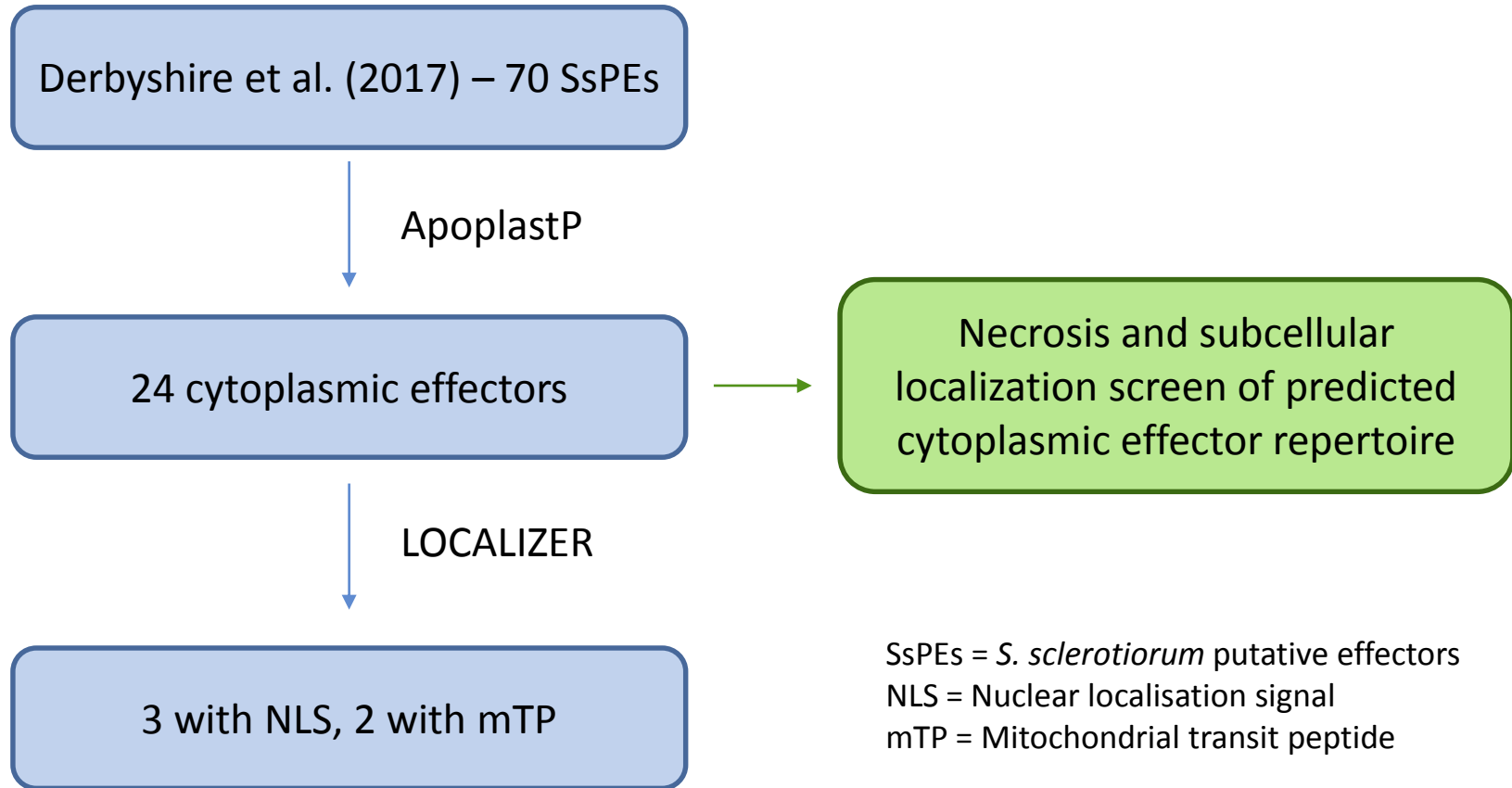
Secretion signal > lack transmembrane domain > lack GPI-anchoring domain > EffectorP

