RNA Interference Technology and Control of Whitefly (*Bemisia tabaci)*

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Abstract

Whiteflies cause significant crop losses both directly as well as indirectly through viral spread. The present control techniques are a collection of varied control approaches, with the majority still depending on hazardous and non-ecofriendly chemicals for control. RNA interference is a type of posttranscriptional silencing approach in which a targeted organism is introduced with dsRNA comparable exactly to a target gene. This method has become known as a potential option for controlling pest insects in agriculture. When whitefly consumes these dsRNA molecules, they activate a silencing process that limits the activity of the target genes, resulting in lower populations of pests as well as crop damage. So far, multiple genes in whiteflies are being addressed using RNAi, and these experiments have proved their potential to control whiteflies. Specificity, efficacy, and environmental safety are all advantages of RNAi-based whitefly control over traditional chemical-based techniques. However, there are certain limitations to using RNAi, such as the possibility of effects that are off-target and the requirement for efficient delivery systems. Nonetheless, RNAi technology shows considerable potential for long-term and successful whitefly in agriculture.

Key words: RNA interference technology, Whitefly, *Bemisia tabaci*

Introduction:

The whitefly, *Bemisia tabaci*, is a global multifarious insect threat that has decimated agricultural output (Abubakar et al., 2022). Whiteflies transmit a significant number of plant viruses very frequently (Navas-Castillo et al., 2011). On a global basis, important vegetable, and fiber crops are under threat, due to whitefly, particularly in underdeveloped nations where dependable food supplies are rare (Rodríguez et al., 2019). The economic harm produced by *B. tabaci* ranges from minor to devastating, with global yearly losses in several crops exceeding billions of dollars (Sani et al., 2020). Infection by the whitefly can cause substantial decreases in the photosynthetic potential of the plant that is being infected, resulting in stunted growth and decreased agricultural output and quality (Gao et al., 2017). RNAi technology has the potential to be a formidable weapon for combating fluid-feeding pests, and it is believed to be extremely specific in the case of target genes (Grover et al., 2019). RNAi is a kind of suppression of genes that is carried out with dsRNA (DAS & BAISAKH). Although RNAi has undergone significant conservation throughout eukaryotes, variations in those elements can be identified across taxonomic groupings (Christiaens & Smagghe, 2014). The RNAi mechanism includes the production of intervening molecules via the action of the dicer enzyme, siRNAs along with miRNAs are two types of intervening molecules (Mamta & Rajam, 2017). The siRNAs are integrated into an ARGONAUTE class protein, and the pairing of bases complementary provides AGO with pattern selectivity that determines the mRNA to target (Hung & Slotkin, 2021).

Cellular absorption, dsRNA deterioration, inter- as well as intracellular transfer, and dsRNA conversion into siRNA all contribute to RNAi effectiveness (Lucena-Leandro et al., 2022). It entails using tiny RNA molecules, generally 21-23 nucleotides long, to suppress or "silence" the production of certain genes. The RNAi mechanism begins with the creation of comparable dsRNA molecules to the mRNA molecule that is being targeted. Those dsRNA molecules undergo degradation using the Dicer enzyme that breaks them down into tiny molecules of RNA known as siRNAs (Munawar et al., 2023). These siRNAs are subsequently incorporated into the RISC, a complex of several proteins in which the Argonaut participates, and it destroys the siRNA's

sense strands. The active RISC, combined with the antisense strand of siRNAs, then breakdown or suppress translation by locating the homologous target mRNA depending on the comparable sequence. As a consequence of the extent of sequence analogy, there is no protein (Rajam, 2020). Because miRNA synthesis varies from the aforementioned siRNA processes, using miRNA to earn RNA silencing necessitates a distinct transgenic design. miRNAs are short RNAs that are produced from transcripts that have a characteristic RNA secondary arrangement of stem-loop. Since the mature and coiled transcript contains dsRNA, the miRNA process doesn't involve an RDR. Primary miRNAs are the original transcripts that are eventually converted to miRNAs (Frizzi & Huang, 2010). Pre-miRNA transcripts are generated and delivered to the cytoplasm where Dicer processes them to produce matured miRNAs. Though the majority of miRNAs need Dicer-like1 (DCL1) (Xie et al., 2005). RNAi has been used to manage pests through the delivery of RNA fragments that target critical genes in insect species. These chemicals are picked up by the insect's cells and activate the RNAi process, causing the target messenger RNA to be degraded and protein production to be reduced. RNAi can therefore be employed to precisely eradicate insect pests while causing no damage to off-target creatures (Haroon et al., 2022).

