

Review Paper

RNAi IN AGRICULTURE: RECENT ADVANCES AND APPLICATIONS IN PLANT PROTECTION

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ABSTRACT

RNA interference (RNAi) is one of the emerging innovative techniques in modern agriculture. It is a natural biological process that silences essential genes in harmful insects and microbes. This occurs through the degradation of target mRNA by complementary double-stranded RNA (dsRNA). The process ultimately leads to the death of the target organisms, thereby reducing infestation. Furthermore, the cell-free technique proposed by GreenLight Biosciences enhances field applicability by significantly reducing the cost of dsRNA production. In addition, multiple strategies for delivery of dsRNA, such as foliar spraying, trunk injection, irrigation, nanotechnology-mediated systems, and bacteria-mediated transfer, have enhanced the feasibility of RNAi for field-level applications. MON87411 and SmartStax Pro are among the first RNAi mediated maize varieties for insect resistance against *Diabrotica virgifera virgifera* and tolerance to herbicides like Glufosinate, Glyphosate and 2,4-Dichlorophenoxyacetic acid whereas Calantha is a first sprayable dsRNA-based bio-pesticide approved by United States Environmental Protection Agency (EPA) against Colorado potato beetle (*Leptinotarsa decemlineata*) on potatoes in USA. Thus, RNAi represents a sustainable and environmentally safe approach for pest and disease management. However, a key challenge lies in creating awareness among farmers and regulatory acceptance of RNAi technology.

Key words: RNA interference, Plant protection, Bio-pesticide, insect resistance and herbicide tolerance.

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INTRODUCTION

Global agriculture is under increasing pressure as the world population is projected to reach nearly 9 billion by 2050 (Hamad and Tayel, 2026). As the population grows, we must grow more food, even though crops are under increasing environmental stress. Biotic stresses, such as insect pests and phytopathogenic microbes, severely impact plant health by causing tissue damage, trigger disease and substantial reductions in crop productivity, thereby raising serious threat to global food security (Gai and Wang 2024). Research has revealed that, at the global level, pests and diseases cause significant primary yield losses of approximately 26%, with secondary yield losses reaching up to 38% (Cerdeira *et al.*, 2017). To mitigate these losses, conventional approaches rely heavily on chemical pesticides due to their immediate effectiveness and ease of use. Currently, around 2 million tonnes of pesticides are utilized globally each year (Sharma *et al.*, 2019). However, over reliance on use of chemical pesticides has grown concerns about their impacts on human and environmental health and widespread development of pesticide resistance among the insect pests and phytopathogenic microbes (Sparks *et al.*, 2021).

In response to these challenges, there is a need for sustainable and precise alternatives to conventional pest management. One such emerging strategy is RNAi, a highly specific, eco-friendly gene-silencing mechanism (Cagliari *et al.*, 2019). RNAi has been found in all eukaryotic organisms, including protozoans, invertebrates, vertebrates, fungi, algae, and plants and is thought to have originally evolved as an antiviral innate immune response (Cullen, 2014). Its utilization in plant protection offers the potential to suppress pest and pathogen genes with minimal off-target effects, making it a promising tool for next-generation crop protection when incorporated into Integrated Pest Management (IPM) and Integrated Disease Management (IDM) strategies.

What Is RNAi? RNA interference (RNAi) is a natural regulatory mechanism in plants that suppresses gene expression at post-transcriptional levels (Hung and Slotkin 2021). The RNAi functions at the mRNA level, exploiting a sequence-specific approach that ensures high target precision using complementary double-stranded RNA (dsRNA) molecules. This averts the synthesis of pathogenic proteins in the plant (Dos Santos *et al.*, 2023). RNAi initiates with the introduction of dsRNA into a cell. This gene knockdown process is known as RNAi in animals, while in plants, it is commonly known as post-transcriptional gene silencing (PTGS) (Hannon, 2002; Baulcombe, 2004). RNAi has emerged as a powerful tool for studying gene function by enabling sequence-specific gene silencing through the degradation of target mRNA (Chen *et al.*, 2018).

