Review Article

RNA Interference: A Functional Genomics Approach for Plant Disease Management

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ABSTRACT

RNA interference machinery is most widely explored by biologist for its conserved mechanism triggered by double stranded RNA (dsRNA), results in silencing of specific genes. It is a process in which small RNAs sequence of 21–30 nucleotides are produced, that regulates the gene expression in a sequence-specific manner. It is a valuable tool for functional genomic studies. Crop plants are subjected to several plant pathogens viz. fungi, bacteria and viruses which not only affect the growth and development of plants but also results in huge yield loss. Thus, plant breeders have adopted various methods to engineer disease resistant plants for effective management of plant diseases. Among them, RNA silencing or RNA interference is used as a powerful tool during past two decades for engineering disease resistant crops. Host-induced gene silencing is mostly used for preventing the plant from disease by silencing the target genes of invading pathogen. Another RNAi-based gene silencing known as (SIGS) spray-induced gene silencing is an innovative disease control strategy used successfully for dicots and monocots against disease causing fungus and past. The present review updates the RNAi technologies and discusses the different strategies that can be adapted towards plant disease management in an eco-friendly manner.

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

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INTRODUCTION

Plants are continuously attacked by several pathogens which lead to many diseases and thereby reduce the productivity. The global food production has been threatened and it necessitates the need of novel approaches for plant disease management. Several conventional and genetic engineering approaches have been used all to reduce the yield loss. Among them, RNA based silencing has been a powerful tool which is used to engineer resistant crops. RNA interference (RNAi) is a biological process which is used for silencing a specific gene post transcriptionally by triggering double stranded RNA molecule. It is also known as gene quelling and RNA interference (RNAi) in fungi and animals, in eukaryotic organisms it is widely characterised and is conserved regulatory mechanism for expression of gene. RNAi-based silencing of gene can be triggered by two major classes of (sRNAs) small RNAs like miRNAs and siRNAs for targeting the organism. Micro RNAs (MiRNAs) are derived endogenously while small interfering RNA (siRNAs) can be endogenous or exogenous in origin and are involved in gene regulation.[1] The mechanism of RNA silencing comprises of different series of components: a double stranded (ds)RNA trigger; a Dicer which work as processor or a Dicer-like (DCL) protein; the product of processor, (siRNAs or miRNAs) of 21 to 24 sequence nucleotide in length; an effector RISC complex in which a protein called Argonaute (AGO) is the key player. The target RNA which is cleaved by siRNA guided AGO is recognised by RDR (RNA dependent RNA polymerase amplifies the double stranded RNA and (SGS) Suppressor of Gene Silencing, which stabilizes the substrate of double stranded RNA for DCLs for

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production of siRNAs strengthen the silencing process of RNA.HD-RNAi and HIGS also called Host-Delivered RNAi and Host-induced gene silencing involves production of dsRNA which targets genes of pathogen in host where they are processed into siRNAs. The siRNAs which are produced are taken by pathogen upon infection which induced RNAi process and silence targeted gene in pathogen. HD-RNAi (Host-Delivered RNAi) is successful for engineering resistance against nematodes, fungi, viruses, parasitic plants and insects (Table 1). The main advantage which is played by HD-RNAi (Host-Delivered RNAi) technology is protection against pathogens and pests without introduction of new proteins in the food products. HD-RNAi (Host-Delivered RNAi) has been successfully implemented against different fungal diseases like barley powdery mildew fungus (*Blumeria graminis*), *Fusarium* and *Puccinia* genus. In the present review we discuss the concept and components of RNAi and the approaches used to induce RNAi. We have also summarized the RNAi mediated silencing of different pathogens towards disease resistance in plants.

RNAi: The concept and its components

RNAi is a cellular mechanism that has the ability to silence a gene or protein function, a process widely known as gene silencing. It is triggered by double-stranded RNA (dsRNA) and regulates a variety of biological functions in animals, plants and fungi etc. The scientists Andrew Fire and Craig C. Mello discovered the mechanism of RNAi for the first time in the nematode worm *Caenorhabditis elegans* in 1998 for which they shared the 2006 Nobel Prize in Physiology or Medicine. The RNA silencing pathway consists of several components like an effector complex called RNA induced silencing complex (RISC), a dsRNA processor called Dicer or a Dicer-like (DCL) protein and small RNAs (siRNAs or miRNAs) of 21 to 24 nt in length.[2] Argonaute (AGO) protein is the key player in the RISC complex and has the ability to degrade the target mRNA.

