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RNAi FOR THE MANAGEMENT OF INSECT PESTS: A REVIEW

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ABSTRACT

RNA interference (RNAi) is a biological mechanism that involves the inhibition of gene expression by the introduction of double-stranded RNA (dsRNA) molecules that are complementary to specific target genes. This technique has emerged as a promising strategy for the management of insect pests in crops. The application of RNAi to insect pest management involves the use of dsRNA molecules that are specific to essential genes in the target insect species. When these dsRNA molecules are ingested by the pests, they trigger a silencing mechanism that inhibits the expression of the target genes, leading to reduced pest populations and damage to crops. RNAi-based insect pest management has several advantages over traditional chemical-based methods, including its specificity, effectiveness, and environmental safety. However, there are still some challenges associated with the use of RNAi, such as the potential for off-target effects and the need for effective delivery methods. Nonetheless, RNAi technology holds great promise for sustainable and effective management of insect pests in agriculture.

Keywords: RNA Interference (RNAi), IPM, Gene Silencing, Sustainable Agriculture

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1. INTRODUCTION

RNA interference (RNAi) is a cellular mechanism in which Argonaute family proteins bind small RNAs, leading to the degradation of longer RNAs through sequence complementarity. RNAi is present in all eukaryotic organisms and is believed to have initially evolved as an antiviral innate immune response. RNAi has been shown to play a critical role in antiviral immunity in insects, and its effectiveness is believed to be correlated with the prevalence of viral infections in insect populations (Fire 2007). RNAi has quickly become a powerful reverse genetics tool for studying gene function, regulation, and interaction at the cellular and organismal levels, and it has also shown promise in pest management (Ren et al. 2019).

RNA interference (RNAi) is a natural biological process that occurs in cells to regulate gene expression. It involves the use of small RNA molecules, typically 21-23 nucleotides in length, to inhibit or "silence" the expression of specific genes. The process of RNAi begins with the production of double-stranded RNA (dsRNA) molecules that are complementary to the target mRNA molecule. These dsRNA molecules are processed by an enzyme called Dicer, which cleaves them into small RNA molecules called short interfering RNAs (siRNAs) (Christiaens et al. 2022). The siRNAs are then incorporated into a complex known as the RNA-induced silencing complex (RISC), which unwinds the double-stranded siRNA and uses one of the strands as a guide to identify and bind to the complementary mRNA molecule. The RISC then cleaves the mRNA molecule, preventing it from being translated into protein. RNAi has many important roles in cells, including regulating gene expression during development, maintaining genome stability, and protecting cells from viral infections (Lucena-Leandro et al. 2022). In addition to siRNAs, there are other types of small RNA molecules involved in RNAi, including microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs). These molecules function in slightly different ways but all play important roles in regulating gene expression. RNA interference (RNAi) is a powerful tool for the management of insect pests. It is a natural process that regulates gene expression by degrading RNA molecules, including mRNA, small interfering RNA (siRNA), and microRNA (miRNA) (Wise et al. 2022). RNAi has been exploited as a means of pest control by introducing RNA molecules that target essential genes in the pest species. These molecules are taken up by the pest's cells and trigger the RNAi pathway, leading to the degradation of the target mRNA and subsequent reduction in protein expression. In this way, RNAi can be used to selectively kill insect pests without harming non-target organisms (Haroon et al. 2022).

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RNAi is effective against a wide range of insect pests, including aphids, thrips, beetles, and moths. It has also been used to control insect vectors of plant viruses, such as whiteflies and leafhoppers. RNAi-based pest control has the potential to reduce the use of chemical insecticides, which can have negative environmental and health impacts. However, there are still some challenges that need to be addressed, such as off-target effects and the development of resistance by the pest population. Nevertheless, RNAi is a promising tool for the management of insect pests and is likely to become increasingly important in the future (Zafar et al. 2020). For insect pest control, dsRNA can be used to target genes that are essential for the pest's survival, reproduction, or development. When the targeted gene is knocked down, the pest may experience reduced fitness, increased mortality, or reduced reproductive capacity, depending on the specific gene and the pest species.

