

Frontiers in insect control

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The role of biotechnology in insect control-both in agriculture and public health.

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Pest control programmes in agriculture and public health have relied on the use of chemicals for the past many decades all over the world. The environmental side effects of indiscriminate use of pesticides have been under scrutiny since the early 1960s when a landmark book of the 20th century, "Silent Spring", authored by Rachel Carson was published. In the last two decades, biotechnology has opened new vistas to enhance pest control that is both ecologically viable and economically sustainable, by providing tools to identify novel and improved pest control strategies.

Microbial entomopathogens (pathogens that exist in nature) such as fungi, bacteria, and viruses, are effective agents for controlling pest insects. Characterization of the genomes of these entomopathogens has facilitated the identification and deployment of candidate genes in target crops by genetic engineering. The introduction of *Bacillus thuringiensis* insecticidal protein(s) into crop plants is a major milestone in this field. Corn and cotton are the two major crops in the world in which biotechnology tools were applied to develop insect resistance. According to a published report by Brookes and Barfoot in PG Economics Ltd (2008), corn and cotton occupy 37.3 percent of the total area planted with GM crops worldwide, second only to herbicide tolerant soybeans (58%). In India, cotton is the only GM crop commercialized and occupies 66 percent of total

GM crop area in the country. The use of the insect resistant GM crops led to reductions in pesticide usage, increased farm income, improved product quality, and better human health.

Insect-resistant GM crops express insecticidal proteins which include Cry proteins, Cyt proteins and Vip proteins, of which Cry proteins are most widely used in GM crops. Cry proteins are used as a single protein (eg., Bollgard cotton, YieldGard corn, Herculex I corn) or stacked with other Cry proteins (eg., Bollgard II cotton) or as a binary proteins (eg., Herculex RW rootworm corn). All of them target insects belonging to specific groups and not all economic pests are targeted by the Bt proteins. Currently, research focus is on developing resistance to sap sucking insects, stored product insect pests, finding alternatives to Bt proteins and also use the GM crop experience in managing vectors of human diseases. Here we present a few such approaches that can be used in insect control-both in agriculture and public health.

Bt proteins have been exploited for almost a century, and more recently the knowledge about individual protein domains in Bt proteins is being utilized for engineering new functionalities. A domain from one Bt protein which improves insecticidal activity can be transferred into a second Bt protein to create a hybrid. Site-directed mutagenesis of coding regions and use of single construct to express two proteins (fusion protein) are other approaches. Domain engineering and expression of fusion protein enables us to express proteins from different sources and increases the spectrum of action.

The Bt proteins are limited in their activity against certain groups of insects including the sap-sucking insect pests, thus necessitating the discovery of novel insecticidal genes. The characterization of insecticidal proteins, called the toxin complex (Tc) proteins, from bacteria that are symbionts of entomopathogenic nematodes, has increased the arsenal for controlling a wider spectrum of insect pests. The decoding of the complete genomes and proteomes of these entomopathogenic bacteria such as *Photorhabdus* sp., *Xenorhabdus* sp and *Pseudomonas* sp. (Nat Biotechnol 24:673-9 (2006)) and comparative genomics of enteric bacteria associated with insects, such as *Yersinia enterocolitica*, and *Serratia entomophila* has resulted in the identification of Tc protein homologues, which present potential alternatives to the insecticidal proteins derived from *Bacillus thuringiensis*. The toxin complex proteins from *P. luminescens* have also been transferred into plants and tested for their activity on different groups of insect pests.

Genes derived from plants have shown promise for pest control by inducing antifeedant activity in target insects. These include proteins that inhibit insect digestive enzymes (trypsins and amylases) and sugar-binding lectins. The alpha-amylase inhibitors have been specifically effective against stored product pests such as beetles and weevils. Plant lectins derived from snowdrop and garlic and expressed in rice and tobacco, were effective against sap-sucking insects.

The insect genomic data is increasing at an exponential rate with genomes of 24 insect species being deciphered by sequencing. These species represent different classes of insects: model insects (*Drosophila* species and honey bees), medically important insects that vector serious diseases (mosquitoes transmitting malaria, yellow fever, Chikungunya and dengue fever), agriculturally important insects, subdivided into beneficial insects (honey bee-pollinator, silkworm-silk production), agricultural pests (Bollworms, Aphids, Whiteflies), and biocontrol agents (wasps). Insect genomics provides the blueprint for an improved understanding of the insect biology and behaviour and makes possible insights that could aid in finding solutions to the complex agricultural and medical challenges. As most of these data are in the public domain, access to these data and post-genomic tools will impact the innovations in pest control strategies.

Gene functional studies and target discovery by genome wide loss-of-function screening using RNA interference (RNAi) is a well-established technique in insects. Plants producing double stranded RNAs (dsRNAs) directed against specific insect genes/pathways such as V-type ATPase of corn rootworm, cytochrome P450 detoxification enzyme in cotton bollworm conferred plant protection by making the insect more sensitive to plant defense chemicals. Molecular biology and genetics have been used to develop genetically modified insect strains (RIDLs - release of insects with dominant lethals) that improve the efficiency of pest control programs such as the sterile insect technique (SIT), which was used for Screwworm eradication in the Western hemisphere. An UK based company, Oxitec,UK, has been credited with this technological innovation. Field testing is in progress for Pink bollworm control in the US and mosquito control in Malaysia.

Current advances in insect and entomopathogen genomics hold promise for developing novel and ecologically sustainable pest control solutions. These solutions are urgently needed for both food and health security in the developing world.

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