Dicer is an RNase lll enzyme which is specific for dsRNA and cut the dsRNA into 3' overhangs of 2 to 3 nucleotide. It has four distinct domains: dual RNase III motifs, an aminterminal helicase domain, a dsRNA binding domain and a PAZ domain which shares with the RDE1/QDE2/Argonaute family of proteins that has been genetically linked to RNAi by independent studies. Cleavage by Dicer is thought to be catalyzed by its tandem RNase III domains. Dicer homologues from many different sources have been identified; some recombinant dicers are also identified *in vitro*. Short interfering RNAs or siRNAs are formed and accumulate as double-stranded RNA molecules of defined chemical structures, as mentioned later. These were first detected in plants undergoing either co-suppression or virus-induced gene silencing and were not detectable in control plants that were not silenced. In particular, each strand of siRNA has 5–phosphate and 3–hydroxyl termini and 2- to3-nucleotide 3- overhang.[2]

MicroRNAs or miRNAs are about 21–25 nucleotides in length and are a class of naturally occurring, small non-coding RNA molecules. They are partially complementary to one or more messenger RNA (mRNA) molecules, and their prime function is to down regulate gene expression in a variety of ways, including mRNA cleavage, translational repression, and deadenylation. Lee and colleagues were the first to describe miRNAs and the term microRNA was coined in 2001. The miRNA is synthesized from MIR genes. Many miRNAs are known to reside in introns of their pre-mRNA host genes and share their regulatory elements, primary transcript, and have a similar expression profile. After Dicer cleavage, the miRNA pathway is similar to the central steps of RNA interference (RNAi) in animals. Unlike siRNAs, microRNAs can direct RISC to down-regulate gene expression by translational repression (based on lower complementarity between miRNA and mRNA).

RISC is a ribonucleoprotein complex which comprise of members of the Ago protein family. Ago proteins have endonuclease activity directed against mRNA strands that are complementary to their bound miRNA fragment. It is partially responsible for selection of the guide strand and destruction of the passenger strand.[3] The guide strand is the strand which is incorporated and is selected by the Ago protein on the basis of the stability of the 5' end. The other strand called the passenger strand is degraded as a RISC complex substrate. After integration into the active RISC complex, miRNAs exert their regulatory effects by binding to imperfect complementary sites within the 3' untranslated regions (UTRs) of their mRNA targets. The formation of the double-stranded RNA, resulting from the binding of the miRNA, leads to translational repression.

Dicer cleaves the long dsRNAs into 19-23 nucleotides (nt) fragments with 5' phosphorylated ends and 2-nt unpaired and unphosphorylated 3' ends. The resulted small dsRNAs are called small interfering RNAs (siRNAs). Each siRNA duplex comprise of a guide strand and a passenger strand. Ago 2 catalyzes the

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

Crop	Target gene	Insect or pathogen	Function	References
Potato	HCpro	Potato virus Y (PVY)	Increase viral resistance	55
Arabidopsis thaliana	16D10	Meloidogyne species	Silencing parasitism gene	56
Gossipium species	HaHR3	Cotton bollworm	Silencing Molt regulating Transcription factor	57
Arabidopsis thaliana	iaaM and ipt oncogenes	*A.tumefaciens*	Silencing tumor causing gene	58
Soyabean	Pds,Actin	Bean pod mottle virus	Silencing viral genome	59
Arabidopsis thaliana	Gfp,CH42, pds	Cabbage leaf curl virus	Increase viral resistance	60
Wheat	Lr,Sr	*Puccinia titiciana* *Puccinia graminis*	Silence leaf rust and stem rust causing genes	61

unwinding of the siRNA duplex. Once unwound, the guide strand is incorporated into RISC complex, 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.

RNA SILENCING –MEDIATED RESISTANCE IN PLANTS

Resistance to bacterial pathogens

Polygalacturonase-inhibiting proteins (PGIPs) recognize fungal polygalacturonases (PGs), which plays an important role in initiating innate immunity in various plant species. However, the connection between rice OsPGIPs and PGs in *Xanthomonas oryzae pv. oryzicola (Xoc),* which causes bacterial leaf streak (BLS) is still not clear. In a recent study, it was demonstrated that the expression of *OsPGIP1* was strongly induced after inoculating rice with the *Xoc* strain RS105.[4] The researchers generated *OsPGIP1*-overexpressing and *OsPGIP1*-suppressed transgenic rice lines and demonstrated the positive role of *OsPGIP1* in the resistance of rice to BLS. Unlike previous examples of the PGIP-PGs working model, other pathogenicity factors induces *OsPGIP1*-mediated resistance to BLS in addition to the PG of *Xoc*. The results demonstrated the benefits of utilizing *OsPGIP1* in breeding disease-resistant rice that will be resistant to BLS and SB caused by bacterial and fungal pathogens, respectively.

