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RNA Interference: A Tool for Plant Disease Management

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Abstract

The RNA interference mechanism is the most studied by biologists for the conservation mechanism that silences specific genes using double-stranded RNA (dsRNA). This is a process in which small RNA sequences of 21 to 30 nucleotides are produced, which control gene expression in a sequential manner. This is a valuable tool for functional genomic studies. Crops are susceptible to various types of plant diseases: Molds, bacteria and viruses do not affect the growth and development of the plant but cause a lot of damage to crops. Therefore, plant breeders have used various techniques to develop disease resistant plants for effective control of plant diseases. Among them, RNA silencing, or RNA interference, has been used as a powerful tool for creating disease-fighting products over the past two decades. Host gene silencing is used to control plant diseases by suppressing pathogen genes. (SIGS) Another RNAi-based gene silencing, also known as gene silencing, is a novel disease control strategy that has been successfully used in dicots and monocots to combat pathogens.

Key words: Disease management, Gene silencing, miRNA, Plant pathogens, RNAi, siRNA.

Introduction

Plants are attacked by various pathogens, causing many diseases, thus reducing yield. Global food production is under threat, with new ways to control plant diseases. A number of conventional breeding and genetic engineering techniques have been used to reduce crop losses. Among them, RNA-based targeting is a powerful tool to create immune effects. RNA interference (RNAi) is a biological process used to silence specific genes post-transcriptionally by activating double-stranded RNA molecules. Also known as gene silencing and RNA interference (RNAi) in fungi and animals, regulatory mechanisms of gene expression are conserved and widely found in eukaryotes. RNAi-based gene silencing is enabled by two major classes of small RNAs (sRNAs) that target organisms: miRNAs and siRNAs. MicroRNAs (MiRNAs) are produced endogenously, while small interfering RNAs (siRNAs) can be

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produced exogenously and are involved in gene regulation (Majumdar et al., 2017). The RNA hunting mechanism consists of several components: a double-stranded (ds) RNA trigger; Dicer, which acts as a processor or Dicer-like (DCL); The processing products (siRNA or miRNA) of 21 to 24 nucleotide sequences in length; An effective RISC complex involves a protein called Argonaute (AGO).

The target RNA isolated by the AGO siRNA guide is RDR (the RNA-dependent RNA polymerase that amplifies double-stranded RNA, and gene hunting (SGS) stabilizes the DCL master RNA substrate for siRNA production, thereby improving hunting.) RNA processing. HD-RNAi and HIGS are also known as host-directed RNAi, genetic manipulation that involves the production of dsRNA that targets host pathogenic genes and processes them as siRNA. The siRNA produced is taken up by the pathogen during infection. This induces the process of RNAi in the pathogen and silences the target genes. Host-directed RNAi (HD-RNAi) has been successful in increasing resistance to fungi, viruses, and plant pathogens (Table 1). RNAi transfer technology) provides protection against pathogens and pests without introducing new proteins into the diet. Host-directed RNAi (HD-RNAi) has been successful against a variety of fungal diseases such as powdery mildew of barley (Blumeria graminis), Fusarium and Puccinia.

Table 1. List of genes targeted in various crops for RNAi

Crop	Target gene	Pathogen	Function	References
Potato	HCpro	Potato virus Y (PVY)	Increase viral resistance	Missiou et al. (2004)
Arabidopsis thaliana	Gfp,CH42, pds	Cabbage leaf curl virus	Increase viral resistance	Trejo-Saavedra *et al*. (2009)
Soyabean	Pds, Actin	Bean pod mottle virus	Silencing viral genome	Pflieger *et al*. (2014)
Arabidopsis thaliana	iaaM and ipt oncogenes	*Agrobacterium tumefaciens*	Silencing tumor causing gene	Alburquerque *et al*. (2017)
Wheat	Lr, Sr	*Puccinia titiciana Puccinia graminis*	Silence leaf rust and stem rust causing genes	Hanzalová *et al*. (2020)

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RNA interference (RNAi):

It is a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules.