RNAi was first detected in the early 1990s, initially leading to the hypothesis that introduced genes might co-suppress endogenous genes. However, it was later discovered that the presence of homologous RNA sequences was actually responsible for silencing the internal genes. Two types of small single-stranded RNAs i.e., siRNAs (small interfering RNAs) and miRNAs (microRNAs), approximately 21–24 nucleotides long, play vital roles in the two key RNAi pathways in plants (Kim, 2005). RNAi is known as PTGS when it degrades mRNA, and co-suppression when both endogenous and introduced genes with matching sequences are silenced. In fungi like *Neurospora crassa* (Shear and Dodge) this phenomenon is known as Quelling and Virus-Induced Gene Silencing (VIGS) when virus vectors are used to trigger RNAi, targeting both viral genomes and host gene transcripts (Agarwal *et al.*, 2003).

Table 1: Historical background of RNAi

Year	Discovery
1990	Napoli and Jorgensen first reported RNAi in plants through transgene-induced gene silencing. (Xu <i>et al.</i> , 2019)
1992	Romano and Macino discovered "quelling" in fungus <i>Neurospora crassa</i> , where homologous RNA silenced endogenous genes (Romano and Macino,1992)
1992	Guo and Kempthues reported RNA silencing in <i>Caenorhabditis elegans</i> , showing mRNA degradation through sense or antisense RNA (Kyoengwoo and Lee, 2007)
1998	Fire and Mello demonstrated that double-stranded RNA (dsRNA) is the key trigger for gene silencing (Zamore, 2006)
2002	Hannon and colleagues named the RNA-cleaving enzyme as "Dicer" (Merritt <i>et al.</i> , 2010)
2004	Joshua introduced "Slicer," the protein complex responsible for cutting target mRNA in the RNAi pathway (Hammond, 2005).
2006	Fire and Mello were awarded the Nobel Prize in Physiology and Medicine for discovering that dsRNA can silence genes by destroying specific mRNA (Zamore, 2006).

Mechanism of RNAi: Following these historical milestones, significant progress has been made in uncovering the molecular mechanisms underlying RNAi. The process of RNAi initiates with the construction of 20–26 nucleotide small RNAs (sRNAs), including Dicer-like (DCL) proteins, Argonaute (AGO) proteins and RNA-dependent RNA polymerases (RDRs) (Muhammad *et al.*, 2019). Argonaute (AGO) is the core protein that binds small RNAs (siRNA, miRNA) and uses them as guides to recognize and silence complementary target mRNAs. DCL proteins, which are RNase III-type endonucleases, generate sRNAs from precursor dsRNA (Gabriela and Plinio 2008; Muhammad *et al.*, 2019). sRNAs are classified as small interfering RNAs (siRNAs) or microRNAs (miRNAs), based on their origin and structure. AGO proteins bind to these small non-coding RNAs (siRNAs or miRNAs), guiding them to specific target RNAs through sequence complementarity. Together, siRNA, Dicer and Argonaute form the RNA-induced silencing complex (RISC), which acts at the translational level (Hirooki and Yukihide, 2022). Within RISC, AGO proteins interact with homologous target RNAs, leading to outcomes such as DNA methylation, end nucleolytic cleavage, or translational repression of mRNA of the target pests and plant pathogens.

siRNAs originate from diverse external sources such as viruses, transgenes, or experimentally introduced dsRNA. Whereas miRNAs are encoded by endogenous genes and are transcribed by RNA polymerase II as primary miRNAs (pri-miRNAs). Both siRNAs and miRNAs are then incorporated into a multi-protein complex called as the RNA-induced silencing complex (RISC). RISC complex also comprises of AGO proteins that selectively bind one strand of the siRNA or miRNA duplex which is referred to as the guide strand. The guide strand directs the RISC complex to the target messenger RNA (mRNA) through complementary base pairing that result in highly sequence-specific regulation of gene expression (Hirooki and Yukihide, 2022). Once the target mRNA is recognized by the RISC complex, the AGO proteins catalyse various downstream silencing effects depending on the level of sequence complementarity. The siRNAs cleave and subsequently degrade the target mRNA in case of perfect or near perfect complementarity. In

contrast, miRNAs repress translation or promote deadenylation and decay of the target mRNA even with partial complementarity (Neumeier and Meister, 2021).