Resistance to fungal pathogens

Rice sheath blight (ShB) caused by the fungal pathogen *Rhizoctonia solani Kuhn*, is one of the most devastating diseases worldwide and causes approximately 50% yield loss in rice cultivars. In a study, host delivered RNAi technology was successfully used to target two pathogenicity MAP Kinase 1 (*PMK*1) homologues, *RPMK*1-1 and *RPMK*1-2, from *R. solani* to generate sheath blight tolerant rice varieties.[5] The transgenic lines carrying the hybrid RNAi constructs demonstrated a significant decrease in fungal infection levels as compared to non-transformed controls and it was further confirmed through microscopic studies. This was the first report indicating the effectiveness of HD-RNAi against sheath blight and also provide new opportunities for durable control of the disease. In this study, host delivered RNAi (HD-RNAi) was used to produce rice varieties tolerant to the important pathogen, *R. solani*.

Magnaporthe oryzae (*M. oryzae*) is the causal organism of rice blast disease and poses a major threat to global rice production. In recent years, and host-induced gene silencing and siRNAs have been effectively used to control fungal diseases and proved a potential tool to study gene function in pathogens. The emergence of new pathotypes has necessitated the need to develop novel strategies to generate long lasting resistant rice cultivars. In a recent study, Brome mosaic virus (BMV)-induced RNA interference (RNAi) has been used to target three predicted pathogenicity genes, *MoABC1*, *MoMAC1* and *MoPMK1*.[6] BMV viral vectors were used to introduce the fungal gene sequences both in sense and antisense orientation which significantly enhanced the efficiency of this host-generated trans-specific RNAi, indicating the involvement of these fungal genes in pathogenicity. The overall study demonstrates the potential of BMV-HIGS system was an important strategy for protecting host plants against pathogenic fungi. This strategy can be used as an effective tool to study host-resistance genes for rice blast protection. Most recently, a study was carried out to study the effect of *in vitro* artificial siRNAs (asiRNA) and *in vivo* HIGS in improving blast resistance in rice.[7] The data indicated that the feeding of asiRNAs inhibited the fungal growth *by* targeting *MoAP1* (i.e., asiR1245,

asiR1362, and asiR1115) which resulted in the silencing of *M. oryzae MoAP1*. Transgenic rice plants expressing RNA hairpins targeting *MoAP1* exhibited improved resistance to 11 tested *M. oryzae* strains. Microscopic results showed profoundly restricted appressoria and mycelia in rice blast-infected transgenic rice plants. The study showed that *in vitro* asiRNA and *in vivo* HIGS can be used as important approaches towards enhanced blast resistance in rice.

Resistance to viral pathogens

RNA interference (RNAi) is a novel strategy for producing viral resistant plants. Most recently, RNAi strategy was used to target the conserved domain of the Papaya ring spot virus (PRSV) CP gene to develop a broader-spectrum transgenic resistance to the Hainan PRSV isolates that limits papaya (*Carica papaya* L.) production.[8] Previous reports say that the genetic divergence among Hainan isolates of PRSV has allowed the virus to overcome the CP-mediated transgenic resistance. In this study, RNAi-CP mutant papaya lines were generated. Southern blot analysis and Droplet Digital PCR revealed that line 474 contained a single transgene insert. Northern blot analysis detected the siRNAs products in virus-free transgenic papaya tissue culture seedlings. The siRNAs were also accumulated in the mutant papaya lines. Overall study indicates that the transgenic papaya line i.e. line 474 can be used against PRSV in the major growing area of Hainan, China.

