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Molecular and Genetic Advancement in Insect Pest Management

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Abstract

Scientific advancement in the molecular and genetic field has offered new opportunities to address many of the productivity constraints and holds great potential to be included in pest management programmes. Successful application of the technologies has leveraged in the saving of time, effort and money. Molecular tools are being used in the entomological field especially in the areas of insect taxonomy for species discovery; insect genetic relationship studies; development of resistant crops that release insect-specific toxins; improvement in breeding programmes; enhancement in the performance of natural enemies; design of microbial agents with increased potency; genomic studies to identify desirable genes to trace the insecticide resistance mechanism and development of bio-rational insecticides with a novel mode of action. Nascent approaches like gene editing, RNA interference (RNAi); gene drives and recently CRISPR-Cas9 system-based pest management tools are being developed. The current scenario in the molecular and genetic field in relevance to insect pest management is discussed here.

Introduction

Many constraints severely affect the global agricultural production and productivity which include ever-increasing population growth, degradation of natural resources, climate changes and emerging pests. Of which, biotic constraints in particular the pests are known to cause 25-50% or complete loss of crop production. There is a huge loss on major crops mainly due to ravages of insect pests. A recent estimate from the FAO of the United Nations has shown that the invasive pests caused an annual loss of at least \$70 billion. Invasive pests are one of the main drivers of biodiversity loss and a potential threat to plant biosecurity. Also, increasing awareness about environmental consequences due to indiscriminate use of chemical pesticides has delivered new momentum in search of alternative ways for managing agricultural pests. Integrated pest management (IPM) is one such concept that received a lot of attention which emphasizes the growth of a healthy crop with the least possible disruption to the agroecosystems. It encourages natural pest control and strives to minimize the use of precarious chemicals by integrating the different components of pest management tactics.

The Advent of the Genomic Era

With the advancement of science and technology, the appropriate uses of modern biotechnological tools in different components of IPM are gaining significance in recent years. Numerous factors determine the effectiveness of a pest control strategy. Various plant protection technologies are being deployed for crop pest management but the use of modern biotechnological tools

that are proven to be most effective becomes mandatory. Molecular biology and genetics are essential to understand entomological problems. Informational molecules such as nucleic acids and proteins are now believed to have high taxonomic information, suggesting increasingly familiar molecular approaches to systematics. The application of biotechnology in plant protection can be broadly divided into two categories namely characterization of pests and management of pests which made significant advances and has a huge influence on IPM.

Insect Genomics

For a better understanding of biology and to identify desirable genes of pest species large numbers of insect genomes have been wholly sequenced in the last decades viz. *Henosepilachna pusillanima* (Mulsant), *Pseudonemorphus versteegi* (Ritsema), *Aristobia reticulator* (Voet), *Bactrocera zonata* (Saunders), *Samia canningi* (Hutton), etc. Such sequence information serves as a tool for development of molecular markers. Also, the draft genome of rice yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) has been studied. The genomic analysis led to the identification of genes related to its specific behaviour. Similarly, the genome of flower thrips, *Thrips hawaiiensis* (Morgan) has been sequenced and characterized by using the next-generation sequencing technique. It is the common thysanopteran insect distributed widely in Asia and the Pacific with varied morphology. The complete mitochondrial genome sequence provided the information for mitochondrial genome research on Thysanoptera (Wang et al., 2021). Apart from nucleic acid-based information, there is fast-growing information coming from proteomics and metabolomics studies too.

In addition, various molecular markers are being utilized to study diversity among populations of pest species. For example, amplified fragment length polymorphism (AFLP) markers were used to study biodiversity among populations of rice gall midge across Asian countries. Likewise, genetic variation in potential pests like *Plutella xylostella* (Linnaeus) and *Pectinophora gossypiella* (Saunders) has also been distinguished through sequence analysis. The understanding of diversity among the populations of crop pests helps in devising effective pest management strategies i.e., the factors responsible for the occurrence of differential response in their populations to various selection pressures (insecticides, pheromones, parasitoids etc.) can be identified.

Insect Taxonomy

Accurate identification of the pest species is the major concern across the World and it is the basis for any studies or effective implementation of management programmes. Earlier taxonomy and species descriptions were based on morphology alone. Hence several consequences occurred, misidentification is one such issue. Also, the major

problems in taxonomic research are lack of taxonomists, time-consuming, cryptic species issue, difficulties of the taxonomically challenging orders and modern taxonomic keys for the revised taxa. Thus, DNA-based identification has now gained general acceptance as a transformative technology, wherein the species of an individual organism is identified by using small regions of the mitochondrial genome (cytochrome c oxidase subunit or COI gene) to discriminate species (Hebert et al., 2003). It made the Linnaean taxonomic system more accessible, with benefits to ecologists, conservationists and agencies for the control of pests.