2. Methods to Deliver dsRNA to Insect Pests

2.1. Ingestion

Ingestion is the most common method for delivering dsRNA to insect pests. dsRNA can be incorporated into the pest's diet by feeding the pest on artificial diets that contain dsRNA, or by treating plant material with dsRNA and feeding the pest on the treated plants. When the pest ingests the dsRNA, it enters the gut and is taken up by gut cells, which then transport the dsRNA to other tissues (Yu et al. 2013).

2.2. Injection

The injection is a more invasive method for delivering dsRNA to insects, but it can be effective for targeting specific tissues or organs. dsRNA can be directly injected into the insect's body, either into the hemocoel or other tissues. The injected dsRNA can then be taken up by cells and trigger RNAi. A dsRNA microinjection is a promising tool for controlling insect pests (Joga et al. 2016). In this technique, small pieces of double-stranded RNA (dsRNA) that are complementary to specific target genes are injected into the insect. Once inside the insect cells, the dsRNA is processed by the RNA interference (RNAi) pathway, leading to the degradation or inhibition of target mRNA molecules and ultimately, the suppression of the target gene's function. The use of dsRNA microinjection for insect control has several advantages over traditional chemical insecticides. First, it is highly specific and can be targeted to specific genes in the insect pest, avoiding off-target effects on non-target organisms. Second, it can be effective against insect pests that have developed resistance to traditional insecticides. Third, it is environmentally friendly, as it does not involve the use of harmful chemicals that can pollute the environment (Bento et al. 2020).

Several studies have demonstrated the effectiveness of dsRNA microinjection in controlling insect pests. For example, dsRNA targeting the vitellogenin gene has been shown to reduce egg production in the mosquito Aedes aegypti, which is a vector for diseases such as dengue and Zika. Similarly, dsRNA targeting the chitin synthase gene has been shown to reduce survival and growth in the agricultural pest *Spodoptera frugiperda* (Ren et al. 2019). Despite its potential, there are some challenges associated with the use of dsRNA microinjection for insect control. One challenge is the development of resistance to the dsRNA, which can occur if the target gene mutates or if the insect develops mechanisms to degrade the dsRNA. Another challenge is the delivery of the dsRNA to the target insect populations, which can be difficult in field settings.

2.3. Topical Application

The topical application involves applying dsRNA to the external surface of the insect's body, either as a solution or in a carrier such as liposomes or nanoparticles. The dsRNA can then enter the insect's body through the cuticle and trigger RNAi in internal tissues (Yan et al. 2020; Yan et al. 2021).

2.4. Transgenic Plants

Transgenic plants can be engineered to express dsRNA-targeting genes that are essential for the survival, development, or reproduction of insect pests. When the pest feeds on the transgenic plant, it ingests the dsRNA and experiences knockdown of the targeted gene. Each of these delivery methods has its advantages and limitations. Ingestion is a non-invasive and cost-effective method that can be used to deliver dsRNA to large populations of pests. The injection is a more targeted method that can deliver dsRNA to specific tissues or organs, but it is more labor-intensive and can be harmful to the insect. Topical application is a less invasive method that can be used to deliver dsRNA to the external surface of the insect's body, but it may be less efficient than ingestion or injection. Transgenic plants can provide a continuous source of dsRNA for pest control, but the development and regulatory approval of transgenic crops can be time-consuming and expensive (Sharif et al. 2022).

Several studies have demonstrated the effectiveness of RNAi as a pest control strategy. For example, in the agricultural pest western corn rootworm (*Diabrotica virgifera virgifera*), RNAi-mediated silencing of essential genes involved in larval development and survival led to significant reductions in pest fitness and survival rates.

Similarly, RNAi-mediated silencing of genes involved in the reproduction and egg-laying of the malaria mosquito (Anopheles gambiae) resulted in a significant reduction in the number of viable offspring (Fishilevich et al. 2016).

However, the efficiency of RNAi as a pest control strategy can be influenced by a range of factors, including the delivery method, the stability and persistence of the siRNA molecules, and the specificity and potency of the siRNA molecules. In addition, the potential for off-target effects and unintended gene silencing can also be a concern.

