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Using RNA interference to protect *Brassica napus* (canola) against fungal pathogens

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Background:

Sclerotinia sclerotiorum, the causal agent of white mould, infects over 600 species of plants worldwide and is particularly devasting to *Brassica napus* (canola). Sclerotinia is a persistent problem for global food production that has traditionally been managed using broad-spectrum fungicides. Thus, there is an immediate need to manage Sclerotinia using novel species-specific control methods.

Objective:

The objective of our research is to develop next generation molecular technologies that specifically targets *S. sclerotiorum* at the RNA level. Our strategy exploits the inherent cellular defence process known as RNA interference (RNAi) using Spray Induced Gene Silencing (SIGS) and genetically through Host Induced Gene Silencing (HIGS). Upon encountering a double stranded RNA (dsRNA) molecule, the fungus processes the dsRNA specifically targeting transcripts with sequence homology.

Methods:

Using a re-designed bioinformatics approach, we identified Sclerotinia-specific target genes. For spray applications, RNAi knockdown was confirmed using quantitative real-time PCR on RNA isolated from fungal liquid cultures or leaf tissues. dsRNA molecules were screened for growth inhibition on *B. napus* using a system representative of field conditions that showed up to 85% reduction in lesion spread. Next, we engineered *B. napus* to constitutively express a hairpin (hp)RNA molecule to silence ABHYRDOLASE-3 in *S. sclerotiorum*.

Results:

The interaction between the host plant and invading pathogen was further characterized at the molecular level using dual-RNA sequencing and at the anatomical level through microscopy to understand the processes and possible mechanisms leading to increased tolerance to this damaging necrotroph. We observed significant shifts in the expression of genes relating to plant defence as well as cellular differences in the form of structural barriers around the site of infection of both SIGS and HIGS approaches. Using live cell imaging, transgenic fungal cultures and endocytic inhibitors, we determined that the uptake mechanism of dsRNAs in *S. sclerotiorum* occurs through clathrin-mediated endocytosis.

Conclusions:

Understanding the mode of dsRNA entry into the fungus proves useful in designing and optimizing future dsRNA-based control methods and in anticipating possible mechanisms by which phytopathogens may develop resistance to this novel category of fungicides. Our results provide proof-of-concept that both SIGS and HIGS are effective means of limiting damage caused by *S. sclerotiorum* to *B. napus* and demonstrates the utility of this biotechnology in the development of resistance against fungal pathogens.