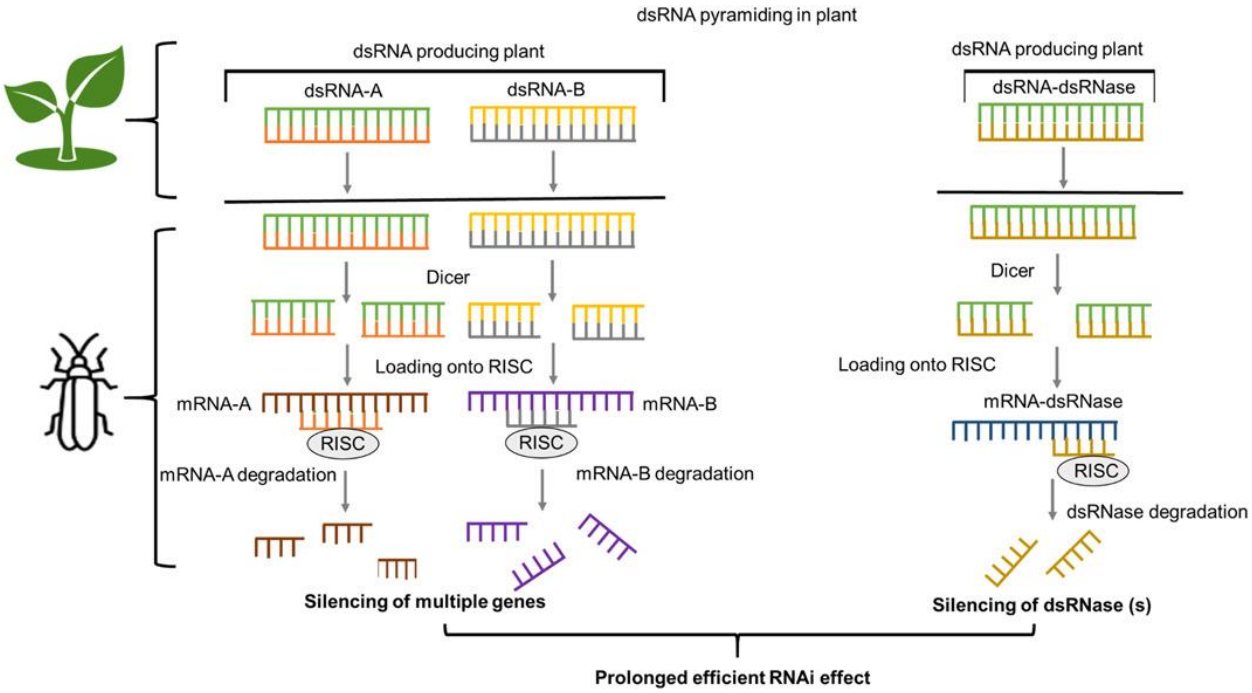

Figure 1 illustrates dsRNAs building in crops to control pests by suppressing insect genes. Figure adopted from (Grover et al., 2019).

The application of RNAi is proving to be an effective crop protection strategy for combating whiteflies (Jain et al., 2021). Scientists have started to look at the practicality of using RNAi to combat pests that affect agriculture (Shelby et al., 2020). The ultimate objective of enhancing whitefly control is to enable crop production that is sustainable in regional agroecosystems (Simmons & Riley, 2021). Pest management with RNAi can minimize the usage of conventional pesticides, which can have significant environmental as well as medical consequences. However, several issues have to be solved, such as impacts that are off-target as well as the growth of the insect population have developed resistance. Nonetheless, RNAi is a potential method for controlling insect pests that are expected to grow more essential in the years to come (Zafar et al., 2020).

