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RNA INTERFERENCE IN THRIPS VECTORS: A STEP FORWARD TOWARD SUSTAINABLE MANAGEMENT

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ABSTRACT

Thrips are major pests of agricultural and horticultural crops worldwide. In addition to causing direct damage, thrips are the most common vector of tospoviruses. Currently, thrips control is mostly based on chemical insecticides, but effective control can be difficult to achieve because it has developed resistance to various insecticides. Therefore, there is an urgent need for novel alternative management options. A viable strategy for the management of the virus-vector complex involves understanding the molecular links between thrips and viruses and disrupting their association. RNA interference, a sequence-specific method to suppress target gene expression, is a conserved process in all eukaryotic organisms and has emerged as a new tool for reverse genetics and potential pest control. Advancements in RNA interference-based gene silencing techniques in the past few years could potentially be utilized in the management of the thrips-tospovirus complexes. RNA interference-mediated plant protection strategies have the potential to revolutionize pest management practices in a sustainable and effective way if development is carried out profoundly by discerning bioinformatics identification, in-silico designing of RNAi trigger molecules, laboratory and field-based toxicity and biosafety investigations.

Key words: RNA interference, thrips, tospoviruses, dsRNA delivery methods, dsRNA uptake and spread, Antisense oligonucleotide (ASO)-mediated silencing

Thrips are small, soft-bodied insects of the order Thysanoptera with fringed wings and asymmetrical mouthparts. Some species of thrips are helpful as predators and pollinators, while a few others are phytophagous and fungal feeders (phlaeothripids). At present, there are approximately 7,700 species within the two suborders Terebrantia and Tubulifera, of which only 1% have been reported to cause economic losses (Morse and Hoddle, 2005**;** Mound, 2015). Most of these economically important thrips belong to the family Thripidae (Terebrantia), which have become a serious global concern in agriculture and horticulture. Thrips cause damage either by directly feeding on leaves, flowers, fruits, and tender parts or indirectly by transmitting plant viruses (Rotenberg et al., 2015**;** Ghosh et al., 2017; Ghosh et al., 2019). The silvery patch appearance is a prominent phenotypic symptom expressed at a later stage during major thrips attacks and if the infestation persists during the early stage of crop growth, it results in the complete deformation of the affected parts (Steenbergen et al., 2018). *Frankliniella occidentalis* (western flower thrips) feeds on floral parts like petals and pollen, causing spotting and deformation of flower buds. In addition, they are also found in crop foliage (Riley and Pappu, 2004). *Thrips tabaci* (onion thrips) actively feed on the foliage of plants belonging to the family Alliaceae; they also occur on cotton, tomatoes, tobacco, and wheat (Mo et al., 2008; Nault and Shelton, 2010). Feeding damages associated with *Frankliniella fusca* (tobacco thrips) include scarring, distortion of leaves and stunting of many weeds and economically important crops (Joost and Riley, 2004). *Thrips palmi* (melon thrips) are primarily reported as pests of plants belonging to the families Cucurbitaceae and Solanaceae (Riley et al., 2011). Increased worldwide trade in fruits, vegetables, and ornamentals has hastened the spread of highly invasive and polyphagous species, viz., *F. occidentalis* and *T. palmi*. Earlier, *Scirtothrips dorsalis* and *Thrips hawaiiensis* had been the common insects affecting chilli crops. *Thrips parvispinous*, a new species that originated in Indonesia and was first seen in India in 2015, has made a comeback in several Indian states, causing heavy yield losses (Kurmanath, 2021**;** Vivek Bhoomi, 2021**;** Rachana et al., 2022). On the list of thrips-transmitted viruses, tospoviruses are considered the most destructive. Among them, the *Tomato spotted wilt virus* (TSWV) is considered to be the most significant virus species globally. Over the

