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Unleashing Host-Induced Gene Silencing (HIGS) for Effective Management of Fungal Diseases in Plants

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Abstract

Plant pathogens pose a significant threat to global agricultural productivity, causing substantial crop yield losses annually. Conventional fungicides have been extensively employed to combat these diseases, but the emergence of fungicide-resistant strains underscores the need for alternative strategies. Host-induced gene silencing (HIGS) offers a promising, environmentally friendly approach to crop protection by harnessing RNA interference (RNAi) mechanisms. In this review, we delve into the historical context and mechanisms of gene silencing, tracing its development from early discoveries to modern applications. We explore the intricacies of HIGS, detailing its mechanism of action and its efficacy against a range of plant pathogens. Furthermore, we highlight the potential target genes utilized in HIGS across various fungal species and discuss the strategies for implementing HIGS effectively to control fungal diseases. Overall, HIGS holds immense promise as a sustainable strategy for crop protection, offering a viable solution to mitigate fungal diseases and enhance global food security.

Introduction

Plant pathogenic fungi and fungi-like oomycetes currently account for perennial crop yield losses of up to 20 per cent world-wide and an additional 10 per cent loss occurs due to post-harvest fungal diseases (Fisher *et al*., 2018). Fungicides are widely applied to manage plant diseases caused by pathogenic fungi, but fungicide-resistant fungal populations have been increasingly reported. Recent techniques using RNA interference (RNAi), which define the ability of double-stranded RNA (dsRNA) to inhibit the expression of homologous genes, have been suggested for crop protection in an environmental-friendly way by using technique called host-induced gene silencing (HIGS) is the innovative strategy to control plant diseases.

Gene silencing is a molecular mechanism that knocks down the gene expression in plants both in nature and in response to external stimuli (Wassenegger, 2002). Gene silencing is a negative feedback mechanism that regulates gene expression to define cell fate and also regulates metabolism and gene expression throughout the life of an organism (El-Sappah*et al*., 2021). Gene silencing is a technique that aims to reduce or eliminate the production of protein from its corresponding gene. It is a switching off mechanism of gene; it operates either at transcriptional level or translational level. The gene silencing phenomenon was unfolded accidentally in Petunia flowers when scientists they are experimenting to deepen the color of petunia flowers by upregulating the gene coding for pigment production, which surprisingly resulted in variegated flowers instead of expected deep purple flowers. It was due to gene silencing mechanism. Since the expression of a homologous endogenous gene as they will as a transgene was suppressed, the phenomenon was called "co-suppression".

History of Gene silencing

- During 1936 Position effect studies in Drosophila by Dohzhansky.
- 1956- Paramutation byAlexander Brink in Maize.
- 1970-Genome imprinting studies in maize by Kermicle.
- During 1982 Monsanto company developed transgenic plants through Antibiotic resistance.
- 1987 - Antisense effect by Rothstein *et al* in tobacco.

- 1989 - Matzke *et al* first identified Transgenic silencing in transgenic tobacco.
- 1990-Post transcriptional gene silencing first discovered in petunia by Jorgensen.
- 1993- RNA interference- Fire and Craig Mello first discovered in *C. elegans.*
- 2002 is considered RNA interference as the Technology of the year.
- 2013- CRISPR *Cas*-Zhang *et al*., first adopt for genome editing.

Gene silencing can be broadly divided into two, they are transcriptional gene silencing and post transcriptional gene silencing. Some examples of transcriptional gene silencing are, Genomic imprinting, Para mutation and Position effect. Genomic imprinting or parental imprinting is the inheritance of permanently inactivated gene from parent to off spring. Inactivation is caused by DNA methylation in the regulatory sequence of inactivated gene. E.g., insulin like growth factor gene (Igf2) in mice. Para mutation is an interaction between two alleles of single locus. In which one allele silences another allele. E.g., anthocyanin pigment production in corn plant. Position effect is the effect on the expression of a gene when its location in chromosome is changed often by translocation. E.g., drosophila eye color.

Post transcriptional gene silencing mechanisms are more important as the topic is concerned, which include anti sense RNA technology and RNAi. Anti-sense RNA complimentary to sense RNA in the same cell can lead to the formation of a stable duplex which interferes with gene expression at the level of RNA processing or possible translation. This technology is widely used in plants. RNAi is the most important and widely used strategy among post transcriptional gene silencing mechanisms. It involves sequence specific degradation of mRNA by complimentary small RNAs. It forms the basis of host-induced gene silencing (HIGS).

RNA interference (RNAi)

RNAi can be defined as sequence specific and homology-dependent gene silencing through a complex mechanism, in which double stranded RNA (dsRNA) is recognized which leads to a chain of events resulting in the degradation of both the dsRNA and homologous RNA. The term RNAi is usually used in animals. It is commonly known as post-transcriptional gene silencing (PTGS) or co-suppression in plants and quelling in fungi. RNAi is a natural phenomenon in eukaryotes. It takes place in all eukaryotic organisms as a cellular defense mechanism. While parallel advanced strategy, CRISPR-CAS9 is a natural phenomenon in procaryotes. It is reversible process, while CRISPR-CAS9 is irreversible process. It shows high target specificity, even though there is a chance of off target effect. RNAi was first demonstrated in the model free living nematode, *Caenorhabditis elegans* in 1998. The experiment was carried out by injecting nematode with dsRNA corresponding to a gene (important for muscle function) involved in wiggling movement (unc-22). For this finding Andrew Fire and Craig C. Mello received a Nobel Prize award in Physiology and Medicine in 2006.