Whiteflies and their Host's Plants

Whiteflies have a diverse host plant range; for example, B. tabaci has been recorded as infecting more than 900 plant hosts. Only a few whiteflies spread over hundreds of plant-infecting viruses including genera Begomovirus, Carlavirus, Crinivirus, Ipomovirus, and Torradovirus (Fiallo-Olivé et al., 2020). Some commercially significant whiteflies from throughout the world are listed in the table below. The table shows that the pest may infest nearly all plant species. Considering that B. tabaci is capable of withstanding for a long time elevated temperatures stress, the threat of whiteflies appears to be exacerbated by a rise in average world temperature. To illustrate the whitefly-inflicted danger faced by agriculture, we are studying a few significant cash crops, namely cotton, cucurbits, and tomatoes.

Common Host Plants Attacked by Various Whitefly Species

Host Plant	Common Name	Scientific Name	References
Rice	Rice whitefly	*Aleurocybotus occiduus*	(Pokhrel & Thapa, 2011)
Sugarcane	Sugarcane whitefly	*Aleurolobus barodensis*	(Pandya, 2005)
Cotton	Sycamore whitefly	*Bemisia afer*	(Abd-Rabou, 2008)

Tomato	Banded winged whitefly	*Trialeurodes abutiloneus*	(Malumphy et al., 2010)
Lemon	Citrus blackfly	*Aleurocanthus woglumi*	(Nguyen et al., 2007)
Coffee	Citrus whitefly	*Dialeurodes citri*	(Uygun et al., 1990)
Cardamom	Cardamom whitefly	*Singhiella cardamomi*	(Sundararaj & Dubey, 2019)
Olive	Olive whitefly	*Aleurolobus olivinus*	(Abd-Raboou & Ahmed, 2011)
Mango	Rugose spiraling whitefly	*Aleurodicus rugioperculatus*	(Stocks & Hodges, 2012)
Peach	Orange spiny whitefly	*Aleurocanthus spiniferus*	(Yamashita & Hayashida, 2006)
Maize	Rice whitefly	*Aleurocybotus occiduus*	(Pokhrel & Thapa, 2011)
Potato	Iris whitefly	*Aleyrodes spiraeoides*	(Saurabh et al., 2021)
Oak	Crown whitefly	*Aleuroplatus coronate*	(Dreistadt, 2016)
Rose	Orange spiny whitefly	*Aleurocanthus spiniferus*	(Yamashita & Hayashida, 2006)

Table 1: Shows various whitefly and their host plant

dsRNA Delivery Methods to Insect Pests

Ingestion

The most prevalent way for introducing dsRNA into parasitic insects is ingestion. dsRNA can be introduced into the insect's diet by serving the pest dsRNA-containing

artificial meals or by treatment of crops with dsRNA as well as feeding the insect the plants that have been treated (Yu et al., 2013). Although orally given dsRNA can be destroyed by the midgut enzyme known as endonucleases, it may also be picked up by midgut cells and transferred to other regions. Oral ingestion-induced RNAi is more efficient for genes transcribed in the midgut than in other organs (Gurusamy et al., 2020). Because of midgut endonuclease deterioration, ingestion results in lower RNAi effectiveness than microinjection, and the genes being targeted ought to be transcribed in the midgut because of inadequate transport of orally administered dsRNA over the midgut epithelium (Castellanos et al., 2019). Due to the fact that dsRNA molecules are significantly bigger than standard chemical pesticides, the administration technique must be tailored to promote their absorption by insect pests, which is commonly by oral intake. The ingestion serves as a simple and inexpensive way for delivering dsRNA to big populations of pests. (Nwokeoji et al., 2019).