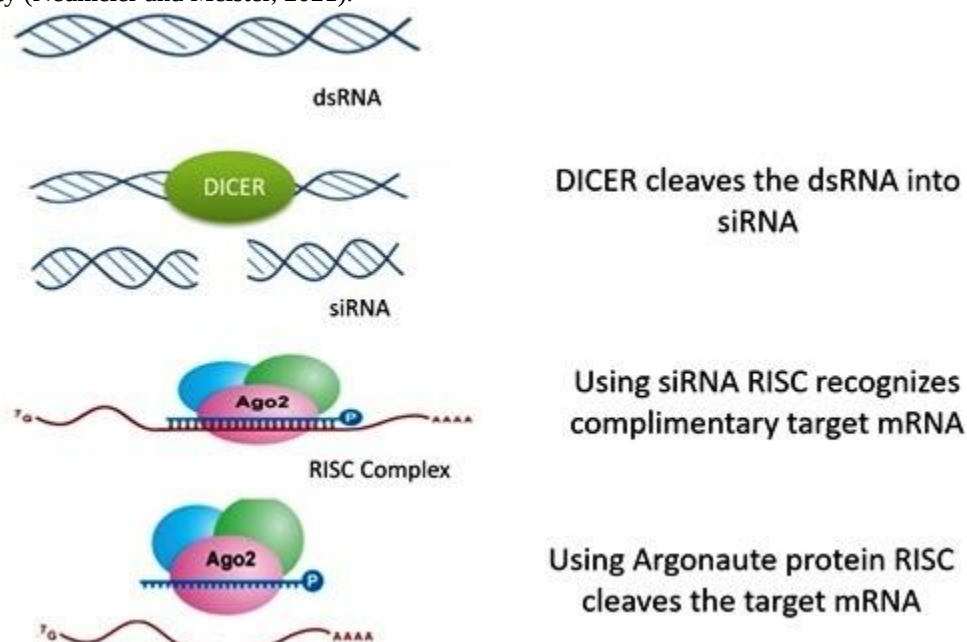


Fig 1: Diagrammatic summary of RNAi-mediated mRNA degradation

Beyond mRNA cleavage and repression of translation, RNAi machinery can also initiate transcriptional gene silencing (TGS) in plants through DNA methylation and histone modifications. This regulation is important for maintaining genome stability, silencing transposable elements, and adapting to biotic and abiotic stress. Additionally, RNA-dependent RNA polymerases (RDRs) present in plants and some invertebrates, can amplify the RNAi response by synthesizing new dsRNAs using the cleaved mRNA as a template, thereby enhancing the silencing signal systemically (Khan *et al.*, 2026).

Pathways of RNAi: siRNA-mediated pathway used for defense against viral infections and plant protection (Mondal *et al.*, 2018; Swevers *et al.*, 2018).

1. miRNA-mediated regulation of gene expression in plant growth and development (Yang *et al.*, 2014; Ylla *et al.*, 2016; Song *et al.*, 2019).
2. piRNA-mediated suppression of germline transposon expression in insect germline to maintain genome stability (Nandety *et al.*, 2015; Van *et al.*, 2018).

RNAi from laboratory to land

Revolutionizing cost-effective dsRNA manufacturing: Although RNAi offers significant potential as a reliable and eco-friendly approach for managing pests and diseases in plants, its broad field application is frequently constrained by challenges in cost-effective production and efficient delivery methods. Traditionally, *in vitro* transcription kits which utilize purified RNA polymerases and nucleotides have been widely used in laboratory settings. However, their high cost (approximately \$700 per mg of dsRNA) makes them impractical for large-scale agricultural deployment (He *et al.*, 2022).

To address this limitation, GreenLight Biosciences has developed an innovative large-scale, cell-free dsRNA production platform that significantly reduces costs. Instead of relying on externally supplied reagents, this system leverages endogenous cellular RNA, which is first depolymerized into nucleoside monophosphates (NMPs) using nucleases. These NMPs are then phosphorylated into nucleotide triphosphates (NTPs) by kinases and finally polymerized into the target dsRNA using a DNA template and RNA polymerases. This streamlined approach has brought down production costs dramatically from about \$1 per gram in fermentation-based systems to as low as \$0.50 per gram making it highly competitive and a viable solution for application of RNAi at field (Marrone, 2024).

Effective delivery methods for RNAi: The overall efficacy of RNAi in pest management is largely determined by the delivery method of dsRNA, making it a crucial consideration in the design of RNAi-based strategies (Choudry *et al.*,

2024). Broadly, delivery methods can be categorized into transformative and non-transformative. Transformative delivery involves the genetic modification of host plants to endogenously express dsRNA, thus providing continuous, systemic protection against target pests (Hough *et al.*, 2022). While this method ensures precise and highly effective pest control, its widespread adoption is limited due to regulatory challenges and low public acceptance of genetically modified (GM) crops. Non-transformative methods involve external application of dsRNA without altering the plant's genome. These are GMO-free approaches that make them more appropriate for regions having strict regulations for genetically modified crops (Hough *et al.*, 2022). However, it provides temporary protection and requires continuous supply of ds RNA. Delivery methods in this category include foliar spraying, irrigation, root dipping, trunk injection, bacterial-mediated dsRNA delivery and nanotechnological approaches to transfer dsRNA to the target plants. (Joga *et al.*, 2016).