Host Induced Gene Silencing (HIGS)

The biotechnological exploitation of RNAi is termed host-induced gene silencing (Nowara *et al*., 2010). HIGS was first demonstrated in 2010 by Tinoco & co-workers and Nowara & co-workers separately. Tinoco & co-workers engineered tobacco to express a *β-glucuronidase* (*GUS*) hairpin to specifically silence *GUS* transcripts in a *GUS*-expressing strain of *Fusarium verticilloides.* Nowara & co-workers developed powdery mildew (*Blumeriagraminis*)-tolerant wheat and barley. HIGS has also proven effective in protecting plants against biotrophic fungi such as *Puccinia triticina*, *Fusarium oxysporum*, hemibiotrophs such as *F. graminearum* and necrotrophs such as *Botrytis cinerea*.

Mechanisms of HIGS

Mechanisms of HIGS is same as that of general mechanism of RNAi. The dsRNA in host plant is recognized and processed by dicer or Dicer-like (DCL) protein. RNase III domains in dicer is specific for dsRNA and cleave the dsRNA into siRNAs. The Passenger strand unwinds from guide strand with the help of ATP independent helicase. Guide strand or antisense strand of siRNA, then bind to Argonaute protein (AGO) and accessory proteins. The guide

strand or antisense strand of siRNAs, argonaute protein (AGO) and accessory proteins combinedly form RISC (RNA induced silencing complex). Guide strand guide the RISC towards the Target mRNA. PAZ domain in the AGO recognizes the targe sequences and PIWI domain which is having endonuclease activity cleaves target mRNA., which inhibits further translation of the target mRNA. It results in silencing of target gene.

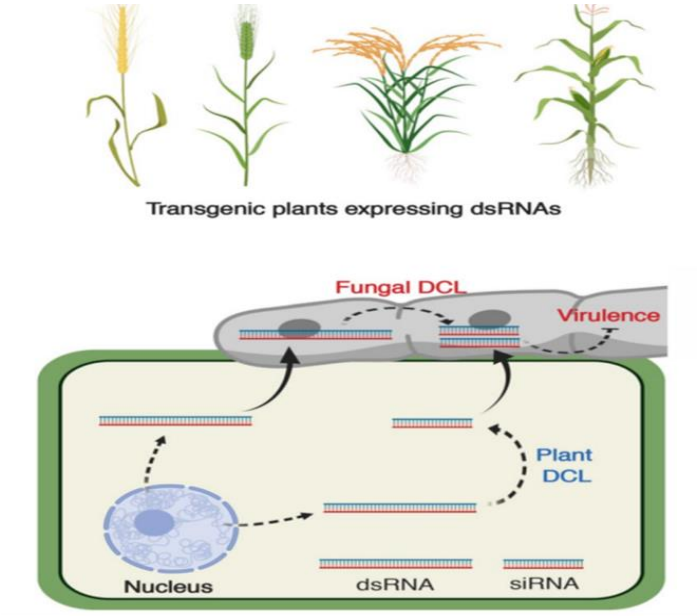

Fig 1

Mechanism of HIGS

HIGS is a somewhat conventional RNAi-based technique as compared to SIGS. It has been there since the 1990s. In this technique sequence-specific dsRNAs are expressed in the host plant to silence target genes of plant pathogens. In another word, dsRNA or a hairpin structured dsRNA construct targeting a specific pathogen gene is transformed into the host plant. The transgenic plant produces dsRNAs and siRNAs, which find their entry into the plant pathogens during host–pathogen interactions. Transgenic plants (e.g., wheat, barley, rice, and maize) expressing sequence-specific dsRNAs targeting fungal gene(s) are generated. The dsRNAs produced by transgenic plants are cleaved into siRNAs by the plant Dicer-like (DCL) proteins and both uncleared dsRNAs and siRNAs are transferred into fungal cells when a fungal pathogen infects. dsRNAs are also cleaved into siRNAs by the fungal DCL proteins. The siRNAs in the fungal cells degrade the fungal pathogen mRNAs to counteract pathogen virulence (or mycotoxin). Methods such as micro-bombardment (coating of gold or tungsten particles with DNA), agroinfiltration (injection of suspension of *Agrobacterium tumefaciens* into a plant), Virus Induced Gene Silencing (VIGS) (Viral vectors carrying a target gene fragment to produce dsRNA which trigger RNA-mediated gene silencing), etc. can be utilized.

