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The RNA Revolution: A New Era of Residue-Free Crop Protection in Horticulture

K. A. Mumithra Kamatchi[1*]
Tamil Nadu Agricultural University, Coimbatore, India
Corresponding author email: mumithra1996@gmail.com

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Chemical pesticides are widely used in horticulture to produce high-quality fruits and vegetables, including flowers, for the global market. A wide range of insecticides and acaricides are used to control lepidopteran larvae, aphids, whiteflies, thrips, mites and fruit flies, while fungicides and bactericides are commonly applied to control *Fusarium*, *Alternaria*, *Phytophthora*, *Botrytis* and powdery mildew on various horticultural crops. However, the frequent and extensive use of these pesticides on vegetable and fruit crops can give rise to problems such as pesticide resistance, pesticide residues on fresh produce, adverse effects on pollinators and beneficial organisms in the agro-ecosystem, environmental contamination, and health problems for farm workers and consumers. Currently, with the strict regulations of Maximum Residue Limits (MRLs) on fresh produce in both domestic and foreign markets, there is a growing need for horticulture to develop accurate, environmentally friendly, and residue-free methods for crop protection. Next-generation RNAi-based biopesticides are likely to meet these requirements and to reduce the use of chemical pesticides for sustainable horticulture.

RNA- Based Biopesticides

Ribonucleic acid (RNA), in particular double-stranded RNA (dsRNA), is the key to next-generation crop protection. In addition to its role in gene expression, dsRNA can also elicit the highly conserved RNA interference (RNAi) pathway, which acts by silencing essential genes that are vital for pest's and pathogen's survival, development, reproduction or pathogenicity. The action of RNA-based biopesticides is based on sequence-specific recognition of target genes based on sequence complementarity rather than non-specific recognition in conventional broad-spectrum pesticides. This allows for highly specific targeting of genes in pests/ pathogens with the possibility of reduced impact on beneficial and non-target organisms.

One Technology, Multiple Targets

The use of dsRNA sequences has been demonstrated to protect horticultural crops from several agricultural issues concerning diseases and pests through RNA interference technology. The five major target categories are provided in Table 1.

Table 1. Gene Target Categories for Precision RNAi-Based Crop Protection Studies

Target category	Representative genes	Examples	Importance
RNAi machinery	DCL1, DCL2, Ago2	*Botrytis, Podosphaera, Plasmopara, Sclerotinia*	Disrupts the pathogen's own RNA-silencing machinery
Essential metabolism	CYP51/ERG11, ATPD, ATPG	*Fusarium, Botrytis, aphids*	Targets essential metabolic processes
Structural/ cytoskeletal genes	TUB2, CHS	*Podosphaera, fungal pathogens*	Disrupts cell structure, growth and development
Virulence-associated genes	CgLAC, pgxB, Tup1	*Colletotrichum, Aspergillus, Fusarium*	Directly interferes with pathogenicity
Insect survival/ development genes	AChE, PBAN, TPS, chitinase, Snf7/mesh	Lepidopterans, aphids, whiteflies, beetles	Cause mortality, developmental defects or reduced reproduction

Cellular Process of RNAi Technology

RNAi-based horticultural protection relies on a highly coordinated process within the pest cell which involves five steps: (i) The application of the dsRNA and its subsequent uptake by the pest, (ii) an enzyme called dicer, which cuts the long dsRNA into small pieces called small interfering RNAs (siRNAs), (iii) loading the small pieces of dsRNA (siRNAs) into the RNA-induced silencing complex (RISC), (iv) the RISC using one strand of the siRNA to guide the targeting of the pest's mRNA, and (v) the RISC complex cleaving the mRNA, thereby preventing the pest from producing a protein that is essential for their survival, reproduction, or virulence (Figure 1).

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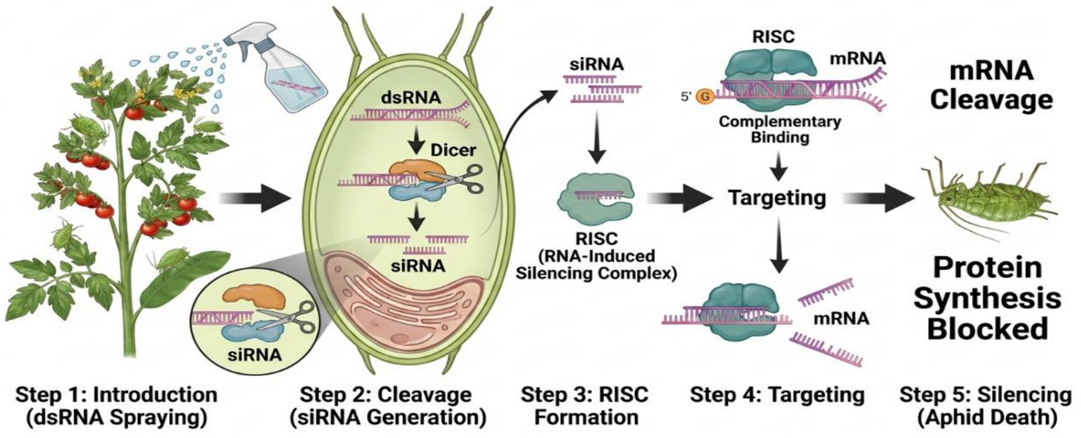

Figure 1: Foliar spray of dsRNA inhibits protein synthesis in aphids

Smart Delivery for Better Protection: Reaching the Right Target

The success of RNA-based bio-pesticides depends on the survival of the RNA, its delivery to the target, and its activity in the target. The activity of dsRNA can be greatly diminished by UV light, rain, nucleases, gut enzymes of insects, and the inability to enter cells. Smart delivery systems are thus being developed to facilitate stability, transport, and precision of RNA.