last ten years, the tomato spotted wilt virus has caused an estimated $1.4 billion in annual losses in the United States (Riley et al., 2011). In the Asia-Pacific region, *Groundnut bud necrosis virus* (GBNV) disease alone causes US$ 89 million in economic losses each year, with 70-90% yield loss in groundnuts and 30% in potatoes in India (Reddy et al., 1995**;** Buiel et al., 1995**;** Daimei et al., 2017**;** Ghosh et al., 2019). Several control measures have been implemented worldwide, with insecticides and host plant resistance being the primary components for thrips and tospovirus management. Indiscriminate use of insecticides to control thrips has various negative ecosystem consequences, such as severe effects on beneficial organisms and non-target insects and health risks. Genetic host plant resistance is not available for all the crop species that are infected by thrips-tospoviruses. The use of transgenic plants is not feasible considering the broad host range and ethical issues related to GM crops (Mahanta et al., 2022). RNA interference (RNAi) represents an attractive avenue for pest control. Advances in molecular techniques in recent years could potentially be used to develop RNAi-based gene silencing techniques in thrips, and they have enormous potential for a non-chemical, non-residual, and environmentally friendly approach against thrips management. In this review, we summarize the current knowledge of RNAi in thrips vectors and highlight the existing gaps in the development of RNAi-based thrips vector control strategies.

RNAi in the functional genomics of thrips vectors

RNAi is a post-transcriptional gene silencing mechanism driven by double-stranded RNA (dsRNA). It is found in a wide variety of eukaryotic species as an anti-viral defence mechanism to protect the organism. This technology has been widely utilized for genetic studies in insects. RNAi-based gene silencing inhibits the targeted gene's activity, allowing for that gene's specific function to be validated (functional genomic studies). Before the discovery of RNAi, gene function research was limited to insect models like *Drosophila melanogaster*. RNAi has aided researchers in conducting extensive functional genomics studies in a variety of model insects and economically significant non-model insects. As a result, we have gained a better insight into insect biology (Zhu and Palli, 2020). The first report on RNAi tools for functional genomic assays in thrips *F. occidentalis* was given by Badillo-Vargas et al., (2015). Furthermore, the functional validations of various genes were performed in different thrips species (Table 1) with the aid of RNAi.

RNAi in the management of thrips vectors

Thrips act as a vector for the viruses belonging to the genera *Tospovirus, Ilarvirus, Carmovirus, Sobemovirus,* and *Machlomovirus* (Jones, 2005). Tospoviruses, economically important plant pathogens of the Bunyaviridae family, have a worldwide distribution. Tospoviruses cause annual losses in excess of $10 million around the world (Mandal et al., 2012). Among these, the TSWV (type virus) is regarded as one of the ten highly lethal plant viruses attributed to the thrips vector's widespread distribution and the virus's vast host range (Scholthof et al., 2011**;** Ertunc, 2020). GBNV is Asia's most commercially significant tospovirus species, impacting a wide range of crops. In India, GBNV causes 70 to 90% yield losses in peanuts and up to 29% yield losses in potatoes (Singh et al., 1997). GBNV also affects soybean (Bhat et al., 2002), peas (Akram M, 2010), mungbean (Jain et al., 2002; Thien et al., 2003), and cowpea (Jain et al., 2002). Outbreaks of GBNV in tomatoes in India have reached 100% disease incidence from 2003 to 2011 (Kunkalikar et al., 2011). They are challenging to manage because of their wide host range, insecticide resistance of thrips, and lack of long-term resistance in crop hosts (Jones, 2005).

The molecular understanding of the thrips-tospovirus interactions would aid in disrupting their association and limiting the spread of the thrips-tospovirus complexes. Computational analysis predicted potential interactions of GBNV glycoproteins with *T. palmi enolase*, *cathepsin*, *C-type lectin*, *clathrin*, and *vacuolar ATP synthase subunit E* (Jagdale and Ghosh, 2019). *Endocuticle structural glycoprotein*, *cyclophilin*, and *E3 ubiquitin-protein ligase UBR7* of *F. occidentalis* are known to interact with TSWV surface proteins (Shi et al., 2022; Badillo-Vargas et al., 2019). Transcriptome-wide responses of *T. palmi* associated with CaCV infection identified significant upregulation of *UHRF1-binding protein 1, nephrin, PFAS* and *hsp70* genes (Widana Gamage et al., 2018). More recently, the investigation of the transcriptome-wide response of *T. palmi* to GBNV infection found 2,363 significant transcripts (1,383 upregulated and 980 downregulated). These differentially expressed genes (DEGs) are involved in innate immune response, endocytosis, cuticle formation, receptor binding, and signalling, which all contribute to viral invasion and replication in the vector system (Mahanta et al., 2022). These studies shed light on potential genetic targets for thrips-tospovirus management.