Topical Application

For some insect pests, dsRNA can also be expressed in microbes or is chemically synthesized and then applied topically at their typical feeding sites (Niu et al., 2023). Topical application entails putting dsRNA into the insect's body's external surface, either as a paste or within a carrier that includes liposomes or nanoparticles. The double-stranded RNA (dsRNA) can then reach the body of the insect through the cuticle and cause RNAi in interior tissues. Regardless of the cuticle barriers, dsRNA may be applied topically to insects. Topically treated dsRNA solutions have been found to pierce insect cuticles and kill a variety of insects (Lu et al., 2023). Topical administration is a less intrusive way of delivering dsRNA to the insect's body's exterior surface; however, it might be less effective than swallowing or injection. (Yan et al., 2021).

Transgenic Plants

It is feasible to express dsRNA in plants that are transgenic for herbivorous insects, allowing molecules to be eaten while the pests eat plant tissue or drink the sap (Gong et al., 2022). Transgenic crops can be created to produce dsRNA-targeting genes that are required for pests such as insects' survival, growth, or reproduction. Once an insect eats the modified plant, it consumes the dsRNA and the targeted gene is silenced. Transgenic plants that produce dsRNA through nuclear and chloroplast

conversion are successful in suppressing pest genes (Wu et al., 2023). The combination of dsRNAs targeting distinct genes or diverse sites on the same gene can offer long-term resistance against several insects, but a single dsRNA is generally insufficient (Cagliari et al., 2019). The key difficulty with using RNAi in insect pest management is avoiding off-target impacts of dsRNAs affecting non target organisms like pollinating organism's predators, and other helpful creatures. Transgenic crops may offer an uninterrupted supply of dsRNA for pest management; however, transgenic cultivation and regulatory licensing may prove time-consuming and costly. (Sharif et al., 2022).

Microinjection

Injecting dsRNA into insects is a more intrusive procedure; however, it can be useful for targeting particular tissues or particular organs. Direct injection of dsRNA inside the insect's body, either inside the hemocoel or other tissues, is possible. Cells can then take up the injected dsRNA and initiate RNAi. Double-strand microinjection is a potential method for insect pest management (Joga et al., 2016). T7 RNA polymerase is commonly used in vitro to manufacture dsRNAs for microinjection (Garbatti Factor et al., 2022). Small bits of double-stranded RNA (dsRNA) complementary to certain targeted genes are introduced into the insect in this approach. Once within the insect cells, the dsRNA gets processed via the RNAi route, which results in the destruction or suppression of the target gene, target mRNA molecules, and, eventually, the reduction of the activity of the targeted gene (Bento et al., 2020). The microinjection approach for administering dsRNA is labor-demanding, requires skill, and is not suitable for usage in the field (Yu et al., 2013). Microinjection, on the other hand, can deliver a precise quantity of dsRNA to the target site instantaneously, resulting in considerably better RNAi efficiency than previous approaches. It is a powerful research tool for analyzing the function of genes in most pests (Pinheiro et al., 2020).

