

# Application of Host-Induced RNA Interference as a means of Silencing *Puccinia graminis* Genes and Conferring Resistance in Wheat

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## Introduction

*Puccinia graminis* f. sp. *tritici* (*pgt*), commonly known as stem rust, presents a crucial challenge to the future of many agricultural crops as it infects and causes disease in many important cereals such as wheat and barley (Fig. 1). Rust causes significant reductions in crop yield. As obligate biotrophic plant pathogens, rust fungi form haustorial cells that become invaginated in plant cells. These haustorial cells are tasked with nutrient uptake and suppressing host defense responses [1].



Fig. 1. Stem rust caused by *Puccinia graminis*

Traditional resistance to rust largely depends on 'gene-for-gene' resistance in which a resistant gene product in the plant detects a corresponding effector molecule produced by the pathogen and ultimately works to suppress its growth [2]. Since the outbreak of Ug99, a relatively new strain of extremely virulent stem rust in Uganda that is impervious to many resistant genes in wheat, the call for better means of resistance to this fungal pathogen has become ever more pressing [2].

Previous research has investigated silencing rust genes via RNA interference (RNAi) using virus-induced gene silencing (VIGS) [3]. VIGS prevents translation by using a viral vector to introduce complementary RNA, forming double-stranded RNA (dsRNA) (Fig. 2). The objective of this study was to use VIGS to silence stem rust genes highly expressed in haustorial cells. Ideally, this method will bring forth a superior means of enhancing wheat resistance to stem rust.