In the current agricultural scenario, invasive insect pests hold great socio-economic and ecological importance as they have risen worldwide significantly. Adopting molecular techniques along with traditional taxonomical tools, researchers rapidly detect several invasive pests and formulate immediate control strategies. The use of molecular and genomic tools facilitates understanding the genetic makeup of individuals. Moreover, the growing availability of genomic tools is advantageous for the use of alternative methods of controlling the invasive insect pests and also helps to understand the pest demography and reconstruction of the invasion routes.

Development of Resistant Crop Varieties

The transgenic *Bacillus thuringiensis* (Bt) has revolutionized the concept of the genetic manipulation approach in the field of crop protection. The Bt technology offered great benefits soon after its implementation with a distinct reduction in insecticide use. But, later on, insect resistance and pest emergence (crop susceptibility to sucking pests) increased and problems of new pests and viral disease emerged in cotton. To overcome the resistance to other insects, during 2016 refuge in bag (RIB) concept was introduced by the government to promote non-Bt cotton cultivation. Today, Bt cotton is under intense surveillance by all the stakeholders of the technology, to ensure the implementation of available management options on time to manage biotic stress. Recently, a synthetic *vip3A* gene in transgenic cotton against chewing insect pests has been developed and evaluated. PCR amplification and southern hybridization revealed successful *vip3A* transgene integration into plant nuclear genome. The enhanced insecticidal expression of the synthetic *vip3A* gene has shown great potential for its inclusion in the list of promising genes that can be used to develop insect-resistant transgenic plants. On the other hand, one of the major constraints in the host plant resistance programme is durability. The single gene-based resistance is often overcome by the development of virulent biotypes of the insect. This concern will be effectively addressed by the use of molecular markers by developing superior crop varieties.

Improvement in Bio-Control Programmes

Biological control is an eco-friendly, technically feasible and economically viable method of pest management. Various technologies have been adapted to improve the performance of natural enemies (predators, parasitoids and pathogens) against the pest population. Significant progress has been made in the development of temperature tolerant and pesticide-resistant strains of natural enemies like *Trichogramma chilonis* (Ishii) and *Chrysoperla zastrowi arabica* (Henry *et al.*) through artificial selection and hybridization. However, advances in molecular research have widened the available techniques for genetic manipulation of arthropods for a variety of traits of the species *viz.* improved climatic tolerance, improved host finding ability, host preference, pesticide and disease resistance, non-diapause, high fecundity and female-biasness. The genetic manipulation of natural enemies offers the promise of enhancing their efficacy in agricultural cropping systems. Moreover, molecular approaches facilitate the identification of the host/ prey range of natural enemies and also help in determining the parasitization levels and type of parasitism under field conditions.

Furthermore, the development of hypervirulent strains of entomopathogenic microbes is possible through the advanced approach. For example, to increase the effectiveness of entomopathogenic virus, the genes that code for neurotoxin in scorpion, *Androctonus australis* (Hector) and funnel-web spider, *Atrax robustus* (O. Pickard-Cambridge) have been engineered into baculovirus *AcNPV* (specific for *Autographa californica* Speyer) and *HaNPV* (specific for *Helicoverpa armigera* Hübner), respectively. Similarly, the deletion of the ecdysteroid UDP-glucosyltransferase (*egt*) gene from NPV reduces the feeding efficacy of the larvae and causes earlier mortality. The *vip3A* and *cyt2Ba* genes from *Bt* were used to improve virulence in EPF like *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschn.). Thethermo-tolerant strains of *B. bassiana* (Balsamo) Vuillemin and *Lecanicillium lecanii* (Zimmermann) by using physical mutagens *viz.* moist heat stress (35 °C) and UV radiation have also been achieved. However, the use of recombinant microbial bio-control agents against a particular organism or outside needs regulatory clearance.

Development in Insect Toxicological Studies

In nature, plants itself known to synthesize a wide range of compounds that disrupt the normal growth and development of insects. Recently, the molecule 1'S-1'-acetoxychavicol acetate (ACA) was identified and isolated from the rhizomes of an edible plant, *Alpinia galangal* (L.) which was tested against *Spodoptera frugiperda* (JE Smith).

The molecule was found to cause abnormalities in the larvae which affects cell proliferation and induces cell death. It is due to the expression of caspase genes which was identified through real time quantitative PCR (RT-qPCR) studies. Genomic techniques allowed the mining of the draft genome of insect pests. In *Leucinodes orbonalis* (Guenee), a large number of genes belonging to the carboxylesterase (CE) and glutathione S-transferases (GSTs) gene families with a known history of insecticide resistance in other insects were revealed. This information will facilitate the development of novel resistance management strategies against insect pests.