Secretion signal > lack transmembrane domain > lack GPI-anchoring domain > *in planta* expression >

- 1) Sequence/motif/predicted function conserved in fungal effectors
- 2) Belong to duplicated gene families and signatures of positive selection
- 3) Analogous to known protein folds

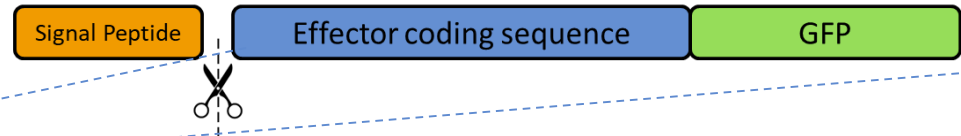
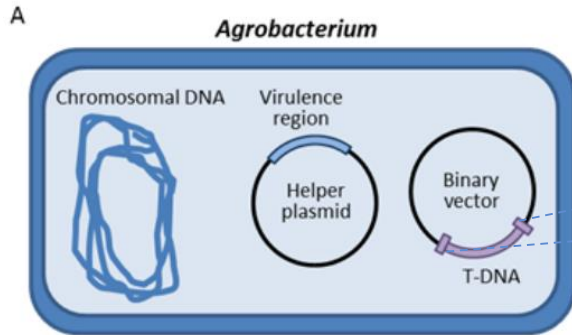
Secretion signal > small > cysteine rich > lack transmembrane domain > lack GPI-anchoring domain

Cytoplasmic effector candidates

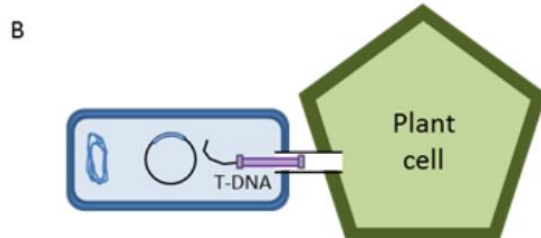


SsPEs = *S. sclerotiorum* putative effectors
NLS = Nuclear localisation signal
mTP = Mitochondrial transit peptide

Effector cloning

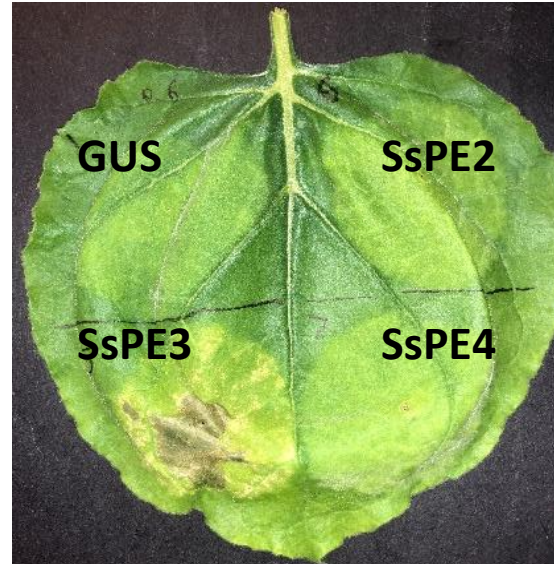
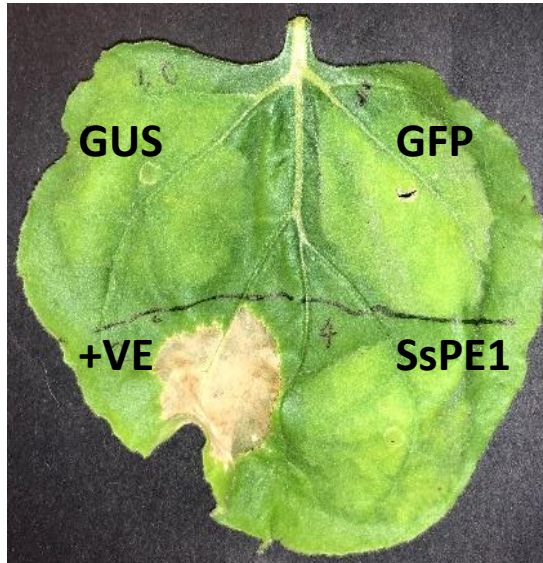


- Clone coding sequences from isolate CU8.24 gDNA/cDNA.
- Screen for necrosis and localization in *Nicotiana benthamiana*.
- GFP fusion enables detection of expression and subcellular localization analysis.