Table 2: Efficacy of RNAi against various agricultural pests and pathogens through targeted gene silencing

Target Organism	Target Site	Effects	References
<i>Spodoptera exigua</i> (Hübner)	Trehalase genes (SeTre-1 and SeTre-2)	RNAi of SeTre-1 & SeTre-2, resulted in higher mortality rates during larva-pupa and pupa-adult stage.	(Chen <i>et al.</i> , 2010)
<i>Spodoptera exigua</i> (Hübner)	Chitin synthase gene A (CHSA)	Disordered cuticle in treated insects, reduced CHSA transcript level	(Chen <i>et al.</i> , 2008)
<i>Ostrinia nubilalis</i> (Hübner)	Chitinase gene (OnCht)	Chitin transcript level reduced by 63-64%.	(Khajuria <i>et al.</i> , 2010)
<i>Helicoverpa armigera</i> (Hübner)	AchE	Reduction in larval growth, pupal weight and fecundity. Mortality and malformation in larvae.	(Kumar <i>et al.</i> , 2009)
<i>Leptinotarsa decemlineata</i> (Say)	Actin, vATPase, Sec 23	Reduced body weight and significant mortality in the treated beetles	(Zhu <i>et al.</i> , 2011)
<i>Verticillium dahlia</i> (Klebahn)	HiC-15 (a hydroxylase) and Clp-1 (a cysteine protease)	Transcripts of HiC-15 and Clp-1 were targeted, reducing their expression in infected fungal hyphae leading to reduced wilting.	(Zhang <i>et al.</i> , 2016)
<i>Blumeria graminis</i> (de Candolle) Speer	Effectors Avra10 and AvrK1	Reduced haustorium and fungal development	Novara <i>et al.</i> (2010)
<i>Meloidogyne incognita</i> (Kofoid and White Chitwood)	16D10 peptide	Decreased gall development and fewer <i>M. incognita</i> eggs were produced	Huang <i>et al.</i> (2006)
<i>Puccinia triticina</i> (Eriksson)	MAPK, CYC1 and CNB	Suppression of leaf rust disease marked by reductions in fungal growth, sporulation, and disease symptoms.	(Panwar <i>et al.</i> , 2013)

Foliar spray: Spray induced gene silencing (SIGS) is an emerging, promising and environmentally friendly strategy. It involves external application of double-stranded or siRNAs to plant surfaces. This RNA can act in two ways: it may penetrate the insect's cuticle when sprayed directly onto pests, leading to lethal effects, or it can be applied to crops, where it is ingested by pests during feeding or contact. In a study conducted by Gong *et al.* (2013), six siRNAs targeting genes responsible for production of acetylcholine esterase in *Plutella xylostella* (Linnaeus) were tested and the studies revealed that the best insecticidal activity with 89% mortality rate was observed when second instar *P. xylostella* were fed with *Brassica* spp. leaves sprayed with siRNAs. However, the ability of dsRNA to penetrate into the cuticle is different among insects, and the topical application is not fit for all insect pests (Li *et al.*, 2015).

Exogenous application of these RNAs has demonstrated defensive effects in various crops such as barley, tomato, strawberry, grape, rapeseed, wheat, onion, rose, lettuce, cucumber, soybean, and *Arabidopsis* against various fungal pathogens such as *Fusarium graminearum*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Fusarium asiaticum* (Ray *et al.*, 2022). According to Koch *et al.* (2016), spray applications of dsRNAs and siRNAs onto barley detached leaves decreased fungal diseases by inhibiting fungal growth and suppressing three fungal cytochrome P450 genes CYP51A, CYP51B and CYP51C of *F. graminearum*. Exogenous application of dsRNAs was also found effective in conferring protection against Tobacco Mosaic Virus (TMV) and Pepper Mild Mottle Virus (PMMoV) in tobacco; Zucchini Yellow Mosaic Virus (ZYMV) in cucumber, watermelon, and squash plants (Kaldis *et al.*, 2018). Vadlamudi *et*

al. (2020) reported that topical application of dsRNAs targeting the CP and HC-Pro genes of PRSV-Tirupati conferred complete resistance to PRSV-Tirupati and 94% and 81% resistance to PRSV-Delhi in papaya.