Traditionally, it was well-known by other names: Co-suppression and post transcriptional gene silencing (PTGS). Andrew Fire and Craig Mello (1998): First described their work on RNA interference in the nematode worm *Caenorhabditis elegans*, (shared the 2006 Nobel prize)

Mechanism

- ✓ Long dsRNAs is cleaved by the RNase III family member, Dicer, into 19-23 nucleotides (nt) fragments with 5' phosphorylated ends and 2-nt unpaired and unphosphorylated 3' ends (Fig. 1).
- ✓ These small dsRNAs are called small interfering RNAs (siRNAs).
- ✓ Each siRNA duplex is formed by a guide strand and a passenger strand.
- ✓ The endonuclease Argonaute 2 catalyzes the unwinding of the siRNA duplex.
- ✓ Once unwound, the guide strand is incorporated into the RNA Interference Specificity Complex (RISC), while the passenger strand is released.
- ✓ RISC uses the guide strand to find the mRNA that has a complementary sequence leading to the endonucleolytic cleavage of the target mRNA (Fig. 1).

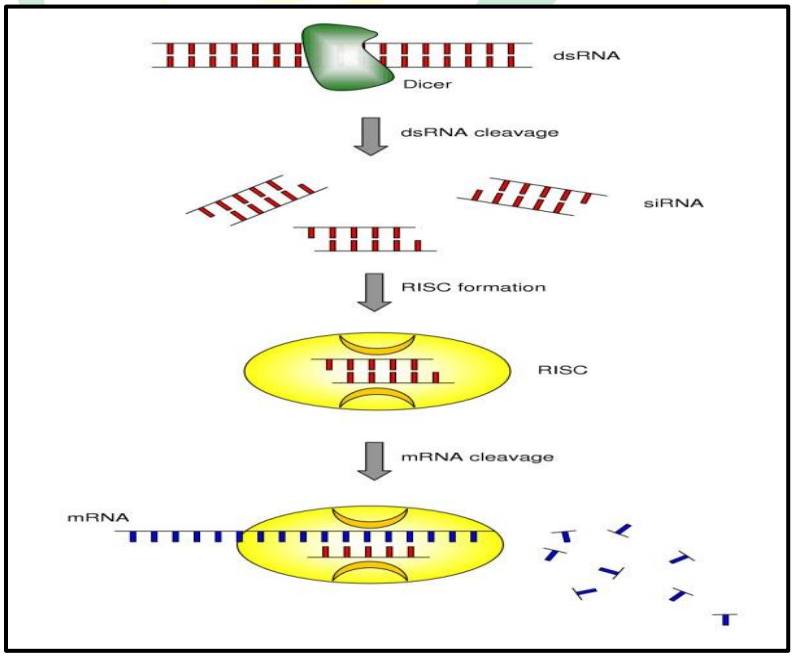

Fig 1. Mechanism of RNA interference (RNAi)

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RNA Silencing –Mediated Resistance in Plants

Resistance to bacterial pathogens

Polygalacturonase inhibitory protein (PGIP) recognizes fungal polygalacturonase (PG), which plays an important role in the initiation of infection in a variety of plant species. However, the relationship between rice OsPGIP and PG in *Xanthomonas oryzae* pv. *oryzicola* (Xoc) which causes bacterial leaf streak (BLS) is still not clear. A recent study showed that OsPGIP1 expression was strongly induced after inoculation of rice with Xoc strain RS105 (Wu et al., 2019). The researchers created transgenic rice lines based on OsPGIP1 and suppressed OsPGIP1 and showed that OsPGIP1 works well in rice resistance to BLS. In contrast to previous models of the PGIP-PG activity model, other pathogenic factors influence OsPGIP1 resistance to BLS in addition to PG in Xoc. The results showed the benefit of using OsPGIP1 for the breeding of disease-resistant rice resistant to BLS and SB caused by bacterial and fungal pathogens.

Resistance to fungal pathogens

Rice sheath blight (ShB), caused by the fungus *Rhizoctonia solani* Kuhn, is one of the most serious diseases in the world and causes a yield loss of about 50% in rice varieties. In one study, host-delivered RNAi technology was successfully used to generate stem-resistant rice cultivars by targeting two pathogenic MAP Kinase 1 (PMK1) homologues, RPMK1-1 and RPMK1-2, from *R. solani* (Tiwari et al., 2017). Transgenic lines harboring hybrid RNAi constructs showed significantly reduced levels of fungal infection compared to untransformed controls, which was also confirmed by mechanistic studies. This is the first report demonstrating the efficacy of HD-RNAi in lung cancer and offers a new opportunity for ongoing disease management. In this study, host-delivered RNAi (HD-RNAi) was used to generate rice cultivars resistant to the major pathogen *R. solani*.

Magnaporthe oryzae i. e., Blast disease of rice is a major threat to rice production worldwide. In recent years, gene silencing and siRNA have been successfully used to control fungal diseases and have been shown to be a potential tool for studying gene function in pathogens. The emergence of new types of diseases requires the development of new strategies to produce long last resistant rice varieties.