Potyvirus sugarcane mosaic virus (SCMV) or *Sorghum mosaic virus* (SrMV) is the causal organism of sugarcane mosaic disease which leads to serious decline in stalk yield and sucrose content. In this study, RNAi strategy was successfully used to target the conserved region of coat protein (CP) genes of SrMV to produce viral resistant lines.[9] The RNAi vector pGII00-HACP was constructed with an expression cassette containing both hairpin interference sequence and *cp4-epsps* herbicide-tolerant gene and was transferred to sugarcane cultivar ROC22 via *Agrobacterium*-mediated transformation. The screening results revealed anti-SrMV positive transgenic lines with enhanced resistance. The study indicates that the modifed SrMV-resistant lines of cultivar ROC22 could be source of resistant germplasms for breeding lines and can also serve as materials for study of resistance mechanisms.

Rice stripe virus (RSV) often leads to severe yield loss in rice in temperate regions of East Asia. A research was carried out to study the impact of RNAi on RSV-resistant transgenic rice, which was generated by introducing an inverted repeat construct that targets RSV nucleocapsid protein (NCP) gene.[10] In this study, three independent RSV-resistant transgenic rice lines were generated. The stable integration of the T-DNA fragment and the expression of siRNAs were confirmed by Southern blotting and Northern blotting analyses. The RSV resistance was stably inherited in the T5 generation. Small RNA (sRNA) high-throughput sequencing method was used to study the expression profile of siRNAs before and after RSV infection in both transgenic and wild type (WT) rice plants. The analysis of siRNA profile in transgenic line indicated that the accumulation of siRNAs derived from the incorporated NCP gene resulted in enhanced disease resistance.

Resistance against insects

The RNAi-based plant protection strategies have been in the limelight from last decade to tackle insect infestations and conferring insect resistance. To realize this, two major ways of the application of dsRNA have been exploited, including expression of dsRNA in transgenic plants and use of dsRNA as an insecticide or its direct application in insects.[11] Several transgenic plants expressing dsRNAs, targeting the genes that negatively regulate plant defenses are reported to exhibit enhanced resistance to insects.[12] One such strategy that is mostly exploited in plants to mitigate insect herbivory is the host-induced gene silencing (HIGS) Although, the transgene expressions in the RNAi-plants have shown promising results in insect pressure mitigation, however, the efficiency and action mechanisms of HIGS are still debatable. For instance, the HIGS efficiency was found to be better in the *dcl* mutants of *Arabidopsis* and *Nicotiana attenuata* as compared to their wild-type plants.[13,14] Similarly, in potato transgenic lines carrying the dsRNAs in the chloroplast instead of nucleus performed well against insect infestations via HIGS; the former lacks any dsRNA processing machinery, while the later has it.[14] Therefore, it is of interest to unravel the mechanism behind the fact that long and unprocessed dsRNA can induce HIGS better than the processed sRNAs. Interestingly, HIGS efficiency also varies depending on the feeding insect group. For example, HIGS has been reported to perform at its best in controlling the insect infestations from the insect order Hemiptera. Hemiptera is one of the most significant insect order as far as the plant-insect interactions are concerned containing some major pests, including aphids and planthoppers. Among the insect groups that feed on plants, HIGS has tremendous potential to control those belonging to the order Hemiptera. Many of these insects,

such as aphids, are agronomically significant pests. Hemipterans mostly feed by using their stylets by sucking the phloem sap causing severe damage and have the potential of causing secondary damage being the vectors of plant viruses.[15,16] Thus, for the RNAi-mediated control of aphid or planthopper infestation, the key insect genes, including mouth parts encoding genes, sheath proteins, and effectors are used as targets during HIGS. In tobacco, RNAi-plants expressing the hair-pin RNAs (hpRNA) targeting *Myzus persicae* tubulin folding cofactor-D and vacuolar (V)-type proton ATPase subunit E-like (*V-ATPaseE*) conferred improved resistance. Similarly, the ectopic expression of an hpRNA targeting V-ATPaseA improved the tobacco plant resistance against whitefly infestations.[17] Several studies reported the successful control of *M. persicae* infestations by the use of specific dsRNAs targeting key aphid structural genes, such as *hunchback* and *receptor of activated kinase C*.[18,19] Feeding of the grain aphid on the transgenic barley expressing dsRNA, targeting the aphid sheath protein gene resulted in reduced survival and fecundity rates of *Sitobion avenae*.[3] Likewise, *Arabidopsis* and *N. benthamiana* plants expressing dsRNAs targeting MpC002, a well-studied aphid salivary effector molecule, resulted in up to 60% reduction of *M. persicae* performance on them.[20] Moreover, the use of HIGS have been successful in controlling insect infestations in transgenic *Arabidopsis* and tobacco plants against *Helicoverpa armigera* and *Spodoptera exigua*, respectively by targeting the cytochrome P450 gene *CYP6AE14* of *H. armigera* and *ecdysone receptor* gene of *S. Exigua*.[21,22]