Irrigation: The delivery of dsRNA via crop roots can trigger RNAi in insect pests and the irrigation of RNAi-based products seems to be an alternative for suppressing pests feeding/growing in stems and fruits (Yan *et al.*, 2020). A study was conducted for exploring the applications of dsRNA via crop roots against the brown planthopper *Nilaparvata lugens* (Stål), and the Asian corn borer, *Ostrinia furnacalis* (Guenée). (Li *et al.*, 2015). When *Nilaparvata lugens* (Stål) fed on rice plants irrigated with carboxylesterase-targeting dsRNA, mortality reached nearly 50% five days after treatment. Thus, irrigation is a simple yet practical method to deliver dsRNA; however, dsRNA may be degraded within approximately 2 days after the application to soil, regardless of texture, pH, clay content and other soil differences (Dubelman *et al.*, 2014). Hence, the success of this delivery strategy relies on the advances of formulations to protect dsRNA from degradation.

Trunk injection: The efficiency of foliar spray and irrigation of dsRNA are relatively low for trees. In contrast, trunk injection has emerged as a promising technique for delivering dsRNA in various tree species, offering advantages such as reduced losses and negligible consumer exposure (Wise *et al.*, 2014; Berger *et al.*, 2019). Phloem is considered as the most suitable channel for the transport of dsRNA/siRNA where it can remain viable for long periods, because of the RNase free environment in phloem sap (Doering *et al.*, 2002; Melnyk *et al.*, 2011). Citrus trees and grapevines were treated with dsRNA via root drench and trunk injection, and the dsRNA was taken up into the whole plant system over 3 months to suppress insect pests (Hunter *et al.*, 2012). The control of some insect pests has been difficult, especially for underground root feeding pests and the trunk injection may solve this problem (Shang *et al.*, 2024). This strategy can prove to be more effective for sap-sucking pests than for chewing pests feeding largely on leaves (Joga *et al.*, 2016).

Bacteria- expressed dsRNA: The delivery of dsRNA using bacteria has many advantages when compared with plant-mediated dsRNA delivery or *in-vitro* synthesized dsRNA delivery (transgenic crop). The application of bacteria-expressed dsRNA is low cost than *in-vitro* synthesized dsRNA. The bacteria expressed dsRNA pesticides can be sprayed on crops at any time because of the ease of mass multiplication of dsRNA expressing bacteria. Colorado potato beetle (*Leptinotarsa decemlineata*), which were fed on different *Escherichia coli* transformations were controlled as dsRNA targets five different mRNAs (Zhu *et al.*, 2020). The RNAase III - deficient *Escherichia coli* used for dsRNA production caused significant mortality and loss of body weight after the beetles ingested the bacteria.

Nanotechnology mediated RNAi: Nanoparticles are defined as any particle within the range of 1 and 100 nm diameter (Bhoi *et al.*, 2024). Various entities in cellular environment adversely affect dsRNA by reacting with it and hence reducing its effectivity in targeting site. Nanoparticles, in addition to protecting and shielding the dsRNA from environmental degradation also promote the translocation and penetration of dsRNA across the peritrophic membrane, cell membrane and insect cuticle (Shen *et al.*, 2014; Zheng *et al.*, 2019). In most cases, the nanoparticle combines with dsRNA to form the nanoparticle-dsRNA complex through the electrostatic interactions between the cationic groups of nano-particle and the phosphate groups in the dsRNA (Yin *et al.*, 2011; He *et al.*, 2013). The complex usually possesses a net positive charge that facilitates the interaction with negatively charged cell membrane. Upon binding to the cell membrane, the complex is internalized into the cytoplasm via endocytosis (Benjaminsen *et al.*, 2013; Cappelle *et al.*, 2016). Association between the nanoparticle and dsRNA can prevent degradation within the endocytic vesicles, a phenomenon referred to as the sponge effect (Selby *et al.*, 2017).

Virus- Induced Gene Silencing (VIGS) in Plants: Virus- induced gene silencing (VIGS) exploits the innate plant defense system of posttranscriptional gene silencing (PTGS) against intracellular viral proliferation and extracellular viral movement. The modified viral genomes combined with part of the plants target gene are transformed into the plant via *Agrobacterium tumefaciens* TI plasmid- based VIGS (Krenek *et al.*, 2015).