In the past few years, RNAi-mediated approaches

Table 1. RNAi in thrips an overview

Sl. No	Targeted gene	Associated function	Delivery approach	Outcomes	References
1.	*Vacuolar ATPase - B synthesis*	Involved in the transport of solute molecules across plasma membranes as well as the pH stabilization in the lumen.	Microinjection method	25% reduction in V-ATPase-B protein expression 2 days after injection resulted in higher mortality and lower fertility (number of viable progenies generated).	Badillo-Vargas et al., 2015
2.	*Alpha-tubulin*	Important in cell division, structural support, and transport pathways.	Symbiont-mediated delivery	Resulted in a high rate of mortality, particularly among those in the first larval instar stage, than adults.	Whitten et al., 2016
3.	*Toll-like receptor 6*, *apolipophorin*, *coatomer protein subunit epsilon*, and *sorting* and *assembly machinery component*	These genes fall under the functional category of cell signalling, digestion, excretion, immune response, metabolism, metamorphosis, protein degradation, protein expression, insect structure, transport, etc.	Leaf disc-mediated dsRNA delivery	Reduced transcription levels of targeted genes	Han et al., 2019
4.	*V-ATPase-B gene*	Involved in the transport of solute molecules across plasma membranes and the pH stabilization in the lumen.	Oral delivery	Recorded with a mortality of 88.7%.	Andongma et al., 2020
5.	*SNF7* (Sucrose Non-fermented) and *AQP* (Aquaporins)	SNF7 is a constituent of the ESCRT pathway (Endosomal sorting complex required for transport). AQP is involved in water regulation.	Oral delivery	Recorded 62 and 72% mortality, respectively.	Singh et al., 2019
6.	*Cytochrome P450, ABC transporter G, glutathione S transferase S1* and *UDP glucuronosyl transferases*	Detoxification genes	Oral delivery	Recorded with increased mortalities.	Gao et al., 2020

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7.	*ACT, TUB, VATPase-B gene* and *SNF7*	The genes are for the cytoskeletal proteins actin and tubulin; *VATPase-B* is involved in the transport of solute molecules across plasma membranes as well as the pH stabilization in the lumen; SNF7 is a constituent of the ESCRT pathway (Endosomal sorting complex required for transport III).	Plasmid-mediated RNA interference (PM-RNAi).	It is causing efficient suppression of the targeted genes and high insect mortality.	Wu et al., 2022
8.	*FoHSP11.6* and *FoHSP28.0*	Small heat shock proteins (sHSPs) belonging to an atypical and typical sHSP family, respectively. Involved in response to temperature and drought and may also function in growth and development.	Oral delivery	Survival rates showed a significant decline.	Yuan et al., 2022

have found application in thrips management owing to their specificity and eco-friendly nature. Through the silencing of critical genes involved in thrips fitness and tospovirus transmission, RNAi can be used as biopesticide molecules without compromising biosafety or environmental sustainability. Badillo-Vargas et al., (2015) targeted the *V-ATPase-B* gene of *F. occidentalis* for silencing. *V-ATPase-B* dsRNA microinjection in the thrips resulted in a 25% reduction of protein expression 2 days after the application, with higher mortality and lower fertility. The silencing of the *alpha-tubulin* gene, which is important in cell division, structural support, and transport pathways of *F. occidentalis*, resulted in a high rate of mortality, particularly among those in the first larval instar stage compared with adults (Whitten et al., 2016). Han et al. (2019) screened 57 genes for lethality against the *F. occidentalis* insecticide-susceptible strain using RNAi. These genes fall under the functional categories of cell signalling, digestion, excretion, immune response, metabolism, metamorphosis, protein degradation, protein expression, insect structure, transport, etc. Their results revealed a significant decrease in the transcription level of the linked genes during qPCR studies. The genes screened in this study could be used directly as prospective target genes for developing RNAi-based thrips-resistant transgenic crops. Oral administration of dsRNA targeting SNF7 (sucrose non-fermented) and AQP (aquaporins) genes responsible for the ESCRT pathway (endosomal sorting complex required for transport) and water regulation in *T. tabaci* resulted in 62 and 72% mortality, respectively (Singh et al., 2019). Andongma et al. (2020) knocked down the *V-ATPase-B* gene in *F. occidentalis*, with a recorded mortality of 88.7%. Gao et al., (2020) knocked down detoxification genes such as *cytochrome P450, ABC transporter G, glutathione S transferase S1*, and *UDP glucuronosyltransferases* in an insecticide-tolerant *F. occidentalis* population and validated their functional role in the induction of tolerance. A series of transplastomic tobacco plants that expressed dsRNAs and hairpin RNAs were developed that targeted four critical *F. occidentalis* genes (*ACT, TUB, VAT*, and *SNF*), resulting in target gene suppression and high mortality (Wu et al., 2022). The expression of *T. palmi Btk29A* and *COL3A1* genes are highly abundant in response to the infection of tospoviruses (Widana Gamage et al., 2018; Mahanta et al., 2022). The fitness of *T. palmi* was significantly altered post-exposure to *Btk29A* and *COL3A1* dsRNA under controlled laboratory conditions (Vavilapalli Rajesh, 2022).