RNA Interference (RNAi) for Insect Pest Management

RNA interference (RNAi) or gene silencing or gene knockdown approach has opened a new avenue for protecting crops against insect pest damages. It is a sequence-specific post-transcriptional gene silencing process (destroys mRNA of a particular gene) elicited by double-stranded RNA (dsRNA) which was first studied in the nematode, *Caenorhabditis elegans* (Maupas). The use of RNAi in pest management can be made in two distinct ways which include plant transformation to express insect target gene RNAi and non-transformative strategies using formulations of sprayable RNAs as direct control agents. Through host-induced gene silencing, the gossypol resistance to *H. armigera* (Hübner) is reduced in which particular gene expression was reduced by dsRNA, which breaks larval tolerance to gossypol. Similarly, by silencing the corresponding dsRNA genes, it can be possible to increase the susceptibility of the pest to insecticides or validate the insecticide target genes.

Also, RNAi insecticide was developed by Monsanto and Dow Agrosiences, known as SmartStaxProR. This plant-incorporated protectant (PIP) will employ a pyramid strategy and reduce the chances of resistance development. A deep understanding of the interactions between insects and their viruses is of great value. The use of virus-specific dsRNA aiming to trigger the RNAi pathway against viral infections has been explored in honeybees especially in deformed wing and sacbrood viruses. Similarly, transgenic *Bombax mori* (Linnaeus) silkworms expressing virus-specific dsRNA have been reported to exhibit higher survival rates upon NPV and cytoplasmic virus infections.

In addition to the above, non-transformative strategies (feeding or topical application) using formulations of sprayables RNA as direct control agents has also been found effective against *Leptinotarsa decemlineata* (Say) and *Tribolium castaneum* (Herbst). In India, this technique has been exploited in tobacco and tomato for the development of resistance against *Bemisia tabaci* (Gennadius) and *H. armigera* (Hübner) by targeting *V-ATPase A* and chitinase (*HaCHI*) genes, respectively. But, the success of RNAi on

target insects depends upon several factors viz. target gene, nucleotide sequence, length of the dsRNA fragment, mode of delivery, the life stage of the target insects and the stability of dsRNA molecule after ingestion. However, RNAi technology has been an effective tool in functional genomics studies and will provide innovative breakthrough applications for the management of pest insects with a unique mode of action. To accelerate its practical application in crop protection, further study on dsRNA uptake mechanisms based on the knowledge of insect physiology and biochemistry is needed.

CRISPR/Cas9: A Gene Drive Approach to Tackle Insect Pests

Genome editing by CRISPR/Cas9 has been identified as a potential new biotechnological strategy for insect pest management, especially for disease vectoring insects of agricultural importance and other invasive insect species. It is faster and more efficient, the system allows precise gene changes in an organism (intentionally) by the way of insertion, deletion or replacement of gene by the use of molecular scissors and manipulating the cells repair mechanism. The feasibility of the 'gene drive' mechanism to the subsequent generations in CRISPR/Cas9 is found to be interesting which is not possible in RNA interference (RNAi) unless the dsRNA is supplied continuously.

By employing CRISPR/Cas9, it is possible to generate novel economic insect strains more rapidly and with greater efficiency than traditional breeding methods. Silencing off the genes confer resistance against insecticide, using this technique will enable to use of the same insecticides which already got resistance at the field level. The functional abnormalities can be induced on economically important insect pests. For example, *S. litura* (Fabricius), CRISPR/Cas9 mediated gene targeting on abdominal gene-A makes the abnormal segmentation more vulnerable. Besides, a new silkworm strain with a high degree of resistance to *B. mori* (Linnaeus) nuclear polyhedrovirus (*BmNPV*) infection is possible by gene knockdown. Female lethality by the introduction of embryonic lethal gene knockdown in the W chromosome is another environmentally friendly Lepidoptera pest control strategy. CRISPR/Cas9 technology has been proven

as an effective and precise tool in functional genomics studies (Rajeshwaran and Sankarganesh, 2020). Its promising effects on insect pests opened a new window for pest management. However, the knowledge about the gene drive on the ecology is not completely understood. There is a need for the systemic scientific approach to move from uncertain risks on ecology to quantifiable hazards. Further, there is a need for biosafety guidelines for this new technology, especially in developing countries.

Conclusion

The biotechnological approaches have tremendous potential for pest management and these techniques can be successfully deployed, which led to a decrease in pesticide usage and that they are environmentally benign. But, there are considerable debates about the environmental risks associated with the use of such technologies including human health risks, depletion in the gene pool and gene flow to the closely related wild relatives. However, such effects are not scientifically proven and still inconclusive. Equally important is the need to present the benefits of the biotechnological tools and products to the public and farming community in a balanced manner to achieve the maximum benefits in pest management. To exploit the enormous potential benefits of advanced techniques, appropriate biosafety regulatory frameworks and stewardship programmes are needed to be effectively implemented.

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