RNA or DNA plant viruses can elicit VIGS: Several plant viruses have been adopted as VIGS vectors to down-regulate an endogenous plant's target gene after inoculating the plant with the VIGS vector. Most of these viral vectors are derived from positive-strand RNA viruses such as Potato virus X (PVX), Tobacco mosaic virus (TMV) and Tobacco rattle virus (TRV) which contain either mono-, bi- or tripartite genomes. These vectors are usually engineered so that the modified cDNA copy of the viral genome is inserted into a binary vector system, transformed into *Agrobacterium tumefaciens* strains, and used for subsequent agroinoculation. However, alternative methods for delivering the viral vectors to the plant have been developed, including mechanical or biolistic inoculation using *in vitro* synthesized modified viral transcripts delivered directly. A different set of VIGS vectors are bipartite single- stand DNA viruses and the monopartite DNA viruses some of which may require a helper virus or satellite DNA for disease symptom induction. For example, Tomato yellow leaf curl China virus (TYLCCV) with its associated DNA β satellite can deliver the sequence without causing typical effects of virus infection. RNA virus-delivered VIGS systems with satellite and helper

RNAs have also been used, particularly in *Nicotiana tabacum*, where viral replication and movement provided by the satellite RNA are uncoupled from gene silencing induction by the helper RNA. This can result in stronger silencing phenotypes compared to satellite RNA virus alone, a system also known as satellite virus-induced silencing system (SVISS) (Valentine *et al.*, 2004; Zulficar *et al.*, 2023).

Virus systems used for silencing target genes in monocotyledonous plants: The Barley stripe mosaic virus (BSMV), a tripartite RNA virus, has been a leading system for gene silencing in monocotyledonous (monocot) plants. BSMV can infect several important crops like barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*) and is widely used to silence endogenous genes in these species, including both above-ground and root tissues. Additional virus systems have been explored, such as Foxtail mosaic virus (FoMV), which has been engineered to silence genes effectively in barley, wheat, and foxtail millet (*Setaria italica*), expanding functional genomics capabilities to more monocot models (Liu *et al.*, 2016; Beernink and Whitham, 2023). The FoMV-based vectors can silence genes, such as PDS and magnesium chelatase, resulting in visible phenotypes like photobleaching or chlorosis. Chinese wheat mosaic virus (CWMV) is another example known for its efficiency in gene silencing at lower temperatures compared to BSMV and FoMV. CWMV vectors have been shown to efficiently silence both mRNA and miRNAs in wheat and tobacco at 17°C, surpassing the efficiency of other systems at this temperature (Yang *et al.*, 2018). Other virus systems used for monocot VIGS include Bamboo mosaic virus (BaMV) and its satellite RNA, which have been applied to species like *Brachypodium distachyon* (Liou *et al.*, 2014). The importance of these virus systems is their ability to down-regulate target genes rapidly and transiently, bypassing the need for stable transformation or mutant collections, which are often difficult in monocot species. This makes VIGS approaches central for functional genomics, particularly in crops with complex or poorly characterized genomes.

Virus systems used for silencing target genes in dicot plant species: Basal Eudicot Species- Tobacco rattle virus (TRV)-based bipartite vectors have established VIGS as a powerful tool for functional studies in basal eudicots. Species such as *Aquilegia formosa*, *Eschscholzia californica* (California poppy), *Papaver somniferum* (Opium poppy), and *Thalictrum* spp. have all been successfully targeted. For example, TRV-mediated silencing of the PHYTOENE DESATURASE (PDS) gene results in visible photobleaching and significant mRNA reduction—demonstrating effective gene knockdown without affecting plant growth or fertility (Hileman *et al.*, 2005). The TRV system typically requires two vector components: one for viral replication and movement, and another for encoding proteins necessary for virion formation. Both are delivered (often by *Agrobacterium* or direct infiltration) for successful infection and gene silencing in basal eudicot cells (Hidalgo *et al.*, 2012).

