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Pest Control through RNAi

(*Pinki Sharma[1], Kavita Kansotia[1], Kiran Kumawat[1], Susila Yadav[1] and Jhabar Mal Tetarwal[2])
[1]Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan
[2]Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj
*Corresponding Author's email: pinki982996@gmail.com

Abstract

Insect pests are a major threat to meeting the food requirements of future generations. Current pest control strategies, including existing transgenic approaches, have some limitations and are not entirely successful in controlling insect pests. However, sequence-specific gene silencing through RNA interference (RNAi) holds great promise for effective management of agricultural pests. RNAi is a naturally occurring conserved process responsible for gene regulation and defense against pathogens. The efficiency of RNAi varies between different insect orders and also depends on various factors, including target gene selection, method of dsRNA delivery, dsRNA expression, and the presence of off-target effects. RNAi-mediated silencing of various insect genes involved in various physiological processes has been found to be detrimental to insect growth, development and survival. In this paper, we reviewed the potential of RNAi-based strategies for effective management of insect pests. We also discussed the various parameters that need to be considered for RNAi host-mediated control of insect pests without any effect on non-target organisms and the environment.

Introduction

Crop losses caused by insects and the use of insecticides to control insects cost billions of dollars worldwide each year; this constant use of insecticides poses a potential threat of the development of insecticide resistance. Insect pest control became a priority in research planning as $3,000 million was allocated to protect five of the most important agricultural crops with the mission of finding durable and cost-effective alternative pest control.

The noncell-autonomous involves an RNAi effect at a site different from the application or production site of the dsRNA, and therefore, it happens exclusively in multicellular organ- isms. Noncell-autonomous RNAi was first noted in *C. elegans* by Fire et al. [31], when injection of dsRNA into the body cavity resulted in targeted gene silencing throughout the injected animal and its progeny. The environmental RNAi in *C. elegans* occurs in the following steps:

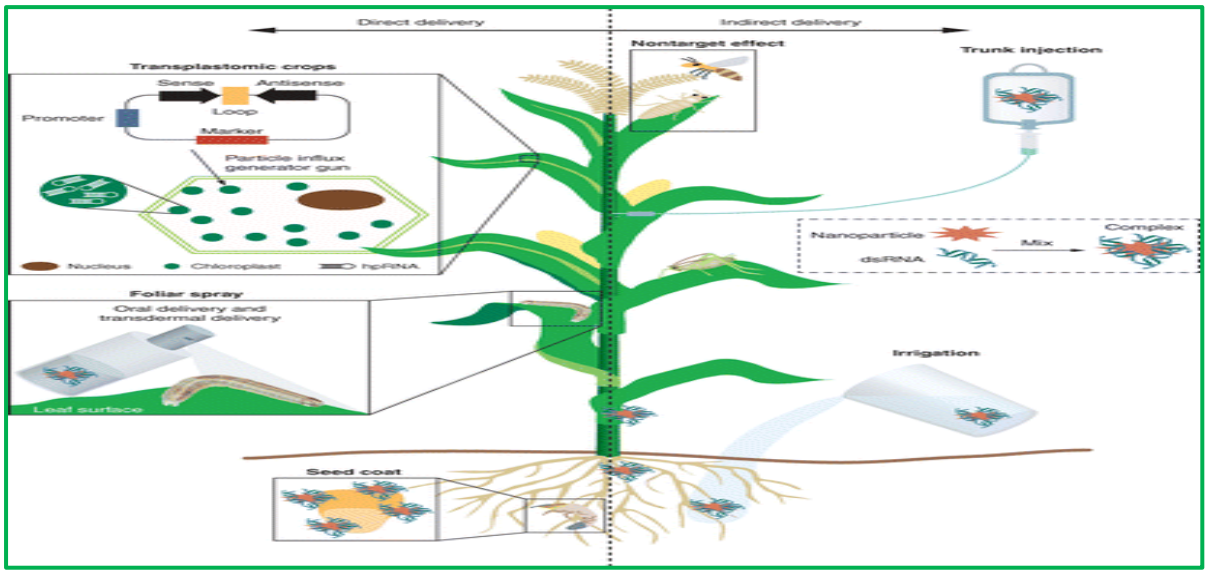
Out Of the latest twenty-first-century approaches developed in this direction, transgenics has emerged as a vital tool for insect pest management based on the practical successes demonstrated by the Bacillus thuringiensis toxin (Bt) in protecting broad categories of crops and, to some extent, replacing chemical insecticides. However, many important insect pests are not covered by Bt protection, and there also remains an imminent threat that at least some species will develop resistance to Bt. Therefore, there is a need to look for another sustainable and environmentally friendly approach to insect pest management, and RNA interference could be one such approach.