Although, the efficiency of HIGS has been well-studied and established across many plant-insect models, another simpler and less controversial method of RNAi was invented and used by the direct application of *in vitro* dsRNAs onto plants and insects.[19,23] In plants, specific dsRNAs can be introduced into the vascular tissues via soil drench, seed dressing, and trunk injection for the RNAi-based crop protection.[24,19,25] On the contrary, introduction of targeted dsRNAs into the insect body often realized by the use of artificial diets or by micro-injections.[19,24] For instance, foliar application of *actin* targeting dsRNA successfully induced RNAi in *Leptinotarsa decemlineata*.[26] Similar results were obtained by the foliar application of specific dsRNAs in western corn rootworm targeting key genes, including *DvSnf7, V-ATPaseA*, and *V-ATPaseC*, which resulted in the increased insect mortality.[27-29] Foliar application of dsRNA is not highly efficient in inducing RNAi in the piercing/sucking insects, as they intake very little amount of plant tissue and largely feeds upon phloem sap. Thus, the use of micro-injections containing specific dsRNAs have yielded great success in inducing RNAi in the piercing/sucking insects; however, limited to laboratory settings, but not in field trials.[30,24] Conversely, use of artificial diets containing dsRNAs has proved to be more effective in inducing RNAi in such insects.[19] For instance, feeding the brown planthopper nymphs on artificial diet containing dsRNA or sRNA targeting *chitin synthase A* effectively induced RNAi.[30] When *M. persicae* nymphs were fed on artificial diets containing dsRNA targeting *MpMIF1* gene, reduced survival and fecundity rates were observed in the *M. persicae* adults on *N. benthamiana*.

Use of exogenous RNA to trigger RNAi in plants was first reported in the Monsanto patent, where the application of *in vitro* synthesized 685-bp dsRNA or 21-nt sRNA achieved the silencing of *phytoene desaturase* in *N. benthamiana*.[31] Subsequently, numerous studies reported the successful RNAi in several plants, including *Arabidopsis*, rice and wheat. [32,29,33] Hunter *et al*. 2012 firstly demonstrated the use exogenous dsRNA in plants against insects by injecting the plant trunk and/or drenching the roots of citrus and grapevine plants with dsRNA targeting *Arginine Kinases* of *Diaphorina citri, Diaphorina citri*, and *Homalodisca vitripennis*. This experiment served as the basic framework for the exogenous dsRNA application mediated RNAi in plants to mitigate insect pressure. For instance, spraying of specific siRNAs, targeting the acetylcholine esterase genes *AChE1* and *AChE2* of *Plutella xylostella*, onto *Brassica oleracea* plants reduced the insect pressure.[34] Similarly, application of dsRNA on tomato plants targeting the vacuolar *ATPase* of western corn rootworm resulted in improved insect resistance.[35] Root absorption of specific dsRNAs in rice and maize resulted in the reduction of brown planthopper and *Ostrinia furnacalis*, respectively.[29] Recently, the root absorption of dsRNA in tomato plants targeting *ryanodine*, *acetylcholinesterase*, and *nicotinic acetylcholine alpha6* genes of *Tuta absoluta* triggered successful RNAi in the insect.[36] Having said that, the efficiency of the exogenous applications of dsRNA or sRNA onto plants to induce RNAi in its attacking pests does differ according to the pest type and order. Therefore, various insecticidal dsRNA formulations, method of application, and dsRNA stability improvement have been explored to improve the overall RNAi efficiency.[37]

Resistance against nematodes

Plant parasitic nematodes (PPNs) pose severe threat to agricultural crops worldwide and RNAi-mediated PPN mitigation is in limelight since last decade. The initial reports of the use of RNAi to control PPNs were

demonstrated in cyst nematodes (CN) and root-knot nematodes (RKN).[38,39] Subsequently, RNAi-mediated resistance was explored in many others, including the migratory nematodes.[40-42] Although, the administration of dsRNA into the nematodes can be possible by both micro-injection and soaking of nematodes in solutions containing dsRNAs; the earlier is not effective for PPNs for their small sizes, whereas the later works fine with the presence of specific chemicals alongside the dsRNAs. For example, successful and effective RNAi were induced in *Meloidogyne incognita* and *Bursaphelenchus xylophilus* by soaking it in dsRNA solutions containing resorcinol and serotonin, and lipofectin, respectively.[39,43,44] Albeit, this method of dsRNA administration and triggering RNAi in PPNs is efficient, it is not suitable for the obligate parasitic nematodes. Therefore, the *in-planta* RNAi technique or host-delivered RNAi (HD-RNAi) edges over the soaking technique and offers new avenues to mitigate the obligate PPNs.

