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Scope of Genetically Engineered Predators and Parasitoids in Biological Control

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Summary/Highlights

- Minimizing the impact of these pests is essential for sustainable crop production and to maintain an economically viable level of output
- Genetic manipulation of natural enemies of insect pests offers promise of enhancing their efficacy in agricultural cropping systems.
- Artificial selection, hybridization or heterosis and recombinant DNA technology are potential genetic manipulation techniques

Introduction

The dynamics of insect pest issues in agriculture have changed significantly due to ecosystem and technological changes. To ensure higher crop yields, it is crucial to control harmful insect pests. While the voluminous application of insecticides can initially provide good results, it can have harmful effects on the environment and human health in the long run. In addition, the use of various pesticides can also reduce the populations of beneficial insects alongside the targeted pests. The remaining traces of these chemical agents lead to food poisoning and environmental pollution. To prevent these harmful effects, law enforcement authorities need to ensure restrictions on the widespread use of chemical components. Proper regulations should be implemented regarding the frequency of spraying, the amount of residue on food, and the action of chemicals. Biological control has been recognized as an effective, environmentally friendly, technically appropriate, economically feasible, and socially acceptable method of pest management. It focuses on reducing insect pests of crops and other harmful organisms by utilizing their natural enemies such as parasites, predators, and pathogens. In India, the earliest successful introduction of a natural enemy against an insect pest was the coccinellid beetle *Cryptolaemus montrouzieri* (Muls.) from Australia in 1898.

Role of biotechnology in biological control

Biotechnology encompasses the use of living systems and organisms to develop products or processes, or any technological application that utilizes biological systems, living organisms, or their derivatives to create or modify products or processes for specific purposes. In the context of insect pest management, biotechnology refers to the deliberate and controlled manipulation of biological systems to achieve effective control of insect pests. The vast array of biological capabilities present in living organisms allows for the meaningful control of insect pest species by selecting organisms with specific capabilities. Biotechnology holds significant potential to contribute sustainable biological components to integrated pest management (IPM) (Joshi et al. 2020). Genetic manipulation of naturel enemies of insect pests offers promise of enhancing their efficacy in agricultural cropping systems (Routray et al., 2016). Indeed the first field trial of a genetically modified arthropod biological control agent was with the predatory mite *Metaseiulus occidentalis* in 1996. Genetic modifications

are carried out on natural enemies of insects and entomopathogens to improve their ability to control pest populations by altering their genetic traits.

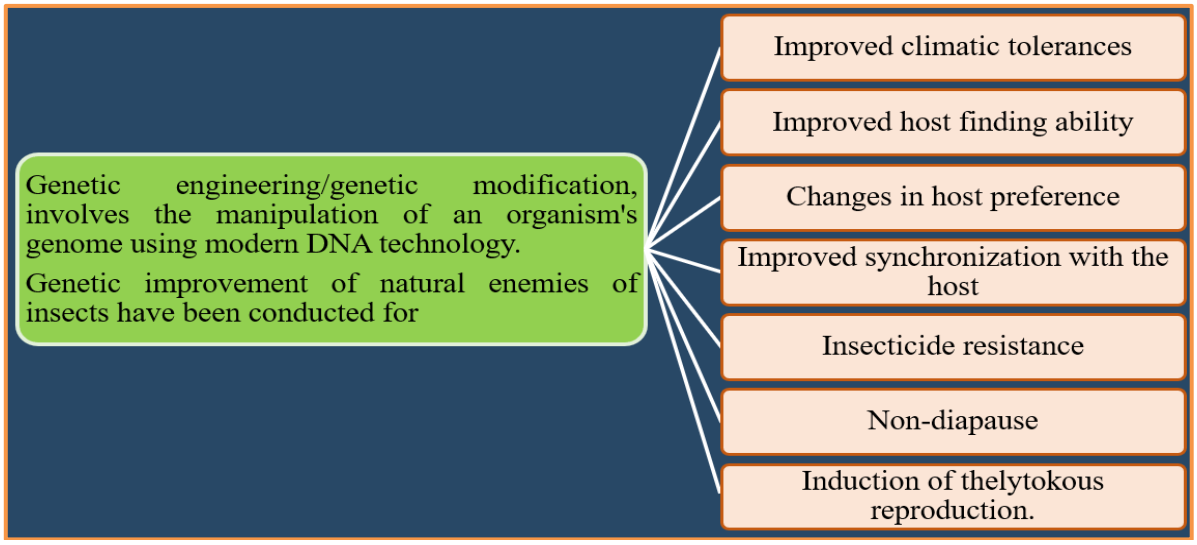

Figure 1: Various objectives of genetic modifications

Prerequisites for genetically improving the natural enemies

1. Accurate identification of parasitoids is a critical initial step in considering their suitability as biological control agents.
2. Understanding who eats whom: The utilization of molecular techniques for species-level identification.
3. Addressing the inadequacies of specific agents