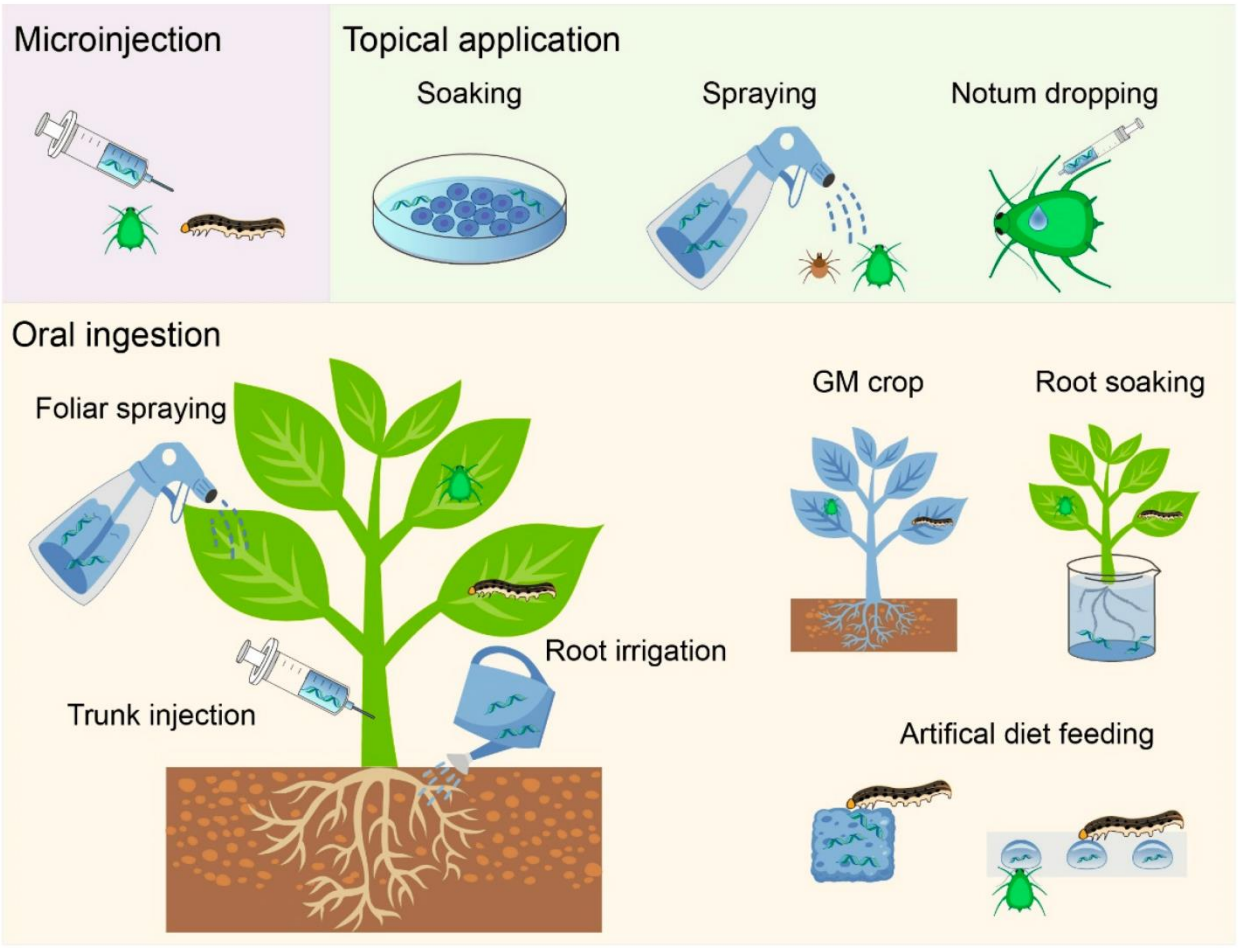

Figure 2: dsRNA delivery techniques. Figure adopted from (Lu et al., 2023).

RNAi as a tool for Whitefly Management

RNAi technology might turn out to be a game changer for combating whitefly, and it is believed to be very target specific. Rapid advancements in the area of RNAi have demonstrated that this approach is a viable and effective preventive tool for insect infestations (Grover et al., 2019). Numerous genes, particularly *BtCG5885*, *BtGATAd*, as well as *BtSnap*, that are expressed primarily in *B. tabaci's* midgut and main salivary glands, were selected as targets by infusing targeted gene-specific double-stranded into the hemolymph. After the administration of dsRNA targeting the *B. tabaci* homolog of the Drosophila *chickadee* gene, abnormalities in follicular cells enveloping the egg cell were detected in *B. tabaci* ovaries due to loss of subcortical actin structures (Ghanim et al., 2007). For whiteflies, a simple RNAi assay approach that allows dsRNA administration via the oral route was designed. *Actin, ADP/ATP translocase, -tubulin, ribosomal protein L9 (RPL9),* and the *V-ATPase A* subunit were