TSWV resistance in transgenic plants expressing

N (the tospovirus nucleocapsid protein, which is a key structural protein and encapsidates the virion RNA) or NS_m (functions as a movement protein in plant hosts and forms tubules in insect and plant cells) genes were found after investigating RNAi-mediated pathogen-derived resistance to TSWV in a gene-specific way (Prins et al., 1996). TSWV RNAi-mediated pathogen resistance is sequence-specific and limited to transgenic plants expressing N or NS_m gene sequences. Exogenous administration of a soluble version of G_N - the virus attachment protein (G_N-S) reduces TSWV binding, uptake (Whitfield et al., 2004) and transmission to a plant host (Whitfield et al., 2008)**.** Debat et al., (2015) developed a broad-spectrum viral resistance method based on a novel and short hairpin-RNA-generating construct (pNhpRNA), which resulted in TSWV, *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) resistance in *Nicotiana benthamiana.* Tabein et al., (2020) demonstrated that exogenous application of dsRNAs analogous to the N gene suppresses viral replication and protects plants against TSWV infection in both *N. benthamiana* and tomato by reducing or significantly delaying symptom development. Mitter et al., (2016) devised an artificial microRNA (amiRNA) technique against TSWV, focusing on the nucleoprotein (N) and silencing suppressor (NS_s) genes. Using an ELISA, plants expressing N-specific amiRNAs remained asymptomatic and tested negative for TSWV. Topical/ Foliar application of dsRNAs of NS_s and N genes on leaves of *Nicotiana tabacum* elicited resistance response against TSWV. The applied dsRNA showed systematic movement from inoculated leaves to younger non-inoculated leaves. (Konakalla et al., 2021). Although RNAi provides remarkable degrees of control, its successful implementation in a practical application depends on two important criteria: (a) selection of suitable delivery strategies by taking care of potential barriers limiting RNAi efficiency (b) systemic distribution of up taken dsRNA for target gene knockdown.

RNAi molecule delivery strategies in thrips: what do we know so far?

Problems with dsRNA delivery frequently limit the practical application of RNAi techniques for insect pest management. To ensure the safe introduction of dsRNA molecules into the insect's biological system a variety of dsRNA delivery protocols are available, including transformative delivery methods (transgenic plants and transplastomic plants), non-transformative delivery methods (micro-injection, oral feeding/ingestion, soaking and spraying/topical application, trunk injection and root absorption/irrigation), cross-kingdom or symbiont-mediated delivery (attenuated strains of bacteria, viruses, yeast, and fungi) and additionally, more recent techniques are also involved (nanoparticle-mediated delivery, synthetic polymer delivery vectors, delivery vehicles from human therapeutics). Among these, only a few have been demonstrated for dsRNA delivery in thrips (Fig. 1) (Zotti et al., 2018**;** Whitten, 2019**;** Wu et al., 2022**;** Jain et al., 2022).

