

Host-induced Gene Silencing in Fungal Pathogens of Cereals

Daniela Nowara, Götz Hensel, Alexandra Gay, Dimitar Douchkov,
Patrick Schweizer and Jochen Kumlehn

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK),
Corrensstrasse 3, 06466 Gatersleben, Germany

RNAi is an established means to knock-down genes in plants and fungi. Both sequence-specificity as well as systemic spreading of gene silencing is essentially mediated by small interfering RNAs. Considering that there is intimate cellular contact between plants and fungal pathogens during infection, we hypothesized that fungal genes may effectively be targeted by small RNAs derived from appropriately designed hairpin constructs expressed by host plant cells. This phenomenon that we called HIGS has already been described in plant parasitic nematodes¹, insects², and parasitic plants³. *Blumeria graminis* is a powdery mildew-causing fungus which infects many plant species including cereals. Intensive research is going on to better understand the barley-powdery mildew pathosystem, which is an established experimental model for biotrophic plant-pathogen interactions. Thirty nine independent transgenic barley lines were generated using a hairpin RNAi construct based on a fragment of the *B. graminis hordei* glucanosyltransferase 1 (*GTF1*) gene. GTFs are specifically found in fungi where they are involved in cell elongation and virulence. We have chosen *GTF1* as a HIGS-target, since it had been found to be significantly upregulated upon infection. In three of the T1-populations obtained, colony formation of *B. graminis* was demonstrated to be significantly reduced, whereas a transgenic control line lacking the hairpin cassette was as susceptible as wild-type control plants. The results suggest uptake of RNA molecules by the powdery mildew fungus from attacked plant cells, which may cause knock-down of targeted fungal genes and reduced disease severity. Results from studies with *Fusarium*⁴ and the rust fungus *Puccinia*⁵ are consistent with this theory. Thanks to its potentially high specificity, the huge array of candidate target genes, and the fact that the protection mechanism acts without any further gene product being required, this novel principle holds great promises for future applications both to validate the function of pathogenesis-related fungal genes especially in untransformable obligatory biotrophs such as *Blumeria graminis*, and to effectively combat fungal diseases of crop plants. The molecular mode of action of HIGS remains yet to be examined in detail.

1. Westwood et al. (2009) Pest Manag Sci 65, 533-539

2. Price & Gatehouse (2008) Trends Biotechnol 26, 393-400

3. Gheysen & Vanholme (2007) Trends Biotechnol 25, 89-92

4. Tinoco et al. (2010) BMC Biol 31, 8-27

5. Yin et al. (2011) MPMI 24, 554-561

for more detailed information see Nowara et al. (2010) Plant Cell 22, 3130-3141