all selected for a knockdown in this work. Following 6 days of feeding, dsRNAs encoding these genes were administered orally to *B. tabaci*, and death ranged from 29-97%. *RPL9* along with *V-ATPase* expression is reduced (Upadhyay et al., 2011). The viability of dsRNA distribution using a synthetic diet for RNAi experiments in whitefly larvae is tough and complex. A novel and high levels of throughput technique for silencing whitefly genes by leaf-mediated dsRNA feeding were developed. The function of genes involved in the shedding hormone, ecdysone synthesis, and signaling pathways in *B. tabaci* their survival, development, and growth was investigated. Silencing of ecdysone pathways genes showed no influence on adult *B. tabaci* survival and fertility (Luan et al., 2013). In addition, genes involved in osmoregulation, sugar metabolism, and sugar transport, such as *alpha glucosidase 1, aquaporin 1 (AQP1), trehalase 1, and trehalose transporter 1*, were discovered to be essential for whitefly viability(Vyas et al., 2017).

RNAi-targeted genes in *Bemisia tabaci* along with mode of delivery

Genes	**dsRNA delivery method**	**Results**	**References**
BtCG5885, BtGATAd, and *BtSnap*	Injection	70% mortality	(Ghanim et al., 2007)
CAPAr	Oral delivery method	30.74% death rate among adults	(Thakur & Jindal, 2022)
α-glucosidase 1, aquaporin 1 (AQP1)	transgenic plants	70% mortality	(Raza et al., 2016)
v-ATPase	transgenic plants	7.6% mortality	(Pizetta et al., 2022)
Acetylcholinesterase (*AChE*)	transgenic plants	90% Mortality after 4 days	(Malik et al., 2016)
Hsp70 and *fas2*	Topical application	82.22% and 72% mortality	(Chakraborty & Ghosh, 2022)

GST (glutathione *S*-transferase)	Artificial diet	40% mortality	(Asokan et al., 2015)
ADP/ATP translocase	Artificial diet	15% Mortality	(Upadhyay et al., 2016)
Trehalose transporter1	Artificial diet	73% Mortality	(Vyas et al., 2017)
Trehalase1	Artificial diet	70% Mortality	(Vyas et al., 2017)

Table 2: Shows method of delievery of dsRNA into whitefly and targeted gene

Resistance to RNA interference

While RNAi has shown to be an effective research tool and is being investigated as a treatment method, it is not without limits. One of the most difficult aspects of RNAi is the emergence of resistance to the procedure. Cells can acquire resistance to RNAi through a variety of ways, a few of which are discussed here.

Targeted gene Mutation

Mutations in the gene of interest are one of the easiest methods for cells to acquire resistance to RNAi. When the siRNA reaches a mutant area of the mRNA, it may be unable to attach and destroy the mRNA as efficiently, permitting a protein to be generated (Niu et al., 2018).

Off-Target Effects

RNAi can occasionally result in the silence of genes that weren't supposed to be targeted. If the siRNA attaches to a sequence that has characteristics comparable but not identical to the desired target, this might happen. If the effects that are off-target result in the silence of a cell-survival gene, the cell may acquire resistance to RNAi (Zhang et al., 2017).

Overexpression of the Target Gene

If the targeted gene is excessively expressed, the siRNA may have more mRNA molecules to target. This has the potential to overload the RNAi mechanism and hinder efficient gene silencing.

RNAi Machinery Alteration

The RNAi pathway components, particularly the enzymes that are responsible for the processing of siRNA including the RISC complex, can be changed to make them less receptive to siRNAs (Parsons et al., 2018).

Alternative Pathway Activation

Cells can make up for the loss of a specific gene by activating other pathways. If RNAi silences a certain gene, the cell could start alternative pathways to make a comparable protein, successfully circumventing RNAi-induced silencing (Guo et al., 2015).

Degradation of dsRNA by Plant

Another barrier to using RNAi in B. tabaci is the plant's internal RNAi mechanism converting external or self-produced dsRNAs to siRNA that are carried in the phloem (Christiaens & Smagghe, 2014). It is right now unclear if such siRNA particles have any effect on insect RNAi. RNAi efficiency varies between insect species due to differences in cellular absorption of siRNA (Vélez & Fishilevich, 2018).