In a recent study, bromo mosaic virus (BMV)-directed RNA interference (RNAi) was used to target three putative pathogenic genes: MoABC1, MoMAC1, and MoPMK1 (Zhuo et al.,

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2017). BMV viral vectors were used to introduce fungal gene sequences in both sense and antisense methods, which significantly improved the efficiency of this host-specific transgenic RNAi, indicating that these fungal genes have the pathogenicity. The extensive study demonstrates the potential of the BMV-HIGS system as an important strategy to protect host plants from pathogenic fungi. This strategy can be used as a useful tool to study host resistance genes for rice blast protection. Recently, a study was conducted to study the effects of artificial siRNA (asiRNA) *in vitro* and HIGS *in vivo* on improving blast resistance in rice (Guo et al., 2019). The data indicate that asiRNA feeding inhibits fungal growth by targeting MoAP1 (i.e., asiR1245, asiR1362, and asiR1115), resulting in *M. oryzae* MoAP1 silencing. Transgenic rice plants expressing hairpin RNA targeting MoAP1 showed increased resistance to 11 *M. oryzae* strains tested. Microscopic results showed that mycelium supplementation significantly inhibited transgenic rice plants infected with rice blast. This study demonstrated that *in vitro* siRNA and *in vivo* HIGS can be used as important methods to improve blast resistance in rice..

Resistance to viral pathogens

- RNA interference (RNAi) is a new strategy to generate virus-resistant plants. Recently, RNAi strategies have been used to develop broad-spectrum transgenic resistance to Hainan PRSV, which limits papaya (*Carica papaya* L.) production by targeting the conserved region of papaya ring gene spotted virus (PRSV). CP was used (Jia et al., 2017). Previous reports showed that genetic differences between Hainan strains of PRSV enabled the virus to overcome CP-mediated mutational resistance. In this study, RNAi-CP mutant papaya lines were generated. Southern blot analysis and Droplet Digital PCR showed that line 474 was a single transgene insertion. siRNA products were detected in virus-free transgenic papaya tissue culture plants by Northern blot analysis. siRNA also accumulated in mutant papaya lines. Extensive research has shown that a transgenic papaya line, namely line 474, can be used against PRSV in the main growing region of Hainan, China.
- Potyvirus Sugarcane mosaic virus (SCMV) or sorghum mosaic virus (SrMV) is the cause of sugarcane mosaic disease and significantly reduces stem production and sucrose content. In this study, an RNAi strategy was successfully used to target the conserved region of the coat protein (CP) gene of SrMV to generate virus-resistant strains (Jinlong et al., 2015). The RNAi vector pGII00-HACP was constructed with an expression cassette containing both the interfering hairpin sequence and the herbicide resistance gene cp4-epsps and was

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transferred into the sugarcane cultivar ROC22 by Agrobacterium-mediated transformation. Screening showed anti-SrMV positive transgenic lines with increased resistance. This study indicates that SrMV-resistant lines of cultivar ROC22 can be a source of resistant germplasm for breeding lines and can be used as a resource to study resistance mechanism.

- Rice yield is often very low in the temperate regions of East Asia due to Rice stripe virus. A study was conducted to study the effect of RNAi on RSV-resistant transgenic rice plants constructed by inserting an inverted repeat construct targeting the nucleocapsid protein (NCP) gene of RSV (Li et al., 2016). In this study, three independent RSV-resistant transgenic rice lines were developed. Stable integration of the T-DNA fragment and siRNA expression were confirmed by Southern blot analysis and Northern blotting. RSV resistance persisted to the T5 generation. We used small RNA (sRNA) sequencing techniques to study siRNA expression profiles before and after RSV infection in transgenic and wild-type (WT) rice plants. Analysis of siRNA expression of the transfected lines showed that accumulation of siRNAs derived from the integrated NCP gene enhanced disease resistance.

Conclusion

RNAi-based gene expression approaches are a powerful tool for disease control in plants. Researchers have made great strides in this area of research. Host-mediated antifungal agents have been developed for some time. RNAi delivery using transgenic plants is now a reality and many products are now on the market. On the other hand, non-GMO RNA products should be introduced to the market in a short time. For example, spray-induced gene silencing (SIGS) using dsRNA/siRNA is attracting attention due to its low cost and ease of preparation in transgenic plants. Once dsRNA reaches the leaf surface, it can travel directly to pathogen cells (eg pathogens) or be taken up by plant cells and transferred to pathogen cells.

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