3. Resistance Development to RNAi

While RNAi has proven to be a powerful tool in research and is being developed as a therapeutic approach, it is not without limitations. One of the major challenges of RNAi is the development of resistance to this process. There are several mechanisms by which cells can develop resistance to RNAi, some of which are described below.

3.1. Mutations in the Target Gene

One of the most straightforward ways in which cells can develop resistance to RNAi is through mutations in the target gene. If the siRNA targets a region of the mRNA that is mutated, the siRNA may not be able to bind and degrade the mRNA as effectively, allowing the protein to be produced (Niu et al. 2018).

3.2. Off-Target Effects

RNAi can sometimes lead to the unintended silencing of genes that are not the intended target. This can occur if the siRNA binds to a sequence that is similar but not identical to the intended target. If the off-target effects lead to the silencing of a gene that is important for cell survival, the cell may develop resistance to RNAi (Zhang et al. 2017).

3.3. Overexpression of Target Gene

If the target gene is overexpressed, there may be more mRNA molecules available for the siRNA to target. This can overwhelm the RNAi machinery and prevent effective gene silencing.

3.4. Altered RNAi Machinery

The components of the RNAi machinery, including the enzymes involved in siRNA processing and the RISC complex, can be altered in ways that make them less responsive to siRNAs. This can lead to decreased effectiveness of RNAi and the development of resistance (Parsons et al. 2018).

3.5. Activation of Alternative Pathways

Cells can sometimes compensate for the loss of a particular gene by activating alternative pathways. If a particular gene is silenced by RNAi, the cell may activate other pathways to produce a similar protein, effectively bypassing RNAi-induced silencing (Guo et al. 2015). In summary, resistance to RNAi can arise through various mechanisms, including mutations in the target gene, off-target effects, overexpression of the target gene, alterations in the RNAi machinery, and the activation of alternative pathways. Understanding these mechanisms of resistance is important for developing more effective RNAi-based therapies.

4. Environmental RNAi and Insect Pest Control

Environmental RNA interference (RNAi) is a genetic mechanism that can be used to control insect pests. RNAi is a natural process that occurs in many organisms, including insects, and involves the use of small RNA molecules to silence or suppress the expression of specific genes. By targeting key genes essential to the survival and reproduction of insect pests, RNAi can be used to disrupt their development and control their populations. To harness RNAi as a pest control strategy, scientists have developed a range of approaches. One of the most promising is the use of genetically modified crops that produce RNA molecules targeting specific genes in insect pests. When the insects feed on the crop, the RNA molecules are taken up and interfere with the expression of essential genes, leading to reduced pest damage and population control. This approach is known as RNAi-mediated crop protection (Niu et al. 2018; Pugsley et al. 2021; Kim and Zhang 2022).

Another approach to using RNAi for insect pest control is the use of RNA molecules delivered through spray applications. In this method, RNA molecules are synthesized and sprayed directly onto the crops or the insects themselves. When the RNA molecules are taken up by the insects, they interfere with essential gene expression, leading to reduced pest damage and population control. Several studies have shown the potential of RNAi-based insect pest control. For example, RNAi-mediated crop protection has been used to control the diamondback moth, a major pest of brassica crops, such as cabbage and broccoli. In one study, genetically modified broccoli plants producing RNA molecules targeting a key gene in the diamondback moth reduced pest damage by up to 99% (Zhu et al. 2019).

RNAi has also been used to control other insect pests, including the western corn rootworm, a major pest of corn crops. In one study, RNA molecules targeting a key gene in the western corn rootworm were applied to corn plants and led to a reduction in root damage caused by the pest (Fishilevich et al. 2016). While RNAi shows promise as a pest control strategy, there are also potential risks and limitations to its use. For example, RNA molecules could potentially affect non-target species and ecosystems, leading to unintended environmental impacts. Additionally, the effectiveness of RNAi-based pest control can be affected by factors such as the delivery method, the concentration and duration of RNA exposure, and the potential for insects to evolve resistance to the RNA molecules over time.