Emerging technologies in augmentation of natural enemies

As in crop breeding, three potential genetic manipulation techniques are being used to achieve the above goals.

a. Artificial selection

b. Hybridization or, use of Heterosis

c. Use of Biotechnology (recombinant DNA (rDNA) techniques)

Artificial selection

Artificial selection of arthropod natural enemies for resistance to pesticides has been proposed as a method for improving the usefulness of natural enemies in integrated pest management programs. The predatory mite *Metaseialus occidentalis* was genetically modified through artificial selection to acquire resistance to carbaryl and permethrin, and multi-resistant strains of the same mite were obtained via laboratory crosses and additional selections (Routrey et al., 2016). Furthermore, a strain of *Trigramma chilonis* was developed that is tolerant to endosulfan and transferred to a private industry that markets it under the name "Endogram." Additionally, a strain of *T. chilonis*, known as MITS-TC, was developed to be tolerant to multiple insecticides, including endosulfan, monocrotophos, and fenvalarate, and was evaluated in the field against *Helicovarpa armigera* (Routrey et al., 2016).

Genetic modification methods

Direct genome modification

It is the insertion of a desirable gene sequence of DNA into the insect species. This approach, known as the sterile insect technique (SIT) or autocidal control, involves releasing large numbers of sterilized insects, either through irradiation or chemicals, to control insect pests of agricultural and medical importance. SIT is most effective when applied over a broad spatial area, rather than treating fields or locations individually. This method has been successfully used worldwide to manage insect pests such as the tsetse fly, Mediterranean fruit fly, melon fly, oriental fruit fly, Mexican fruit fly, pink bollworm, codling moth, cactus moth, false codling moth, Australian painted apple moth, and *Teia anartoides*.

Recombinant DNA Technology

This technology enables the identification, cutting, and insertion of one or multiple foreign genes into the genome of another organism, expressing new characteristics. After selecting a trait, one or more genetically engineered constructs can be inserted into the insect's genome using transposable elements or viral vectors. . The hsp70 promoter was further utilized in heat-inducible and laser mediated gene expression system in transgenic silkworm (Uhlirova et al., 2002) and butterfly (Ramos et al., 2006)

Homing endonucleases

Genome editing technologies (gene editing) are useful to understand the functions of target genes and allow genetic material to be added/removed/altered at site-specific locations in the genome (Segal and Meckler, 2013). The use of engineered nucleases or homing endonucleases (HEGs) is the common method for such kind of editing which creates double-strand breaks (DSBs) of 15 and 40 bp in length at the desired location in the genome. The induced DSBs are repaired through non-homologous end-joining or homologous recombination, leading to targeted gene editing. Earlier, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) systems were used for genome editing that requires the use of a variety of nucleases, and the off- target effects of nucleases can lead to cellular toxicity. ZFNs and TALENs systems have been recently replaced by the CRISPR/Cas9 system, which is less expensive, more effective and convenient than ZFNs and TALEN

RNA interference

RNA interference (RNAi) is a post-transcriptional gene-silencing mechanism in which double-stranded RNA (dsRNA) down-regulates specific gene expression in a sequence-dependent manner. This technology enables functional genomics studies and offers significant potential for crop improvement and pest management. The first successful application of RNAi for insect control was demonstrated by Baum et al. (2007), who expressed dsRNA targeting the vacuolar ATPase A (V-ATPase A) gene in maize, resulting in mortality of the western corn rootworm (*Diabrotica virgifera* LeConte) and suppression of root feeding. Owing to its high specificity, RNAi has gained increasing attention for use in genetically modified crops as well as spray-based insecticides. RNAi has also shown promise in managing arthropod pests affecting beneficial insects. *Varroa destructor*, an obligate ectoparasite of honeybees, feeds on haemolymph and transmits multiple viruses, leading to colony collapse within 2–3 years if untreated (Rosenkranz et al., 2010). Upon ingestion, IAPV-dsRNA is processed into 20–30 bp small interfering RNAs (siRNAs), which activate the RNA-induced silencing complex and bind to homologous viral mRNA, resulting in protein silencing and reduced viral spread within bee colonies (Hunter et al., 2010).