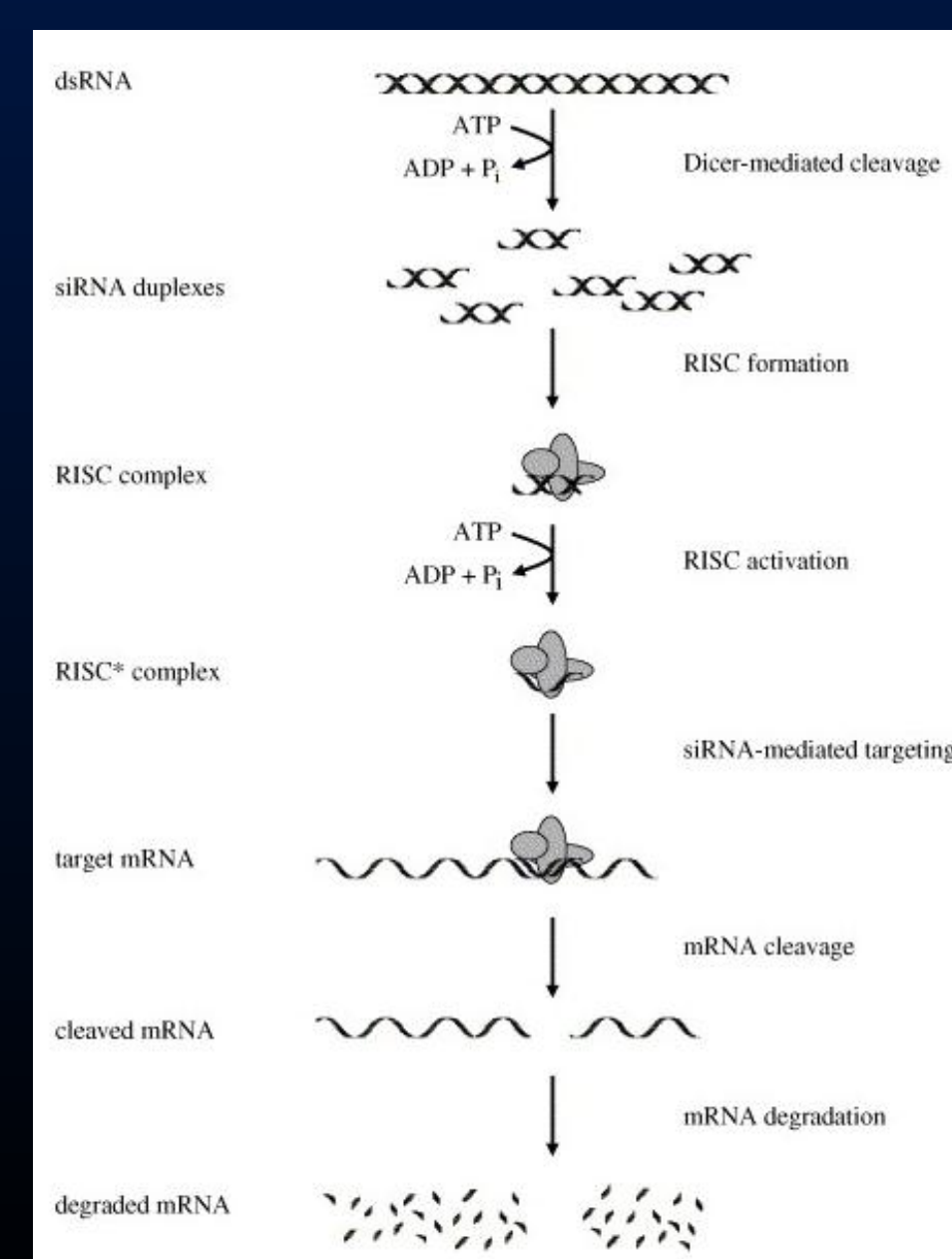


Fig. 2. Schematic mechanism for RNAi, which controls post-transcriptional gene expression via anti-sense gene disruption. Dicer enzyme introduces double-stranded cuts to form smaller fragments called short interfering RNA (siRNA). The RNAi silencing complex (RISC) binds to the short dsRNA and cleaves the sequence apart. The antisense strand triggers mRNA degradation by cellular nucleases in the RISC [4].

## Materials & Methods

- Candidate genes of *Puccinia graminis* were selected primarily based on the following criteria:
  - Conserved regions with leaf rust (*Puccinia triticina*)
  - Homology to other genes in gene family
  - Levels of expression in haustorial cells
  - Presence of predicted secretion signal peptides
  - Significant homology to wheat and mammalian species was avoided to prevent inadvertent gene silencing.
- Construction of Barley Stripe Mosaic Virus (BSMV) -derived vectors
  - TA vector ligation and transformation
  - Gamma vector digestion (Pac1, Not1 restriction enzymes), ligation, and transformation
- *In vitro* transcription of viral RNAs, virus inoculations, rust inoculations, and disease assays
- Extraction of RNA from infected leaves to verify levels of gene silencing
- Assessment of wheat resistance to stem rust through qualitative analysis (Fig. 3)