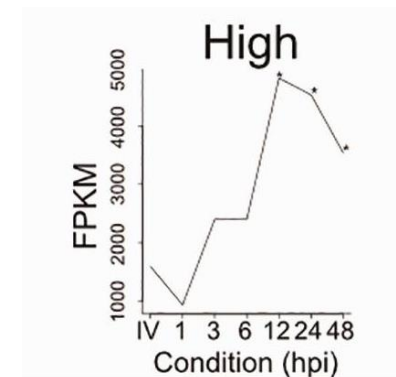
OGTR, 2018

SsPE3 triggers necrosis



Analyse subcellular localisation with confocal microscope

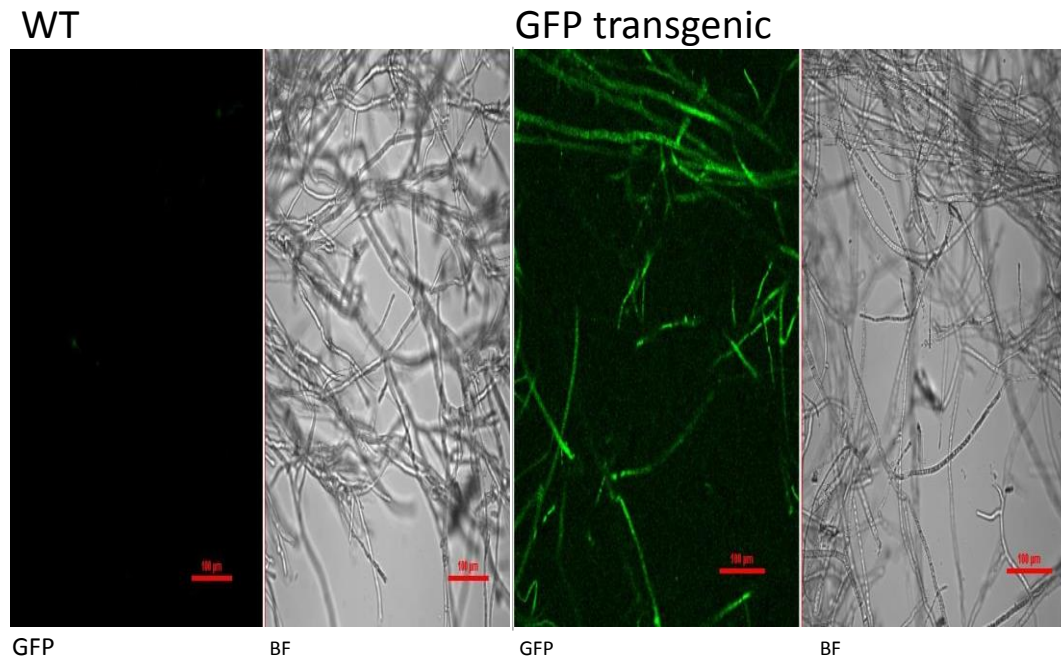
- No predicted domains.
- Upregulated on canola & soybean (Derbyshire et al., 2017; Westrick et al., 2019).
- Conserved in *S. sclerotiorum* isolates found on *Lupinus angustifolius* and *Lupinus mutabilis* (Mousavi-Derazmahalleh et al., 2019).



