Double stranded RNA induced gene silencing in the fungal pathogen, *Fusarium virguliforme*.

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**ABSTRACT**

*Fusarium virguliforme* is a fungal pathogen which causes the disease sudden death syndrome in soybean. So far there are no soybean lines which provide complete resistance to this fungal pathogen. Our goal is to develop transgenic lines which can provide resistance to this devastating soybean disease. Creating lines which produce small interfering RNA (siRNA) and trigger RNA interference (RNAi) response in the pathogen is one possibility in creating resistant lines. RNAs in a process where cellular machinery recognizes double stranded RNA and degrades it. Small interfering RNAs are produced from the double stranded RNA and trigger the degradation of corresponding mRNA. RNAi has been used in many different organisms to knockdown the expression of target genes. The response elicited by the double stranded RNA can spread between cells and has been shown to be able to spread between host and pathogen. Our long term is to apply RNAi to knockdown genes which are important for *F. virguliforme* growth and pathogenicity. In this study, we investigated if the RNAi pathway is conserved in *F. virguliforme*. Here we treated *F. virguliforme* spores with siRNA molecules to show that RNAi-mediated knockdown of polyamine oxidase led to reduced pigment accumulation and sporulation. These results established that RNAi pathway is conserved in the SDS pathogen.

**BACKGROUND**

Sudden Death Syndrome (SDS) is a costly and devastating soil-borne disease, discovered in Arkansas in 1971, that affects soybean plants. The disease is caused by soil fungi from within the group *Fusarium solani* species complex, particularly *F. virguliforme* in the United States (Aoki et al., 2003; Aoki et al., 2005). Distinct symptoms of the disease include yellowing and defoliation of upper leaves and browning and rotting of the plant’s roots. SDS is most severe when soybean crops are planted early in cool, wet soils. Scientists and farmers located in heavily affected areas have been working on effective methods to create transgenic soybean plants that exhibit resistance to the effects of the invading pathogenic fungi. *F. virguliforme* has been shown to be a major contributor to the formation of SDS in soybean plants. Trans-specific gene silencing using the RNA interference (RNAi) pathway is being explored as a possible way to silence key genes that are involved in the formation of SDS.

In fungi, the use of RNAi as a tool for reverse genetics, aimed at the modification of gene expression is constantly growing with more than 40 species already proven to be responsive. RNAi is a naturally occurring post-transcriptional gene-silencing (PTGS) phenomenon, in which sequence-specific double-stranded RNA (dsRNA) initiates the degradation of homologous messenger RNA (mRNA), thus silencing gene expression. dsRNA enters the cell and is recognized by the enzyme Dicer where it is cleaved into smaller fragments called short interfering RNA (siRNA) containing 21-23 nucleotide base pairs. Polyamine oxidase genes are responsible for the breakdown of the cationic molecules spermine and spermidine. The breakdown of these molecules produces free amine groups and free radicals. The free radicals are known to be involved in cell signaling and the free amine groups are available for the cell to use as a nitrogen source on nitrogen limited media.

**MATERIALS AND METHODS**

**Strategy**

- Design siRNAs
- Harvest *Fusarium virguliforme* spores
- Treat spores with siRNAs for 48 hours
- Plate spores on assay plates with spermine as sole nitrogen source
- Observe spore growth, count spores and conduct reverse transcription PCR

**Treatments**

- FvPO1FvPO2 = Wild-type
- fvp01fvp02 = Double knockdown
- fvp01FvPO2 = Single gene knockdown
- FvPO1fvp02 = Single gene knockdown

**RESULTS**

**A**

![Figure 1. Reduced pigment and spore production among the fungal colonies regenerated from individual spores transformed with siRNA molecules specific to FvPO1 and FvPO2 genes. A) Reduced pigment accumulation in the double knockdown mutants for both FvPO1 genes. Photos of individual colonies developed from single transformed spores 14 days after plating on agar plates amended with spermine as the sole nitrogen source are presented. FvPO1FvPO2, is the wild-type Mont-1 isolate; fvp01fvp02 both genes were silenced; fvp01FvPO2, FvPO1 was silenced; FvPO1fvp02, FvPO2 was silenced. B) Reduced sporulation in the double knockdown mutants for both FvPO genes. Sporulation was determined as the spore counts of single colonies.](image)

**B**

![Figure 2. RT-PCR results confirming the reduced expression of FvPO1 and FvPO2. Mycelial samples grown on the plates showed that there was decreased expression in both FvPO1 and FvPO2 genes in the treatment (lane 2) that contained siRNA molecules specific to both FvPO1 and FvPO2 genes. FVTax1 was used as an internal control.](image)

**CONCLUSION**

- We confirmed the presence of the RNAi pathway in *F. virguliforme*.
- We designed a system for testing the affects of siRNAs on *F. virguliforme*.
- Polyamine oxidase genes in *F. virguliforme* are involved in both sporulation and pigmentation.
- Future work will continue to test siRNAs for genes which may be vital to fungal growth or to fungal virulence for use in trans-specific gene silencing.

**REFERENCES**


**ACKNOWLEDGEMENT**

I would like to sincerely thank Jordan Baumbach and Madan K. Bhattacharyyya for assisting me in this research experience. This project was supported by the Agriculture and Food Research Initiative Competitive Grants Program Grant no. 2013-68004-20374 from the USDA National Institute of Food and Agriculture.

**RESEARCH GOAL**

Project goal: Determine if the RNAi pathway is active in *F. virguliforme*.

Approach: Use siRNAs to silence polyamine oxidase genes in *F. virguliforme* spores

![MATERIALS AND METHODS](image)

![CONCLUSION](image)

![ACKNOWLEDGEMENT](image)