4.1. Oral Delivery/Ingestion of dsRNA

Oral delivery/ingestion of double-stranded RNA (dsRNA) is a promising approach for insect pest control. dsRNA is a form of RNA that can silence or downregulate specific genes in insects, leading to reduced pest damage and population control. By delivering dsRNA through oral ingestion, it is possible to target pests that feed on crops and other plants, making it a potentially effective and environmentally safe approach to insect pest control. One of the main advantages of oral delivery of dsRNA is that it is a natural process that occurs in many insects. Insects commonly ingest RNA molecules as part of their diet, and the RNA molecules are taken up by their cells and can interfere with essential gene expression. By providing targeted dsRNA molecules, it is possible to enhance this process and specifically silence genes essential to pest survival and reproduction (Katoch et al. 2013).

Several studies have shown the potential of oral delivery of dsRNA for insect pest control. For example, a study published in the journal Science in 2006 showed that feeding of dsRNA targeting the *Snf7* gene in the western corn rootworm led to a significant reduction in root damage caused by the pest. Another study published in the same journal in 2010 demonstrated the potential of oral delivery of dsRNA for controlling the Colorado potato beetle, a major pest of potato crops (Korb et al. 2010).

One of the main challenges of oral delivery of dsRNA is ensuring that the RNA molecules are stable and effectively delivered to the target pests. Several methods have been developed to overcome this challenge, including the use of nanoparticles, liposomes, and other delivery vehicles to protect the RNA molecules and ensure their effective uptake by the insects. While oral delivery of dsRNA is a promising approach to insect pest control, there are also potential risks and limitations to its use. For example, dsRNA molecules could potentially affect non-target species and ecosystems, leading to unintended environmental impacts. Additionally, the effectiveness of dsRNA-based pest control can be affected by factors such as the delivery method, the concentration and duration of RNA exposure, and the potential for insects to evolve resistance to the RNA molecules over time (Niu et al. 2018).

4.2. Delivery of dsRNA Through Soaking

Delivery of dsRNA (double-stranded RNA) through soaking has emerged as a promising method for agricultural insect control. dsRNA can be used to silence specific genes in insects, leading to reduced expression of target proteins and ultimately insect mortality. Soaking is a method of delivering dsRNA by immersing insects or plant tissues in a solution containing the dsRNA (Arshad et al. 2021).

The effectiveness of dsRNA soaking for insect control depends on several factors, including the concentration and length of the dsRNA molecule, the duration of the soaking period, and the species of the target insect. In general, dsRNA molecules that are longer and more concentrated are more effective at inducing RNA interference (RNAi) and reducing insect survival. However, care must be taken to avoid off-target effects, where unintended genes are silenced, which can lead to unintended consequences for the ecosystem. One advantage of dsRNA soaking is that it can be applied to a variety of insect species, including those that are difficult to control with traditional insecticides. It can also be applied to plant tissues to confer resistance to insect pests. However, the effectiveness of dsRNA soaking may be limited by factors such as the permeability of insect cuticles and the presence of nucleases that can degrade the dsRNA (Zhang et al. 2018). Overall, dsRNA soaking has shown promise as a method for agricultural insect control, but further research is needed to optimize the method and evaluate its long-term effects on insect populations and ecosystems.

To maintain a continuous supply of dsRNA to herbivorous insects, it is necessary to establish a long-term effective approach. This can be achieved through transgenic plants that produce sufficient dsRNA and can serve as a source of food for insects. Two independent studies conducted in 2007 demonstrated the potential of transgenic plants in controlling insect infestations by producing dsRNAs that target specific insect genes. In one study, transgenic maize plants that produced vacuolar adenosine triphosphatase (*V-ATPase*) dsRNA were shown to reduce damage caused by western corn rootworm infestations (Baum et al. 2007). In the other study, transgenic *Arabidopsis thaliana* or *Nicotiana tobacum* leaves expressing dsRNA specific to a cytochrome P450 gene (*CYP6AE14*) suppressed the expression of the gene in cotton bollworm larvae, resulting in reduced tolerance to gossypol-containing food (Mao et al. 2007). This indicated the potential for RNAi technology to be used in genetically modified anti-insect crops, as demonstrated in the enhanced resistance of cotton plants to cotton