Table 1: Successful examples of RNAi-mediated gene knockdown in various insect species

Target insect	Mode of delivery	Concentration of RNA	Target gene
Anopheles gambiae	Feeding nanoparticles	–	*AgCHS1 AgCHS2*
Apis mellifera	Abdominal injection	1 µl	Vitellogenin
Apis mellifera	Mixed with natural diet	0.5	Vitellogenin
Apis mellifera	Soaking	1.26	Toll-related receptor
Diabrotica virgifera virgifera	Artificial diet	5.4 ng/cm^2	Vacuolar ATPase subunit A
Diabrotica virgifera virgifera	Feeding	1,000 ng	DvSnf7
Epiphyas postvittana	Droplet feeding	1.0 µg	Gut carboxylase
Glossina morsitans morsitans	Blood meal	10 µg	Midgut protein TsetseEP
Helicoverpa armigera	Feeding	25–50 nM siRNA	Acetylcholine esterase
Helicoverpa armigera	Transgenic plant	–	CytochromeP450 (CYP6AE14)
Menduca sexata	Injection	100 ng	Moricin
Nilaparvata lugens	Feeding	0.02, 0.1, and 0.5 mg/ml	Trehalose phosphate synthase (TPS)
Phyllotreta striolata	Injection	1 mg/ml	PsOr1
Phyllotreta striolata	Feeding	0.05–3.2 ng/ml	Arginine kinase
Reticulitermes flavipes	Artificial diet	13 µg	Cellulase
Schistocerca gregaria	Injection	5 µg dsRNA	Vitellogenin
Schistocerca gregaria	Injection	5 µg	gapdh
Spodoptera frugiperda	Droplet feeding	0.4 mg	Cytochrome P450 (CYP6BF1v4)
Spodoptera litura	Soaking and in artificial diet		Aminopeptidase N
Spodoptera litura	Injection	1.5 or 2.5 mg/ml	Vitellogenin receptor

RNA interference (RNAi) is a post-transcriptional gene silencing mechanism that is initiated by the introduction of double-stranded RNA (dsRNA) into a cell. This technique is well known in plants as "post-transcriptional gene silencing". This process is closely related to

post-transcriptional gene regulation by microRNAs (miRNAs), which involves the inhibition of translation initiation. RNAi was initially reported in plants, and then there has been tremendous research output on the various components of RNAi. Gene silencing using RNAi technology has been well studied in invertebrates, particularly Caenorhabditis elegant and Drosophila S2 cells.



RNAi effects

RNAi can be well distinguished into intracellular and extracellular RNAi. Intracellular RNAi involves expression of hairpin RNAs in transgenes, introduction of dsRNA into cells by transfection or electroporation, and injection directly into the cell. In extracellular RNAi, dsRNA is delivered by dipping, feeding, or injection into the hemocoel. The latter requires the uptake of dsRNA molecules by cells. The mechanism of RNAi was further categorized into cell-autonomous and non-cell-autonomous systems.

Cell-autonomous RNAi

In cell-autonomous RNAi, the silencing process is restricted to the cell in which the dsRNA is introduced or expressed. Here, dsRNA is cleaved by RNase III (Dicer) into 21–25 nt long siRNAs, and then these siRNAs are incorporated into RISC, which mediates mRNA degradation. Cell-autonomous RNAi uses widely conserved machinery and similar strategies in a wide range of organisms, and many initial studies focused mainly on cell-autonomous RNAi.

Effects of RNAi in different orders of insects

RNAi has proven successful in various insect orders. The successful effects of RNAi in insects have also been reviewed by Katoch and Thakur.

