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BACKGROUND

- Sclerotinia sclerotiorum* (Lib.) de Bary is a polyphagous necrotrophic fungal pathogen that infects over 400 plant species worldwide and in United states, yield loss is over \$200 million/year.
- It is difficult to control due to a lack of high-level resistance in important crops, making traditional breeding approaches difficult to enhance resistance.
- To identify genes contributing to quantitative disease resistance against *S. sclerotiorum*, we have previously conducted a genome-wide association study using a panel of 325 *Arabidopsis thaliana* accession inoculated with two *S. sclerotiorum* isolates that differ in aggressiveness.
- Several WRKY transcription factors, including *AtWRKY3*, *AtWRKY4*, *AtWRKY19*, *AtWRKY27*, and *AtWRKY61*, were present within linkage disequilibrium blocks at loci associated with resistance.

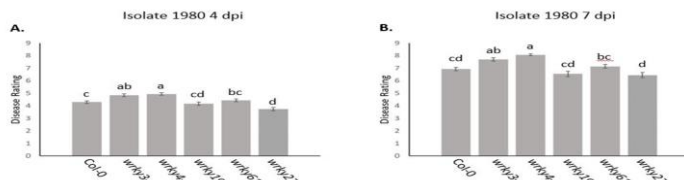


Figure 1. Altered *Sclerotinia* response in several *wrky* T-DNA mutants. A and B showing increased susceptibility of *wrky3* and *wrky4* mutants and B showing increased resistance of *wrky27*

- WRKY transcription factors are one of the largest families of transcriptional regulators in plants and are responsible for the regulation of genes responsive to biotic and abiotic stress
- Preliminary evaluations of T-DNA insertional mutants indicated that *wrky3* and *wrky4* mutants are hypersusceptible to *S. sclerotiorum* while *wrky27* mutants exhibited increased resistance
- The goal of this project is to further characterize the role of *WRKY3*, *4*, and *27* in resistance to *S. sclerotiorum*

Objectives

- Developing transgenic *Arabidopsis* lines overexpressing WRKY from 35s promoter in Zdr-6, Col-0, and Lm-2 genetic backgrounds and evaluating resistance to *Sclerotinia*.
- Conducting expression analysis of *AtWRKY3*, *4*, & *27* at 0, 12, 24, & 48 hours post inoculation (hpi) with *Sclerotinia* in resistance compared to susceptible *Arabidopsis* ecotypes
- Conducting expression analysis of sunflower and canola orthologs of *AtWRKY3*, *4*, & *27* in susceptible and partially resistant lines at 0, 12, 24, & 48 hpi with *S. sclerotiorum*

METHODOLOGY

Developing transgenic *Arabidopsis* lines overexpressing WRKY from 35s promoter in Zdr-6, Col-0, and Lm-2 genetic backgrounds and evaluating resistance to *Sclerotinia*.

- Three *Arabidopsis* ecotypes will be used, one is relatively resistant (Zdr-6), one is moderately susceptible (Col-0), one is highly susceptible (Lm-2)

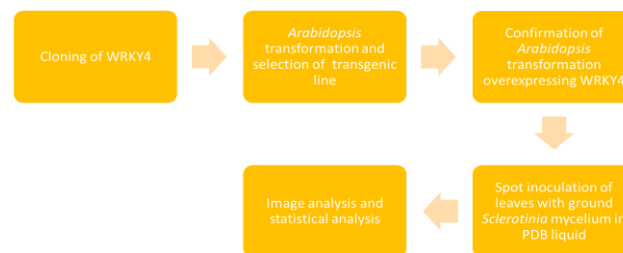
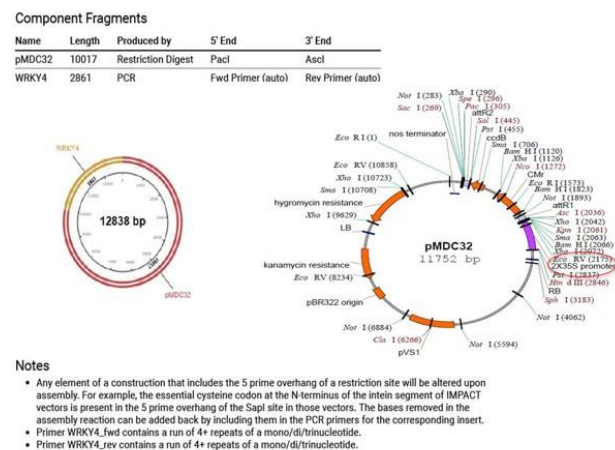


Figure 2. Workflow of the research process.



Name	Primer 5'	Primer 3'	Len	%GC	3' %GC	3' Tm	3' Ta
WRKY4_fwd	taccggccccccccctggaggTTAATTTGGGGATCGATGTC		42	60	41	56.6	60.2
WRKY4_rev	ccgctctagaactagtaATGCAAGAATAATTTGGGTCATAGG		42	41	41	57.1	60.2

Figure 3. Cloning of *wrky4*

Conducting expression analysis of *AtWRKY3*, *4*, & *27* at 0, 12, 24, & 48 hours post inoculation (hpi) with *Sclerotinia* in resistance compared to susceptible *Arabidopsis* ecotypes

- Ten ecotypes of *Arabidopsis* will be used.
- Five of them are susceptible (Wa-1, Lm-2, Bg-2, Shahdara, & Or-0).
- Five of them are partially resistant (Zdr-6, Petergof, Ag-0, Tamm-2, UKSE06-349) ecotypes.
- Evaluation of gene expression for *WRKY3*, *WRKY4*, and *WRKY27* will be done by BioRad CFXconnect instrument
- Analysis of Amplification data will be done following the comparative $\Delta\Delta CT$ method (Schmittgen and Livak, 2008)

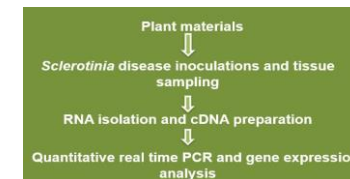


Figure 4. Workflow of the process



Figure 5. BioRad CFXconnect instrument

Conducting expression analysis of sunflower and canola orthologs of *AtWRKY3*, *4*, & *27* in susceptible and partially resistant lines at 0, 12, 24, & 48 hpi with *S. sclerotiorum*

- To identify canola and sunflower orthologs of *WRKY3*, *4* and *27*, the protein sequence for the three genes from *Arabidopsis* will be downloaded and used as a "BLAST" query against the canola and sunflower genome annotations.
- The research workflow will be same as the previous one except the *Sclerotinia* disease inoculation procedure. Instead of *Arabidopsis* leaf inoculation, there will be canola stem inoculation and sunflower root inoculation

CONCLUSION AND FUTURE WORK

- Through the research work, the role of *WRKY3*, *4*, and *27* in resistance to *S. sclerotiorum* will be evaluated
- Current work focuses on the first two objectives

REFERENCES

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Acknowledgements

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