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bollworms in later studies. These promising results suggest that RNAi technology could be an effective approach for insect pest control in the future. Bt toxin transgenic plants have been successful in controlling lepidopteran pests, but phloem sap-sucking insects such as aphids, whiteflies, planthoppers, and plant bugs have become major pests since no Bt toxin effectively controls them. Therefore, alternative methods must be considered to control these pests and plant-mediated RNAi could be a game-changer for plant protection (Zafar et al. 2020). The brown planthopper, *Nilaparvata lugens Stål*, is a major pest for rice crops and Zhao et al. (2011) attempted to use plant-mediated RNAi to control it. They found that genes for the RNAi pathway are present in *N. lugens* and developed dsRNA constructs to transform rice. Although the transcription levels of target genes were reduced, a lethal phenotype was not observed, possibly due to insufficient RNAi effects or compensation from other genes (Zhao et al. 2011). Nonetheless, this study confirmed that transgenic plants expressing dsRNA can trigger RNAi in insect pests, making gene silencing a promising technique for insect pest control, provided that appropriate target genes are selected. To overcome the challenges posed by micro-injection, researchers have developed an artificial feeding system for delivering dsRNA to insects. This approach is gaining popularity as it has the potential to be used in the development of novel methods for pest control, such as using dsRNA transgenic plants (Zhao et al. 2011). Studies have shown that feeding dsRNA to insect larvae can trigger RNAi, reducing transcript levels and resulting in the desired phenotype. For instance, feeding EposCXE1 dsRNA to larvae of the horticultural pest *Epiphyas postvittana* led to a reduction in the expression levels of a larval gut carboxylesterase gene. Similarly, in the triatomine bug *Rhodnius prolixus*, dsRNA was successfully delivered by both injection and ingestion methods (Araujo et al. 2006). In another example, feeding dsRNA to honeybees led to a significant decrease in gene expression levels and caused high mortality and morphological abnormalities (Zhang et al. 2013). The effectiveness of the dsRNA feeding strategy varies from species to species, and while it has been widely used in insects, careful consideration must be given to selecting the appropriate target genes for successful gene silencing. Overall, the success of this approach demonstrates that dsRNA ingestion is a simple and efficient technique for functional genomics and dsRNA application studies.

Bt toxin-expressing transgenic plants are effective against chewing pests, but not against phloem sap-feeding insects such as aphids, planthoppers, whiteflies, and psyllids. Pesticides are still widely used to control these pests, but the oral delivery of dsRNA and its use in transgenic plants could be a powerful tool to control these types of pests. Chen et al. (2010) demonstrated the feasibility of RNAi via feeding in the brown planthopper (*N. lugens*) using a feeding-based technique and the target gene TPS. The silencing of the TPS gene through dsRNA feeding led to significant reductions in mRNA and enzymatic activity, and the development of *N. lugens* larvae fed with dsRNA was disturbed, resulting in lethality. Similar success was observed in the whitefly, where higher mortality of whiteflies was observed following treatment with siRNA for structurally important genes (Upadhyay et al. 2011). However, the more generally accepted method at present is to express dsRNA targeted against insect genes in transgenic plants. Pitino et al. (2011) constructed dsRNA transgenic tobacco and Arabidopsis plants to down-regulate *M. persicae* gene expression. The *Rack1* gene and *C002* genes were selected as targets, resulting in a knockdown of target gene expression by up to 60% in aphids fed with dsRNA transgenic plants, and the aphids produced fewer progenies. While plant-mediated RNAi approaches are feasible for controlling phloem sap-feeding pests at the field level, appropriate target genes must be carefully selected when designing dsRNA transgenic plants. Therefore, feeding experiments, along with genomics and proteomics, can be important research tools for selecting more effective pest control genes.

5. Conclusion

In conclusion, RNA interference (RNAi) is a powerful technology with immense potential for the management of insect pests on crops. RNAi-based approaches are more specific and environmentally friendly than traditional chemical-based methods, making them an attractive alternative for sustainable pest control in agriculture. Although there are still some challenges associated with the use of RNAi, such as the need for effective delivery methods and the potential for off-target effects, ongoing research is aimed at addressing these issues. Overall, the development of RNAi-based strategies for the management of insect pests represents an important area of innovation for the future of agriculture and has the potential to make a significant contribution to the development of sustainable, environmentally friendly crop production systems.

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