The HD-RNAi method has revolutionized the dsRNA/sRNA delivery into PPNs targeting the essential nematode genes. Broadly, three kinds of nematodes genes are targeted via *in-planta* RNAi, such as the housekeeping genes, the effector genes, and the development genes. For instance, the RNAi-mediated silencing of the housekeeping genes, including *ribosomal protein 3a, ribosomal protein 4, spliceosomal SR protein, Mi-Rpn7,* and *Prp-17* resulted in the significant reduction of the PPN load.[45,46] Similarly, nematode parasitism was effectively reduced by targeting some effector genes, including MeTCTP, AF531170, 4G06, 8H07, and 10AO6 via host generated RNAi. [47-49] In the soybean CN, *Heterodera glycines* silencing of a major sperm protein resulted in the reduction of number of eggs produced.[50] Further, the RNAi hampered the *H. glycines* reproduction, which lasted in its progeny exhibiting impaired reproduction ability.[50] Similarly, targeting the FMRF amide like neuropeptides in *M. incognita* via HD-RNAi showed promising results of nematode mitigation.[51] Performance of *M. incognita* on the host transgenic tobacco lines carrying *flp-14* and *flp-18* dsRNAs was reduced up to 80% as compared to the wild-type plants.

Even with its higher success rates of inducing RNAi in the PPNs, the HD-RNAi process is tedious and time consuming. On contrast, development of RNAi construct by adopting the gateway cloning system is an easier, rapid, and effective way out.[51] Usually, the hairy-root transformation method by using *Agrobacterium rhizogenes* is employed to achieve targeted RNAi in the gateway cloning method. This was successfully demonstrated in several crops, including tomato, soybean, and beetroots.[52-54] However, out of all discussed RNAi-inducing strategies, the HD-RNAi method has proven to be the most effective in controlling and mitigating PPN infections. Furthermore, identification of key PPN-target genes based on experimental bioassays are essential for ensuring proper *in planta* dsRNA expressions in the transgenic host and HD-RNAi efficiency. Moreover, further in-depth understanding of the mechanisms on dsRNA uptake by PPNs will facilitate the success of HD-RNAi and be helpful in nematode control.

CONCLUSION AND FUTURE PROSPECTS

The above studies suggest that RNAi mediated gene silencing approach is a powerful tool towards disease management in plants. Researchers have made tremendous progress in this area of research. Host induced gene silencing mediated anti-fungal pathogens are now a days developed. The transfer of RNAi by using transgenic plants is reality now days and some products are currently available in the market. Contrarily, it is expected that non transformative RNA-based products will reach the market soon. For instance, attetion has been attracted towards Spray Induced Gene Silencing(SIGS) by dsRNA/siRNA because of its low cost and flexibility as compared to transgenic plant. Once the dsRNAs reach the leaf surface it can directly move towards target pest cells (e.g., insects or pathogens) or indirectly it can be taken up by plant cells and then can be transferred into the pest cells. An alternative way is Water-soluble formulations which contain pesticidal dsRNA. It is used mostly where plant transformation cannot take place or take long time and more costly to be developed.

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CONFLICT OF INTREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RNAi: RNA interference; **siRNA:** Small interfering RNA; **HD RNAi:** Host delivered RNA interference; **PPNs:** Plant parasitic nematodes; **HIGS:** Host induced gene silencing; **PGIPs:** Polygalacturonase-

inhibiting proteins; **PGs:** polygalacturonases; **SIGS:** Spray induced gene silencing.

SUMMARY

The RNAi is a powerful technique in controlling and mitigating various biotic stresses in plants. Myriads of researches have already been conducted in investigating the efficacy and application of RNAi in conferring plant resistance against dreadful pathogens and pest. Having said that, additional efforts are required to develop new efficient and stable formulations for the dsRNA or siRNA, which could prove to be groundbreaking in the success of RNAi-mediated resistance in plants.

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