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Role of Biotechnology in Biological Control

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Introduction:

Biotechnology has been defined as any technique that uses living organisms or part of organisms to make or modify products, to improve plants or animals or to develop microorganisms for specific uses. It involves the Production, Isolation, modification and use of substances that can be produced from biosynthesis. In Pest management, it is the use of genetically modified organisms in the production of crops or animals including the production of insect suppressive agents.

Methods in Biotechnology

The main biotechnological methods that can be used to produce genetically modified organisms that are of relevance to pest control can be split into two categories, i.e.

1. Those that involve tissue culture techniques.
2. Those that involve the use of recombinant DNA technology.

The former techniques have been extensively developed in relation to the production of crop plants while the latter have been employed with plant pests and natural enemies.

Technique	Application	Example(S)
Agro bacterium-based plant transformation	Ti-plasmid used to carry novel DNA into plants	Bt insect resistant crop plants (tobacco, corn, cotton)
Particle acceleration	DNA coated gold particles fired into growing tissue	Used to produce transgenic Soybean
Electroporation	Electric current used to alter protoplast membranes permitting DNA uptake	Used to produce transgenic Rice

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Microinjection	DNA injected into the nucleus or cytoplasm of a protoplast	Used to produce transgenic Tomato

The recombinant DNA technology involves the use of genetic engineering so that the modified plants carry the functional foreign genes. These novel genes either reinforce the existing functions or add new traits to the transformed plants. These developments have provided the opportunity to develop crops with novel genes for insect resistance.

Steps Involved in Producing a Transgenic Organism

Identify Traits to Alter and Gene(S) For Insertion

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Gene Insertion

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Select Organisms or Cells with Inserted Gene

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Evaluate Gene Expression-is it Stable, is it Passed to Progeny?

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Laboratory Assays with Genetically Transformed Organisms

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Field Trials-Evaluate Risks Associated With Environmental

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Release Commercialization of Transgenic Organism

Transgenic Crop Protection:

Transgenic plant is defined as one that has had foreign genetic material purposefully introduced and stably incorporated into the plant genome through means other than those that naturally occur in the environment. In other words, transgenic plants are those which carry additional stably integrated and expressed foreign gene(s), usually transferred from unrelated organisms. Before taking up any attempt to produce transgenic plants to counter insect attack, the following requirements and priorities need to be identified.

(i) The factors for resistance should be controlled by single genes.

(ii) Standardization of methods for transfer of such genes can easily be accomplished.

(iii) Expression of transferred genes should occur in the desired tissues at the appropriate time.

(iv) The transgenic plant should be safe for consumption.

(v) Inheritance of the gene in the successive generations should be very stable.

(vi) There should be no penalty for yield in terms of other quantity

Remarkable achievements have been made in the production, characterization, and field evaluation of transgenic plants. Both Agro bacterium-mediated gene transfer and direct DNA transfer methods have been used to produce transgenic plants with new genetic properties. Genes conferring resistance to insects have been inserted into crop plants such as maize, rice, wheat, sorghum, sugarcane, cotton, potato, tobacco, broccoli, cabbage, chickpea, pigeonpea, cowpea, groundnut, tomato, brinjal and soybean.

Development of insect resistant transgenic crop cultivars has focused on two distinct approaches:

1. Integration of bacterial genes encoding for production of toxic proteins, especially from Bacillus thuringiensis (BT).
2. Integration of plant genes encoding for production of enzyme inhibitors and sugar binding lectins.

The first approach, based in particular on integration of delta endotoxin genes derived from various subspecies of Bt.

Bt Endotoxin:

Bacillus thuringiensis (Bt) is a gram positive entomocidal spore-forming bacterium. Bt synthesizes an insecticidal crystal protein (Cry) which when ingested by insect larvae is solubilized in the alkaline conditions of the midgut and processed by midgut proteases to produce a protease resistance polypeptide toxic to the insect. The crystal proteins are grouped into different classes based on sequence homology and insecticidal specificity. Bt toxins are thus, highly specific, some strains are specific to Lepidoptera, while others are specific to Diptera and Coleoptera. Bt-toxins from different strains vary widely in their spectrum of activity. The most important are those produced from a strain *kurstaki* called *Btk*, effective against lepidopteron pests of forest trees , vegetables, cotton and ornamentals. These products have been sold since the early 1960's and account for bulk of Bt applications. In the late 1970s, another strain called *Bti (I for israelensis*), was identified, the products of which are effective against Dipteran insects.

Cotton:

Considerable progress has been made in developing cotton cultivars with Bt genes for resistance. The first generation Bt cotton varieties were developed by Monsanto and their seed partners to express the Cry1Ac protein. Worldwide, several different Cry1Ac events are grown commercially. In the USA and some other Countries, the Bollgard event MON531 was used by seed company breeding programmes to develop the first commercial Bt cotton varieties. In Australia and other Countries, the Ingard event MON757 was used. The Tobacco budworm, (*Heliothis virescens)* and Pink bollworm (*Pectinophora gossypiella)* are very well controlled by Bollgard cotton whereas the cotton bollworm, *Helicoverpa zea* is controlled satisfactorily except during the bloom stage when feeding on reproductive parts of flowers which tend to have a lower concentration of cry protein than other plant parts.