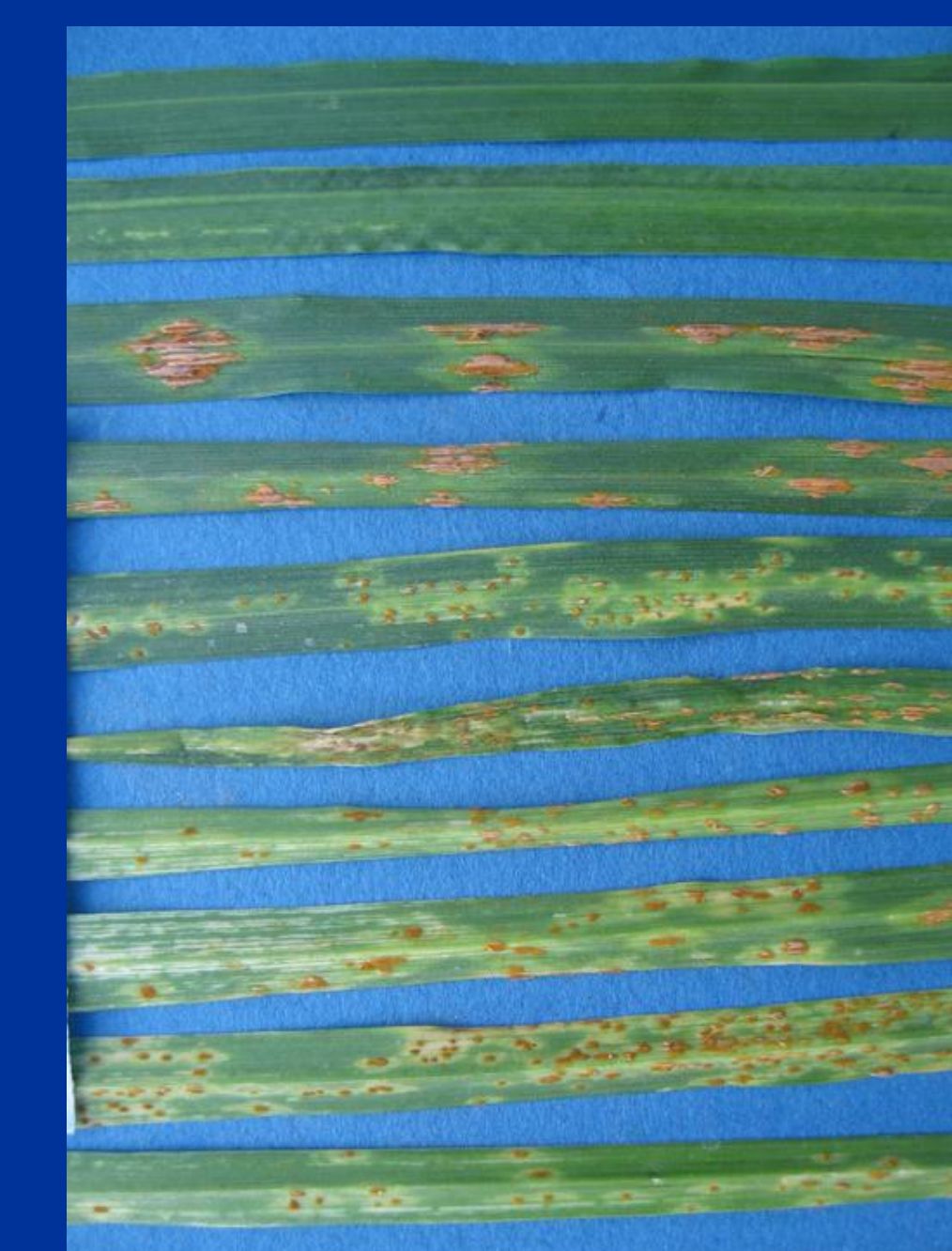
## Results

- Five candidate genes of *Puccinia graminis* were selected for silencing (Table 1).
- Gamma constructs were obtained for four of the five genes.

Table 1. Candidate genes were selected for silencing from stem rust haustoria / infected leaves library.

<sup>a</sup> A ratio of expression in haustoria (Haust) vs. expression in infected leaves (InfW<sup>a</sup>)

| Gene ID    | Function                                  | Expression (Haust/InfW <sup>a</sup> ) | Trans-membrane Domains | Predicted Secretion Signal Peptide | Fragment Length (base pairs) |
|------------|---|---------------------------------------|------------------------|------------------------------------|------------------------------|
| PGTG_16914 | Amino acid permease <i>inda1</i>          | 15.41                                 | 10                     | No                                 | 373                          |
| PGTG_07026 | Amino acid permease <i>inda1</i>          | 9.13                                  | 9                      | No                                 | 370                          |
| PGTG_07327 | Proline-rich ligand binding site motif    | 9.42                                  | 1                      | No                                 | 232                          |
| PGTG_04176 | Glycosyltransferase                       | 8.06                                  | 0                      | Yes                                | 407                          |
| PGTG_15914 | Hypothetical protein similar to chitinase | 12.93                                 | 0                      | Yes                                | 269                          |



Uninoculated wheat  
VIGS empty vector inoculation  
*P. graminis* (PGT7a) inoculation  
PGT7a + VIGS empty vector  
PGT7a on wheat with Sr31  
PGT7a + BSMV::pgt50  
PGT7a + BSMV::pgt64  
PGT7a + BSMV::pgt55  
PGT7a + BSMV::pgt78  
PGT7a + BSMV::pgt72

Fig. 3. Enhanced wheat resistance to rust after infection with several VIGS constructs [5].

## Discussion

Data collection is still in progress. Results are expected to indicate if any of the proposed candidate genes in this study can successfully be silenced. If a successful silencing is achieved, the extent of rust virulence will play a large role in identifying which candidate genes are associated with enhancing wheat resistance to rust. Given the relatively high expression levels of these genes in haustorial cells, the determination of whether any are particularly essential to the haustoria will provide useful insight into the development of a novel means of conferring wheat resistance to even the most virulent strains of rust. Further identification of essential haustorial genes will greatly enhance the prospects of conferring rust resistance in cereal crops, especially geared toward those grown in agricultural regions most susceptible to particularly virulent strains such as Ug99 and its derivatives.

## Acknowledgements

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## References

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