Rosid Species- TRV, Turnip yellow mosaic virus (TYMV), Apple latent spherical virus (ALSV), Bean pod mottle virus (BPMV) and Cabbage leaf curl virus (CaLCuV) are among the vectors employed for VIGS in rosids. Species successfully targeted include *Arabidopsis thaliana*, various cucurbits such as *Cucumis sativus*, *C. melo*, *Cucurbita pepo*, *Citrullus lanatus*, *Fragaria ananassa* (strawberry), *Glycine max* (soybean), *Gossypium hirsutum* (cotton), *Malus domestica* (apple), *Medicago truncatula*, *Phaseolus vulgaris* (common bean), *Pisum sativum* (pea), and *Rosa hybrida* (rose), among others. For *Arabidopsis thaliana*, protocols using both RNA and DNA virus-derived vectors have been established. The ALSV system, for example, facilitates effective downregulation of both endogenous and transgenic genes in a wide host range, including important rosid fruit crops.

Asterid Species- TRV, Tomato mosaic virus (ToMV), Potato virus X (PVX), and DNA virus vectors have enabled VIGS in asterids, particularly within the Solanaceae and Petunia families. Species such as *Nicotiana benthamiana*, *N. tabacum*, *Solanum lycopersicum* (tomato), *Solanum tuberosum* (potato), and *Petunia hybrida* are prominent models, making Solanaceae the most widely used family for VIGS experimentation. *Nicotiana benthamiana* was the first species where VIGS was reported, frequently using TRV for gene silencing. Success has also been reported in several other *Nicotiana* and *Solanum* species, and increasingly in broader asterid clades (Deng *et al.*, 2012).

Commercial applications and regulatory approvals for RNAi-based products: In recent years, RNAi based approaches have significantly advanced crop protection and improvement, resulting in the introduction of several commercial RNAi based products. The first RNAi based products was approved by U.S. regulatory authorities in 2017, followed by Chinese regulators in 2021. On June 15, 2017, the United States Environmental Protection Agency (EPA) approved MON87411, the world's first insect-resistant GM corn that utilizes dsRNA to target the *DvSnf7* gene for controlling rootworms. This milestone marked a major breakthrough in the field of pesticide development (Christiaens *et al.*, 2022; De Schutter *et al.*, 2022). Bayer's "SmartStax Pro" maize (MON87411) integrates multiple traits, including expression of the *Bacillus thuringiensis* (Bt) protein- Cry3Bt1 for insect resistance, glyphosate herbicide tolerance, and dsRNA-mediated silencing of the *Snf7* gene in the western corn rootworm (*Diabrotica virgifera virgifera*). This product is available to farmers in the US since 2022 and in Canada since 2023.