Diptera. Knockdown of the immunoresponsive TsetseEP gene expressed in the midgut has been demonstrated in G. morsitans. This was also the first report of gene knockdown in a blood-sucking insect by including dsRNA in a blood meal.

Lepidoptera Application of dsRNA in artificial diet led to knockdown of targeted genes in E. postvittana and Plutella xylostella Mao et al. genetically engineered Nicotiana tabacum and Arabidopsis thaliana plants with the cytochrome P450 gene from H. armigera. Cytochrome P450 levels were reduced and larval growth was slowed when larvae were fed transgenic leaves. Cottonseed meal uses an antidote to a natural cotton insecticide called gossypol. dsRNA-triggered RNAi of cytochrome P450 monooxygenases reduces insect tolerance to gossypol and exposes insects to the full force of gossypol. In a permethrin-resistant strain of P. xylostella, RNAi-mediated silencing of the cytochrome P450 CYP6BG1

gene after consumption of a droplet of dsRNA solution resulted in increased insect susceptibility to the pyrethroid insecticide.

The effects of RNAi against carboxyesterases were studied in E. postvittana larvae by feeding them an artificial diet supplemented with dsRNA. Repression of the EposCXE1 carboxyesterase gene was observed after 2 days of feeding and max.

Challenges for RNAi as a successful insect control strategy

While the prospects for using RNAi as an insect control strategy look promising, there are still several aspects to be studied before it becomes a practical reality. Although there are logical considerations for success in some experiments, the long-term effectiveness of the approach will need to be determined after field experimentation. dsRNAs administered either in the diet or from transgenic plants would first reach the midgut of the insect. The body of insects is covered by a chitinous exoskeleton, while the midgut of most insects is lined by a peritrophic membrane (PM) or a perimicrovillar membrane (PMM) in Hemipterans. Midgut cells, which are responsible for absorbing nutrients from the gut lumen, are able to take up dsRNA. The midgut region is therefore the only area for exchange between hemolymph and gut contents with exposed cells affecting nutrient absorption. All factors including RNAi efficiency, such as expression levels of the core RNAi machinery, uptake of dsRNA/siRNA from the extracellular medium, and absence/presence of dsRNA-degrading enzymes in the midgut are important for RNAi. dsRNAs are likely mediated by midgut surfaces by exposure of midgut epithelial cells and Malpighian tubules to dsRNAs in intestinal contents. Plant material with hairpin RNA ingested by insects may not provide sufficient levels of intact dsRNA to trigger strong RNAi. Furthermore, dsDNA does not always need to be present in large amounts to have effective RNAi, but several factors are involved in determining the effects of gene silencing.

- Insect nucleases
- Intestinal pH
- Length of dsRNA
- Off target effects

Conclusion

RNAi has enormous potential to become a successful approach to control insect pests. However, several research and ethical issues need to be resolved before this technology can be applied on a commercial scale. To date, Bt transgenic is the main method of biological control of insects, but the pace of RNAi research indicates that RNAi will play a significant role in the control of insect pests in the near future. Transgenic plants producing Bt toxins have been shown to be successful in controlling insect pests of many crop plants, but there have been sporadic reports of the development of resistance to Bt toxin.

References

1. Naranjo, S. E. (2011). Impacts of Bt transgenic cotton on integrated pest management. Journal of Agricultural and Food Chemistry, 59, 5842–5851.
2. Nunes, F. M. F., & Simo˜es, Z. L. P. (2009). A non-invasive method for silencing gene transcription in honeybees maintained under natural conditions. Insect Biochemistry and Molecular Biology, 39, 157– 160.
3. Katoch, R., & Thakur, N. (2013). Advances in RNA interference technology and its impact on nutritional improvement, disease and insect control in plants. Applied Biochemistry and Biotechnology, 169, 1579– 1605.
4. Turner, C. T., Davy, M. W., MacDiarmid, R. M., Plummer, K. M., Birch, N. P., & Newcomb, R. D. (2006). RNA interference in the light brown apple moth, Epiphyas postvittana (Walker) induced by double-stranded RNA feeding. Insect Molecular Biology, 15, 383–391.