Fungal pathogens cause a significant yield loss (more than 70%) of crop plants globally. Currently, RNAi or HIGS based approaches are being used not only for functional genomics studies but also for the development of fungal resistant crop plants (Qi *et al*. 2019). HIGS involves the silencing of vital genes in plant pathogens by expressing dsRNA using hairpin RNAi construct against specific genes of the pathogen in the host plant (Rajam 2012; Qi *et al*. 2019). In other words, siRNAs derived from the expressed dsRNA in the host plant silence the genes of the target pathogens. The concept of HIGS for the control of fungal pathogens in plants is schematically represented in Fig 2.

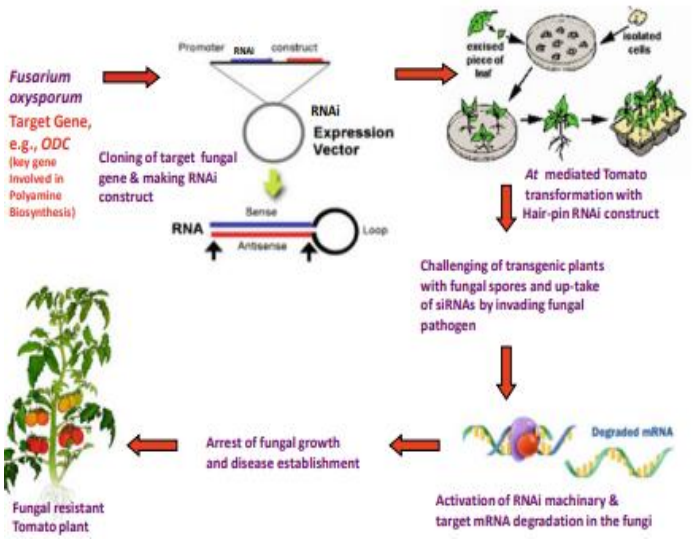

Fig 2. Concept of HIGS for developing fungal resistant crop plants

Best strategies for applying HIGS to control fungal disease

A number of possible strategies are feasible to exploit HIGS for durable resistance.

(i) A silencing construct that targets entire gene families.

(ii) Multiple lines deployed in rotation to minimize the selection pressure on pathogens.

(iii) A 'polygenic' HIGS line conferring resistance to single or multiple fungal pathogens.

(iv) A combination of both classical R genes and HIGS cassettes in the same host, which may synergistically boost resistance.

Table 1: Potential target genes in different fungi, utilized for HIGS

Fungal species	Host	Target gene name	Gene description	References
Puccinia striiformis f. sp. *tritici*	Wheat	*PsCPK1*	Protein kinase A (PKA) catalytic sub unit gene	Qi *et al.* (2018)
Blumeriagraminis	Barley	*Avra10*	Effector	Nowara*et al.* (2010)
Aspergillus flavus	Maize	*afC*	Polyketide synthase, involved in afatoxin biosynthetic pathway	Thakare*et al.* (2017)
Magnaportheoryzae	Rice	*MoAP1*	Transcription factor	Guo *et al.* (2019)
Fusarium oxysporum	Banana	*ERG11A, ERG11B, ERG11C; ERG6A, ERG6B*	Three CYP51 paralogous genes (ergosterol biosynthesis genes); two C-24 sterol methyl transferase paralogs	Dou *et al.* (2019)
Phytophthora infestans	Potato	*PiGPB1*	G protein β-subunit	Jahan *et al.* (2015)

Advantages

- ✓ Avoids application of multiple fungicides.
- ✓ Efficient transformation protocols are available for most of the world's important stable crops, including wheat, barley, rice, maize, potato, soybean, canola.
- ✓ RNAi is sequence specific and therefore is more specific than most fungicides.
- ✓ It helps to provide control of emerging fungicide resistant strains in field populations.
- ✓ A gene that shares nucleotide sequence similarity among two or more pathogens can be used as a target to control multiple diseases.
- ✓ Broad spectrum control of multiple pathogens could be developed by targeting several pathogen genes.
- ✓ Small interfering RNA and double-stranded RNA technologies do not produce heterologous proteins that could lead to concerns about allergies

Disadvantages

- ✓ Consumers' concerns about transgenic crops
- ✓ An efficient transformation protocols is not available for some crop species.
- ✓ Potential instability of HIGS transgene.
- ✓ Potential silencing of off-target genes in plant associated organisms may affect plant beneficial relationships.
- ✓ Not all fungal species may be targeted through HIGS. Some fungi species apparently lack the whole or most of the RNA silencing components in the genome.
- ✓ Some pathogenic species may already possess or could evolve suppressors of the silencing mechanism as a counter- defense strategy.

In conclusion, host-induced gene silencing (HIGS) emerges as a potent tool in the arsenal against

plant fungal diseases, offering targeted and environmentally sustainable solutions to mitigate crop yield losses. By harnessing the RNA interference (RNAi) mechanism, HIGS presents an innovative approach that holds great potential for controlling a wide range of fungal pathogens. While challenges such as consumer acceptance and technical limitations remain, ongoing research and advancements in genetic engineering are poised to address these concerns and further enhance the efficacy and applicability of HIGS in agricultural systems worldwide. As we continue to refine and implement HIGS strategies, we move closer to realizing a future where crop protection is achieved with minimal environmental impact and maximal sustainability, ensuring food security for generations to come.

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