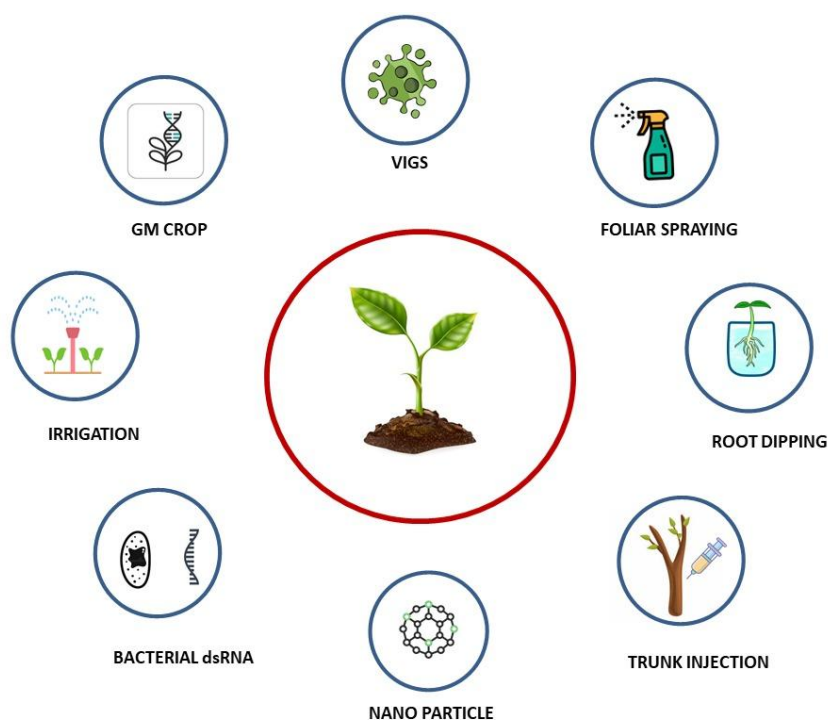


Fig 2- Effective delivery methods for RNAi

Calantha™, the first sprayable dsRNA biopesticide was approved in December 2023 by EPA (Navra *et al.*, 2025) with Ledprona as the dsRNA active ingredient that targets the gene encoding the PSMB5 protein in the Colorado potato beetle (*Leptinotarsa decemlineata*) while infesting potato. (Bramlett *et al.*, 2020; Rodrigues *et al.*, 2021). Bayer's "Vistive Gold" high-oleic soybean (Mon87705), which involves RNAi-mediated silencing of a gene in the fatty acid biosynthesis pathway, has received approval for cultivation, food, and feed use in USA, Canada, and Japan and for food and feed use in the European Union (Shelke *et al.*, 2023). Additionally, Syngenta and Greenlight Biosciences have recently completed successful field trials showing resistance against the Colorado potato beetle in dsRNA-treated plants (Bramlett *et al.*, 2020; Rodrigues *et al.*, 2021). Bayer's SIGS-based "BioDirect" platform has also shown promising results in controlling *Varroa destructor* mites in honeybee colonies through RNAi technology that led to reduced mite populations and improved colony survival (De Schutter *et al.*, 2022). The platform uses specially designed double-stranded RNAs (dsRNAs) that target vital genes in *Varroa* mites. These genes are crucial for mite survival, reproduction, and development. Silencing these essential genes disrupts critical biological functions in the mites, leading to reduced mite viability, decreased reproduction rates, and eventually lower mite populations.

Challenges faced by RNAi

a) Cost and scalability of dsRNA production:

Initially, the production cost of dsRNA and siRNA, the core functional units of RNAi, was high. The introduction of the cell-free dsRNA production technique by GreenLight Biosciences has largely addressed this issue. However, the cost and inefficiency of delivery agents, such as lipid nanoparticles or chemical modifications, remain a major concern for large-scale deployment. These agents are essential to protect the fragile RNA from degradation and to ensure effective cellular uptake, making the complexity and expense of the delivery system the most persistent barrier to commercial scalability.

b) Environmental stability and degradation:

Although RNAi presents a promising alternative to chemical pesticides for insect pest and disease control, further studies are required before it can become a practical solution. The long-term effectiveness of RNAi-based products needs careful evaluation, as minor changes in the target DNA sequence can prevent dsRNA from binding to host mRNA, thereby reducing its lethality. RNAi is applicable only when the genome sequence of the target organism is known, limiting its use for species whose genomes have not yet been sequenced. Additionally, the presence of nucleic acid-degrading enzymes in insects can degrade dsRNA, further diminishing its efficacy in pest control.

c) Risk of resistance and off-target effects:

A small change in the nucleic acid sequence of the target organism can lead to resistance against RNAi, since it relies on recognizing specific gene sequences to silence the target gene effectively. If the target gene sequence changes even slightly, the RNAi mechanism may fail to identify and degrade the intended target mRNA, thus losing its lethal effect. Moreover, RNAi may unintentionally affect non-target organisms that carry similar gene sequences, potentially leading to harmful off-target effects

d) Regulatory hurdles and public acceptance:

The use of genetically modified organisms (GMOs) faces regulatory hurdles and limited public acceptance in many developing countries for example in India tremendous research in GM crops has been done in last two decades but there is lack of acceptance towards them due to lack of coordination across different governments, ministries and departments (Shukla *et al.*, 2018). Government regulations often restrict the approval of GMO technologies, including RNAi based products, due to concerns about safety, environmental impact, and ethical considerations. Additionally, lack of public awareness about advanced biotechnologies like RNAi contribute to resistance against their adoption.

Future directions: Since RNAi relies on sequence-specific gene silencing, future studies should be focused on bioinformatics and genome sequencing that will enable the design of highly specific dsRNA molecules, minimizing off-target effects and reducing the risk of resistance development. Integrating RNAi with other pest management strategies (e.g., CRISPR-based gene editing or microbial biocontrol agents) could also enhance durability of pest control. RNAi should be explored as part of integrated pest management (IPM) systems, in combination with biocontrol agents, natural products and resistant crop varieties, to reduce chemical pesticide dependence. A cost-effective delivery technique that protects the nucleic acid from environmental degradation and efficiently carries it to the target site remains one of the most underexplored yet critical areas in RNAi research. Developing such delivery systems will enhance the stability, accuracy and overall effectiveness of RNAi-based applications. Approaches like nanoparticle encapsulation, liposome carriers, or plant-based expression systems can help maintain RNA stability and improve its bioavailability in the field. Policy development and communication strategies that will improve the public understanding and regulatory acceptance of RNAi technologies, will ensure the smooth translation from lab to land.

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