B. Tabaci uses RNAi to regulate the transmission of viruses

Whiteflies are the carriers for approximately 9% of the total plant viruses. Whiteflies have been documented to transmit around 200 viruses from five viral genera. Begomovirus are among the most dangerous (Kanakala & Ghanim, 2016). The virus penetrates the digestive tract and reaches the insect's hemocoel via the filter chamber alongside the midgut after being consumed by the vector via its stylet while eating viral-infected plants (Leshkowitz et al., 2006). These virions move to the vector's glands of saliva and subsequently attack other crops when the vector is feeding, allowing the virus to propagate throughout other plants (Wei et al., 2014).

The role of knottin genes in the relationship between the tomato yellow leaf curl virus (TYLCV) with its whitefly vector was investigated using a leaf-mediated silencing technique. *Knot-1* to *knot-4*, a set consisting of four knottin-like genes, was discovered to be involved in the transmission of viruses by whiteflies. *Knot-1* RNAi knockdown resulted in a significant increase in viral uptake and transfer to tomato plants (Hariton Shalev et al., 2016).

A defensin-like protein has been discovered and described within the whitefly body, where it was shown to be continuously expressed in the fat body, midgut, ovaries, as well as salivary gland. This protein's expression increased by fungus infection, it was also shown to be elevated in viruliferous whiteflies as well. It might be crucial in controlling immune responses to infections (Wang et al., 2013). Adult whiteflies were given dsRNA containing the gene for the defensin-like antimicrobial peptide (Btdef). There was a remarkable reduction in tomato yellow leaf curl China virus (TYLCCNV) in the dsRNA-fed whiteflies. Although the fundamental mechanism is unidentified it is believed that Btdef affects begomovirus infection, presumably through altering begomovirus-whitefly interactions via symbiont (Wang et al., 2017).

Another study used an indirect technique to lower the burden of the bean golden mosaic virus (BGMV) in whitefly. The knockdown of the viral rep gene using RNAi resulted in the development of a genetically modified (GM) bean that is resistant to BGMV. It resulted in a significant reduction in BGMV DNA quantities in whiteflies consuming GM plants compared to insects eating non-GM plants after 4 (52%) and 8 (84%) days, respectively. This investigation validated the efficacy of GM bean plants in lowering BGMV inoculum (de Paula et al., 2015).

By putting their stylets inside phloem tubes made of cells, whiteflies sucking plant sap. Osmoregulation is recognized to be critical to the survival of sap-sucking insects. *Glucosidases* support viruses by folding glycoproteins properly, which aids in nucleic acid encapsulation. Transgenic *N. tabacum* expressing dsRNA against both *AQP* and *alpha glucosidase* was produced to investigate the potential of targeted plant osmoregulatory machinery for pest management (Raza et al., 2016).

Conclusion

RNAi is a powerful technology for studying gene function as well as pest control, and multiple dsRNA delivery methods have been designed for various species and use. It is still in its early phases, and the field confronts multiple serious obstacles that will require substantial research focus to overcome. Because of dsRNA destruction by nucleases, a an absence of functional RNAi machinery, and a lack of information about dsRNA absorption, transport as well as systemic propagation mechanisms, RNAi efficacy in whitefly can be exceedingly varied. The possible benefits of using

RNAi as an alternative crop-protection method include pest selectivity, minimal toxicity in comparison to conventional pesticides, and little environmental effect. To get community acceptance and avoid regulatory delays, RNAi approaches must be researched further. The poor environmental stability of dsRNA, as well as its absorption and systemic transit in the plant, apparently restricts its use for efficient insect pest management. The problem must be tackled through experimental evaluation of the cellular and molecular foundations of dsRNA uptake in plants, as well as any potential limitations addressed through the use of formulation chemistry along with nanoparticles to enhance the RNAi molecules' perseverance, entry, and transportation in plants and insects. Biopesticides created using RNAi have the potential to transform pest control by being safe, targeted, and highly efficient.

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