Derbyshire et al., 2017

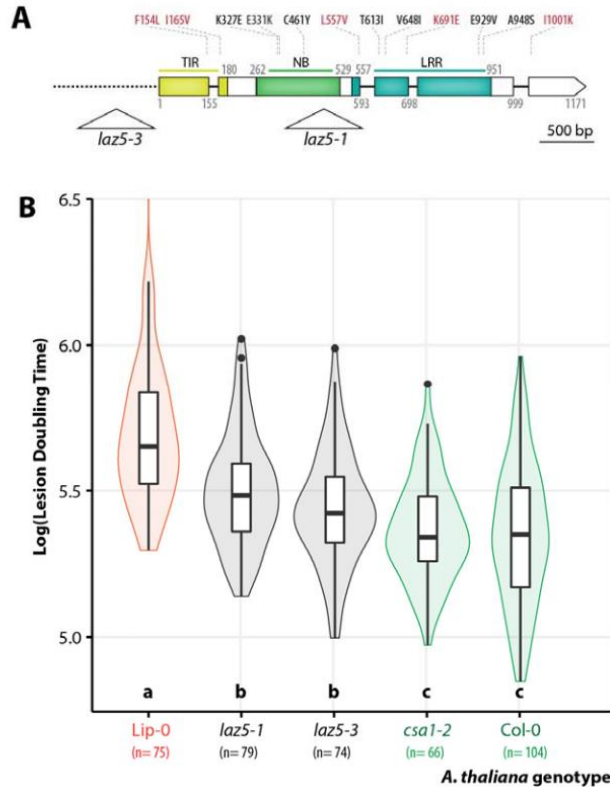
Validate requirement for virulence

- Knockout effector candidates that elicit necrosis and assay for reduced virulence on canola.
- Targeted transformation demonstrated for generation of a GFP-expressing strain.



Roshan Regmi

Do necrosis-inducing effectors hijack the host immune system?



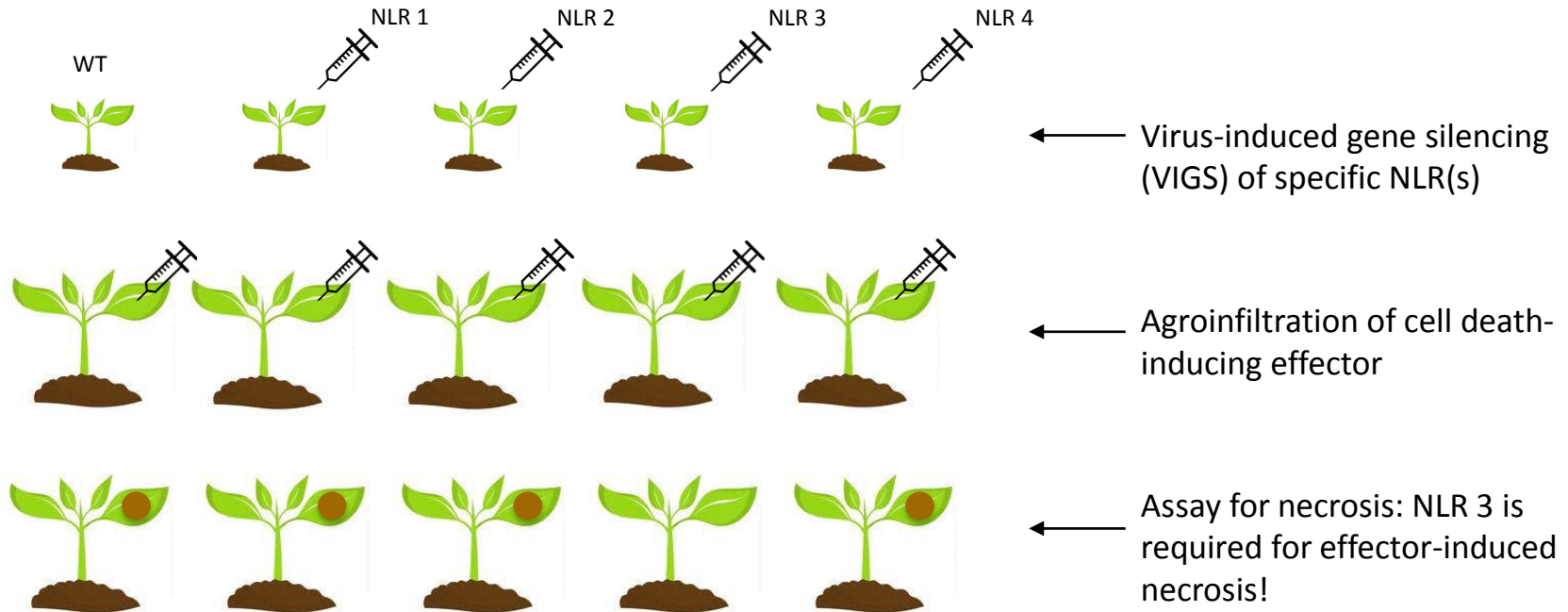
NLR = NB(S)-LRR = Nucleotide binding-site and leucine-rich repeat immune receptor