Badillo-Vargas et al. (2015) used the microinjection-based delivery strategy of dsRNA directly to the hemocoel of female thrips to target the vacuolar ATP synthase subunit B (*V-ATPase-B*) gene. This resulted in increased female mortality and reduced fertility, i.e., the number of viable offspring produced. Whitten et al. (2016) have employed *F. occidentalis* symbiotic bacteria (Gram-negative gamma proteobacterium) to synthesise and deliver dsRNA. When the recombinant bacteria were consumed, they colonized the insects, successfully competed with the wild-type microflora, and produced systemic knockdown. If the bacteria's delivery can be optimized for agricultural applications, symbiont-mediated RNAi might be used to control populations of certain insect species while not impacting beneficial species. Jahani et al. (2018) reported an efficient and effective liquid diet method for the delivery of dsRNA targeting *V-ATPase-B* in *F. occidentalis* adults and larvae. Han et al. (2019) developed ingestion-based RNAi in *F. occidentalis* in which dsRNAs of 57 target genes were delivered via a leaf disc-mediated method using a bioassay chamber optimized by 3D printing. Andongma et al. (2020) developed an artificial feeding diet setup comprised of feeding cups-the lids of sterile 1.5 ml microfuge tubes filled with about 300 µl of feeding solution and covered with stretched alcohol-sterilized para film with which the solution made full contact. Using the ingestion RNAi method, Gao et al. (2020) knocked down lethal genes responsible for insecticide tolerance in *F. occidentalis*. Zhang et al. 2022, evaluated five different feeding solutions-LB, tryptone soy broth yeast, pollen, sucrose, and honey. These solutions were combined with *V-ATPase-B* dsRNA and orally administered to *F. occidentalis* to investigate their impact on RNAi efficiency. They concluded that a pollen and honey solution diet resulted in better knockdown of the *V-ATPase-B* gene. Plastid-mediated RNA interference (PM-RNAi) has emerged as a promising transplastomic technology in the recent past. Wu et al. 2022, have investigated the susceptibility of *F. occidentalis* to PM-RNAi. They generated the transplastomic tobacco plants expressing

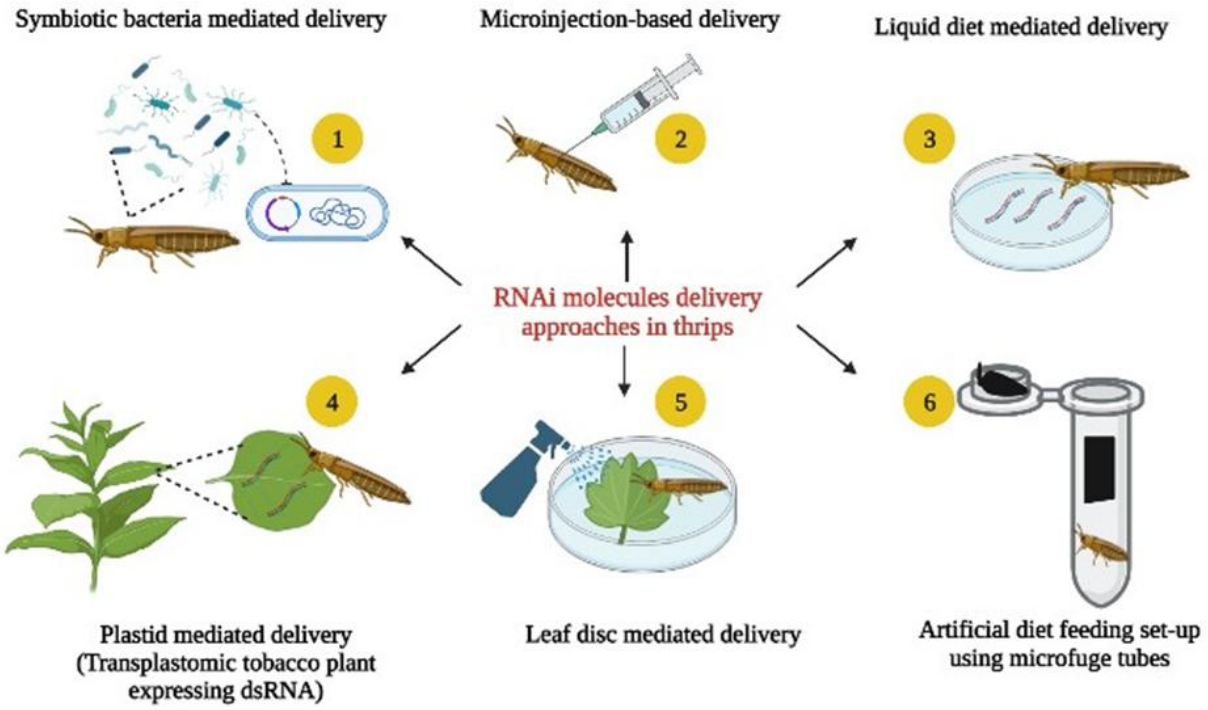

Fig. 1. RNAi molecules delivery approaches in practice for thrips. (Picture created using Biorender.com)