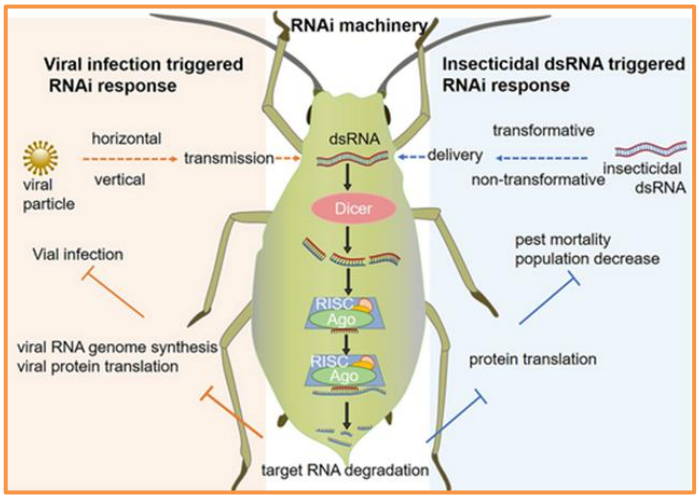

Figure 2: Use of RNAi machinery in insect modification

CRISPR Cas

The application of CRISPR Cas9 gene editing in insects has been extensively investigated through a series of experiments involving model organisms such as *Bombyx mori* and *Drosophila melanogaster*, as well as other economically significant crop-damaging species. The straightforward protocol for its design has facilitated diverse experimental approaches aimed at elucidating gene function. (Zhang et al., 2014). The use of transgenic crops with BT insecticidal protein genes is widespread for protecting crops against harmful pests (Bravo et al., 2011). In a study by Wang et al. (2016), a mixture of Cas9 mRNA and sgRNA was

injected into eggs of *Helicoverpa armigera (H. armigera)* to target the ninth exon of the cadherin gene. Mutating the insect cadherin gene resulted in higher resistance to Cry1Ac compared to the control strain. The results indicate that the cadherin gene is an essential receptor for cry1Ac and plays a role in insect resistance against cry1Ac. Additionally, using CRISPR Cas9, pigment genes of *H. armigera* were mutated, leading to several physical phenotypical changes (Khan et al., 2017). Chang et al. (2017) demonstrated a new mechanism in *H. armigera* to disrupt pest mating using the CRISPR/Cas9 system for insect genome editing. In another study, two sgRNAs were employed to delete a cluster of genes. The entire CYP6AE cluster was edited in *H. armigera* using a dual sgRNA-directed CRISPR-Cas9 system. Injection of the Cas9 protein and two guided RNAs into H. armigera embryos, including 400 eggs, resulted in 125 hatching and 65 developing into adults. These findings illustrate that genome editing of these genes impacts the survival rate of the cotton bollworm (Wang et al., 2018).

Disadvantages

Some of the major disadvantages of using genetically modified insects can be summarized as follows:

a) Due to the alteration of the ecosystem, human health can be at risk indirectly. Direct consumption of genetically modified insects may have some threats to the human. In some cases, the added proteins may also have an allergic reaction. If honeybee was genetically engineered, then the product honey should be verified by the lab test, whether it is appropriate or not for human consumption.

b) By using SIT, the wild population is either eliminated or vastly reduced, the ecosystem is permanently altered, and it is difficult to predict all of the broader ecological impacts of these changes. The elimination of these targeted insects also has negative impacts on other non-targeted beneficial organisms (predators and parasitoids).

Conclusion

Though the use of predator and parasitoids has no hazardous environmental impact but their efficacy under various conditions limits their usages. The improvement of their potency can boost their uses over the chemical mode of pest control. The cost–benefit ratio for classical biological control is highly favourable (1: 250) and for augmentative control is similar to that of insecticides (1 : 2–1 : 5), with much lower development costs. future pest management will depend strongly on biological control because it is the most sustainable, cheapest and environmentally safest system of pest management. A genetic improvement can be useful when the natural enemy is known to be a potentially effective biocontrol agent, the limiting trait in it is primarily influenced by a single major gene, the gene can be obtained by selection, mutagenesis or cloning, the manipulated strain is fit and effective, the released strain can be maintained on some form of reproductive isolation.

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