- NLR identified as a susceptibility gene to *S. sclerotiorum*.
- Is this NLR targeted by an effector?

Barbacci et al., preprint

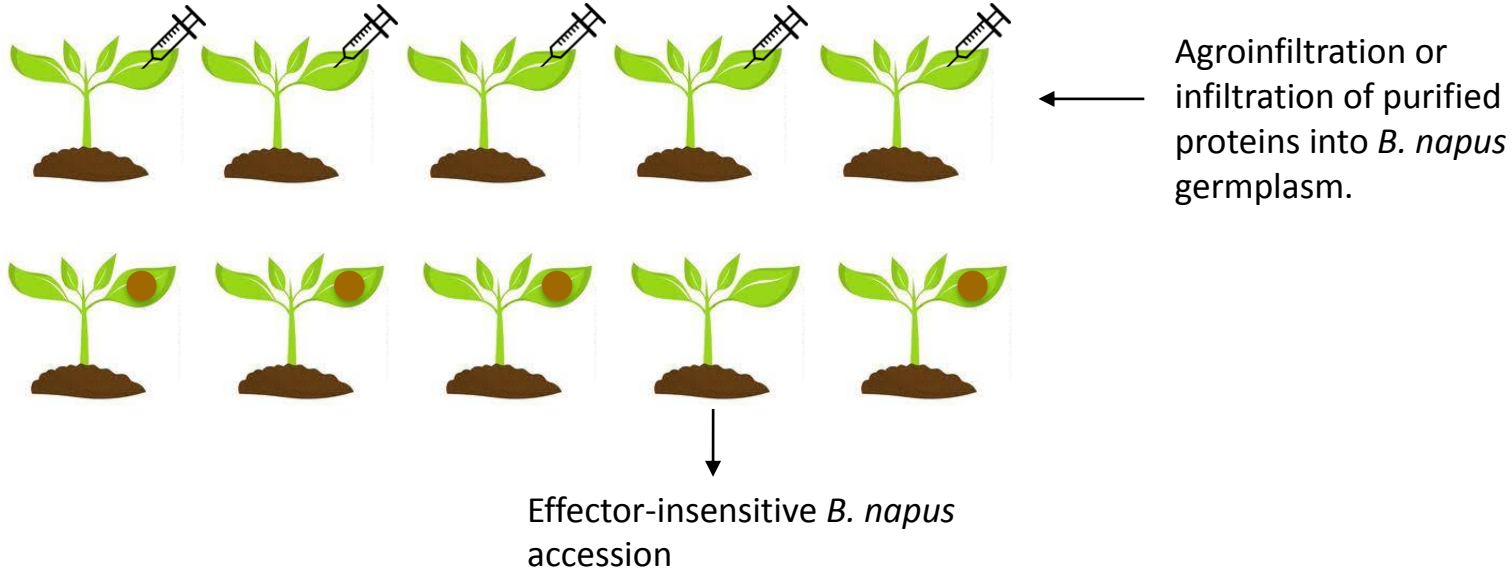
Do necrosis-inducing effectors hijack the host immune system?

N. benthamiana plants



In collaboration with Kee Hoon Sohn lab, POSTECH, South Korea

Diverse collection of ~240 *B. napus* accessions.



- Generate biparental mapping population and screen with effector.
- Develop markers linked to effector (in)sensitivity.

Acknowledgements



CCDM

- Lars Kamphuis
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- Roshan Regmi



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- Shirin Seifbarghi



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THANK YOU



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