dsRNAs and hairpin RNAs (hpRNAs) targeted against four essential *F. occidentalis* genes. Results revealed that transplastomic plants induced a potent RNAi response by causing high insect mortality, unlike nuclear transgenic plants. Vavilapalli Rajesh (2022) evaluated the artificial feeding setup for dsRNA oral delivery to adult *Thrips palmi*, targeting the genes *Btk29A* and *COL3A1*. In this study, a methylene blue dye was added to the prepared diet to track the feeding status of the thrips.

Each of these delivery approaches has its own merits and demerits (Table 2). The microinjection technique can directly produce the systemic RNAi response by ignoring the insect mouth cavity and gut, but it is apparently impracticable to use in the field. The approach of feeding dsRNA-containing fluid is not only appropriate for studying gene function in microscopic insects, but it may also be augmented with nanoparticles to encapsulate dsRNA or symbiotic microorganisms to express dsRNA and enhance RNAi efficacy. Unfortunately, difficulties such as dsRNA degradation during insect feeding, inadequate dsRNA absorption by intestinal epithelial cells, and species variations in RNAi efficacy must be fixed quickly in the development of this technology (Cooper et al., 2019**;** Kunte et al., 2020)**.** Nanoparticles represent a vibrant field in the delivery of dsRNA; when the selection

Table 2. Advantages and disadvantages of dsRNA delivery methods in practice for thrips.

Delivery approaches	Advantages	Disadvantages
Microinjection-based approaches.	✓ Mainly used for Functional genomics studies. ✓ High efficiency in inhibiting gene expression.	✓ Impracticable at the field level. ✓ Cuticular damages occur.
Symbionts mediated delivery.	✓ Exploit natural transmission.	✓ Not cultivable in the lab in most cases.
Oral/Ingestion-based delivery.	✓ Less labour-intensive, less expensive. ✓ Convenient for a large number of target genes.	✓ A higher dose is usually required.

is made wisely, they not only stabilize dsRNA but also protect it from nucleases and pH extremes and enhance its uptake. The cationic groups and phosphate groups of nanoparticles and dsRNA, respectively, form complexes with a net positive charge, which helps in the interaction with the charged cell surfaces of insects (Mitter et al., 2017**;** Nilon et al., 2021). BioClay is a new nanocarrier system that consists of layered double hydroxide clay nanosheets (LDH) developed by Mitter et al. (2017)**.** This engineered formulation benefits from consistent release, and long-term persistence and promotes systemic protection. Spray-on application of dsRNA augmented with nanoparticles (BioClay) to disrupt multiple whitefly developmental stages in planta increases efficiency, persistency, and penetration and offers complete whitefly life cycle control (Jain et al. 2022). The spray-on application of the nano-conjugated dsRNA extends the benefits of RNAi as an alternative, sustainable pesticidal molecule to manage thrips as well as interrupt tospovirus transmission under open field conditions.

Uptake and spread of dsRNA

The current understanding of dsRNA uptake and systemic spread comes from studies in the nematode *C. elegans*. In this nematode, several SID (systemic RNAi defective) proteins can perform absorption, viz. SID-1, SID-2, SID-3, and SID-5 etc. There is no direct evidence for the uptake and transport mechanisms of dsRNA in insects. However, in some cases, homologues of SID proteins are thought to have been involved in these functions in various groups of insects, e.g., Hemiptera, Coleoptera, Lepidoptera, Orthoptera, and Hymenoptera, etc. (Zotti and Smagghe, 2015**;** Niu et al., 2018). In addition, clathrin-dependent endocytosis, receptor-mediated endocytosis, phagocytosis, caveolar endocytosis, and micropinocytosis pathways are also involved in the uptake of dsRNA in insects (Velez & Fishilevich, 2018**;** Christiaens et al., 2020**;** Yan et al., 2020**;** Kunte et al., 2020**;** Jain et al., 2021). Further studies related to the mechanisms of dsRNA uptake and spread in thrips are encouraged to better understand the stability of dsRNA after being taken up from the environment into the insect body.