- **Host-induced gene silencing (HIGS):** Plants as 'RNA Factories' produce dsRNA against invading pests/pathogens. HIGS transgenic plants are commonly produced by Agrobacterium-mediated transformation. For instance, HIGS has been developed to control root-knot nematode and has decreased nematode reproduction by 73.75% in tuberose.
- **Virus-induced gene silencing (VIGS)** is a method that makes use of a modified viral genome as a VIGS vector to produce efficient siRNA for the target gene. Tobacco Rattle virus (TRV) based VIGS has been considered the most popular target system due to its systemic movement and high host range. For instance, TRV-based VIGS silences the *LeCTR1* gene and improves the plant's tolerance to tomato leaf curl virus.
- **Spray-induced gene silencing (SIGS):** It has two pathways: direct uptake when the pest or pathogen is directly absorbed by the plant surface, triggering the RNAi mechanism; and indirect uptake when the plant absorbs the dsRNA and then triggers the RNAi mechanism when fed or infected by a pest or pathogen. Successful applications include control of important diseases like gray mold, white mold, late blight, Asian soybean rust and tobacco mosaic virus, and insect pests like the diamondback moth, whitefly, Asian citrus psyllid, soybean aphid and other leaf-chewing insects. For example, an application of this dsRNA

to the target gene of the pathogen causing powdery mildew resulted in lowered disease severity in rubber.

- **Nanocarrier-Mediated Gene Silencing (NDGS):** One of the major drawbacks of RNA-based biopesticides is the rapid degradation of dsRNA, where nanotechnology can help overcome this challenge. The layered double hydroxides (LDH), chitosan nanoparticles, clay nanosheets, silica nanoparticles, and single-walled carbon nanotubes (SWCNTs) have been studied for RNA delivery and confer their protective properties to enhance the persistence of RNA and the efficiency of silencing. For instance, single-walled carbon nanotubes (SWNTs) enter cell walls and avoid degradation of biomolecules within cells, and are effective cargo transporters into living plant cells when introduced by whitefly and diamondback moth. Clay-based formulations, such as BioClay™, also have been studied to deliver dsRNA to plant viruses within the crops of vegetable plants such as zucchini and capsicum.
- **Microbial-mediated gene silencing:** Engineered microorganisms or insect-associated symbionts can produce or protect dsRNA to achieve more persistent delivery. The symbiotic bacteria can also be continually cultured and provide dsRNA, which greatly improves the efficiency of RNAi. For example, an RNAi biological pesticide platform technology developed by Renaissance BioScience Corp involves the delivery of dsRNA in baker's yeast, which will be sprayed onto the surface of crops to have its effect on Colorado potato beetle and other chewing pests.
- **Extracellular Vesicles:** Plant and microbial EVs are nature's RNA couriers and can be used as biological cargo carriers to protect dsRNA and transport, providing a new method for targeted RNA delivery in the future.

RNAi Technology in Commercial Action

As of 2025, four dsRNA-based crop-protection products had been registered, including three plant-incorporated dsRNA maize products (SmartStax PRO®, MON95275, DP23211) for the control of corn rootworm. Another important milestone for sprayable RNA biopesticides is Calantha™ contain the dsRNA active ingredient Ledprona for control of Colorado beetle in potato.

Challenges in RNAi Technology

Although RNA-based biopesticides have the potential to be very specific in protecting crops, the efficacy of the products depends on delivery requirements, stability issues, and biological barriers:

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- Externally applied dsRNA can rapidly degrade due to UV radiation, rainfall, and temperature changes.
- dsRNA is not very stable at low pH or in the environment, which decreases the quantity of the intact RNA that reaches the target.
- dsRNA is broken down by digestive nucleases and other digestive enzymes before it can get to the target cells in the insect gut.
- Careful selection of sequences with minimal similarity to genes in beneficial and non-target organisms is essential for effective RNAi.
- Traditional foliar sprays may be ineffective for retaining and penetrating waxy leaves or during rainfall. An optimized formulation should include RNA protection, adhesion, controlled release and efficient uptake without causing phytotoxicity.
- The high cost of production can be incurred on large-scale synthesis, purification, formulation and storage of high-quality dsRNA.
- RNA-based products need to be evaluated from a regulatory perspective for environmental fate, persistence and food safety.

Conclusion and Future Perspectives

In the near future, it is not expected that RNAi will replace conventional chemical pesticides. Rather, it is probably to be a key element of integrated precision-based crop protection. Great potential lies in the combination of sequence-specific RNAi and biological control agents, resistant cultivars, beneficial microorganisms, pheromone approaches, and smart farming technologies. Future development of nanotechnology, target selection using artificial intelligence, precision spraying and controlled-release formulations holds promise to further enhance stability and field performance of dsRNA. In parallel, enabling policies, regulatory pathways, affordable production, and farmer awareness will be key for the scaling-up process. The future of crop protection is not RNAi versus chemicals, but RNAi combined with biology and smart technologies for residue-free crop protection and sustainable agriculture.