Future applications

Antisense oligonucleotide (ASO)-mediated silencing

ASOs are generally 16 to 21 nucleotide-modified synthetic, single-stranded DNA molecules that modulate gene expression by binding to target RNAs, including mRNAs, miRNAs, and long noncoding RNAs through complementary base pairing. This is followed by RNase H-mediated cleavage of the RNA-DNA hybrid, thereby causing a significant reduction in the expression of the target gene (Fusco et al., 2019). The cleaved mRNA is further degraded by nucleases, and ASO is recycled within the cell. ASOs are highly efficient, as multiple mRNA copies are degraded by a single ASO molecule. The primary characteristic of ASO is a phosphorothioate backbone and modified flanking nucleotides, where the 2′-OH of the ribose sugar is substituted by a fluorine atom. These modifications prevent ASO from degradation and increase its binding ability to target mRNA. Though ASO and siRNA have many similarities, they also have some differences. So, the selection of either ASO or siRNA mainly depends on the target gene to be silenced. ASO offers few advantages over siRNA as it is single-stranded, so the production cost is lower in the case of ASO. In addition to this, siRNA generally needs a vector for its delivery, while no such vector is needed in the case of ASO.

A single chemical modification makes ASO resistant to nucleases, while siRNAs are vulnerable to cellular nucleases. Even though novel pesticides are developed every year, still the yield losses due to the insect pest remain the same (Oberemok et al., 2018). Recently, ASO-based pesticides have emerged as an alternative to chemical pesticides. Priti et al. 2022, published the first report of antisense oligonucleotide (ASO)-mediated silencing in *T. palm*i. In this investigation, the GBNV-related *T. palmi* genes *UHRF1BP1* and *PFAS* were targeted for silencing using modified ASOs. It induced 93.33% mortality with reduced target mRNA expression. The use of ASO in agriculture is still in its infancy. Since the nuclease activity is weaker in insects as compared to mammals (Tkachev, 2004), the use of this approach to develop novel insecticides is less vulnerable to degradation by nuclease (Dias and Stein, 2002) and thus paves the way toward sustainable pest management.

Genome editing-mediated gene silencing

Genome editing (GE) offers a significant advantage of site-specific DNA modification by employing DNA repair mechanisms through its platforms, viz., ZFNs, TALENs, and CRISPR-Cas. Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) and its associated protein (Cas-9) are an effective method of genome editing that is associated with adaptive immunity in prokaryotes to defend themselves against viruses or bacteriophages (Porteus, 2016**;** Hille and Charpentier, 2016). The mechanism of CRISPR/ Cas-9 genome editing can be generally divided into three

steps: recognition, cleavage, and repair. The designed sgRNA (single guide RNA) directs Cas-9 to recognize and make double-stranded breaks (DSBs) in the target sequence in the gene of interest. Then, the Cas-9 protein is activated for DNA cleavage and cleaves the non-complementary strand of target DNA to produce predominantly blunt-ended DSBs. Finally, the DSB is repaired by the host cellular machinery (Shao et al., 2016; Liu et al., 2019; Yang et al., 2020; Mengstie and Wondimu, 2021). Recently, successful heritable genome editing has been reported in *Bemisia tabaci* through an ovary-targeting peptide ligand fused with the Cas9 enzyme (Heu et al., 2020)**.** In addition to its genome editing activity, CRISPR/ Cas-9 can also be used to artificially activate or repress (silence) certain gene targets through modification of the Cas-9 protein (Jiang and Doudna, 2017). Although, there are no existing genome editing protocols available for the thrips-tospovirus pathosystem, using these novel methods to manage thrips will maximise benefits while maintaining environmental sustainability.

CONCLUSION

RNAi approaches are valuable functional genomic tools to investigate the role of genes in thrips-tospovirus interactions. The development of transcriptomics data will enrich thrips' genetic information and enable more comprehensive functional investigations of commercially relevant thrips. The gene associated with growth, reproduction (vitellogenin), innate immunity, endocytosis, insecticide resistance (cytochrome P450), etc., of the thrip's life cycle, could be used as novel targets for RNAi-based gene silencing to manage thrips and restrict the spread of tospoviruses. Although many articles describe effective suppression of gene expression by dsRNA in insects, whether the knockdown reaches the levels necessary for commercial product development requires more investigation. With all the available knowledge, RNAi has tremendous potential to be developed as a modern pest management technique.

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