Heliothis virescens

Pectinophora gossypiella

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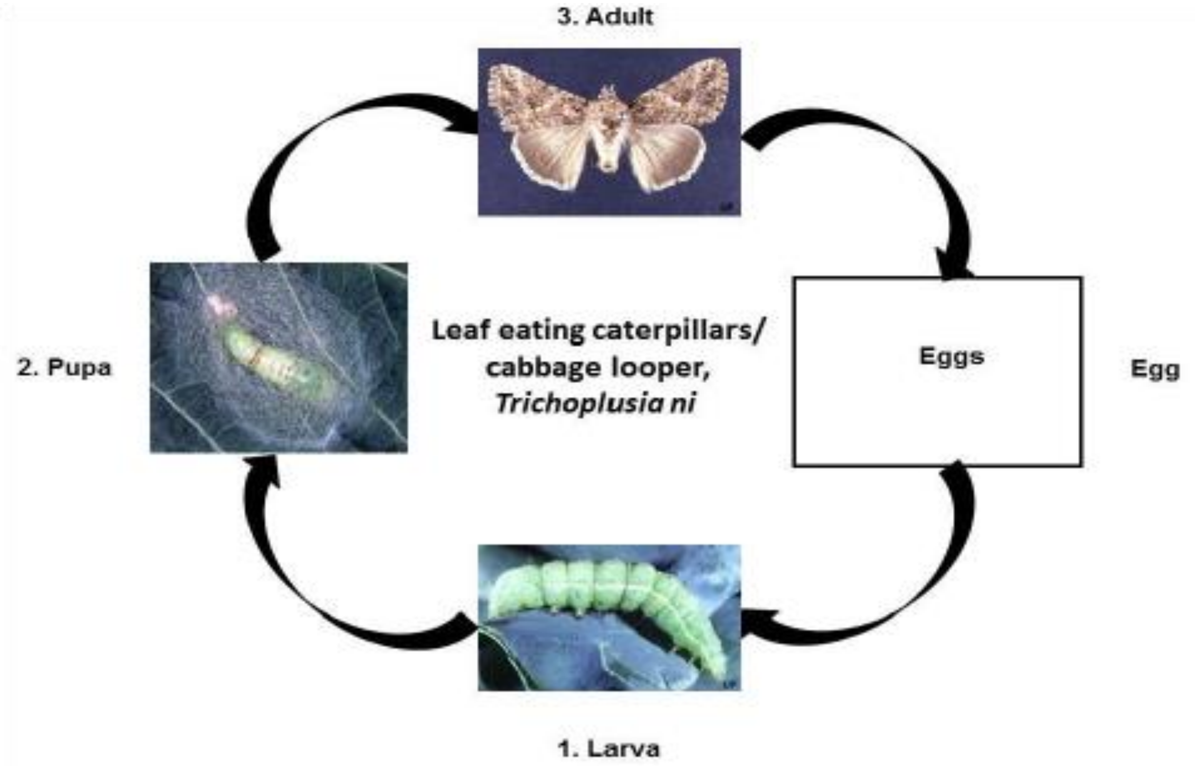
The introduction of Bt Cotton in 1996 and 1997 was very timely since *P. gossypiella* and *H. virescens* in the USA and *H. armigera* in China and Australia had become resistant to many of the conventional insecticides and Bt cotton offered a reasonable solution to the problem. Cotton cultivar Coker 312, transformed with the cry1A(c) gene (having 0.1% toxin protein),has shown high levels of resistance to cabbage looper, (*Trichoplusia ni)*, tobacco caterpillar (*Spodoptera exigua)* and cotton bollworms (*H.zea)*, (*H.virescens).*



Plant-derived Genes:

The search for insect control proteins alternative to Bt has concentrated, with a few exceptions, on those derived from plants. One major advantage of using plant genes as a source of crop resistance is their broad spectrum of activity across several orders, including sap sucking insect pests, such as Hemiptera, in addition to Lepidoptera, Coleoptera and Orthoptera. Thus far, a no. of economically important crop plants, such as oilseeds, potato, rice, sugarcane, tobacco have been genetically transformed to express various genes of plant origin. Focused largely plant transformation involving plant genes has focused on

(i) Protease inhibitors, which disrupt amino acid metabolism and interfere with the processes of digestion in insects.

(ii) Alpha-amylase inhibitors, which target carbohydrate metabolism and alpha amylases are required for the digestion of starch, a major energy source particularly for the weevils. E.g. Transgenic tobacco plants expressing amylase inhibitors from wheat (wheat alpha-amylase inhibitor) increase the mortality of lepidopteran larvae by 30 - 40 per cent.

(iii) Lectins, which cause agglutination and cell aggregation. E.g. Lectins from wheat (wheat germ agglutinin and WGA) and the Snowdrop plant (Galanthus nivalis agglutinin and GNA) rae inhibitory to the sap sucking homopteran pests such as aphids, leafhoppers and planthoppers, which feed on the phloem exudates and against which there are no known *cry* proteins.

(iv) Enzymes such as chitinase, which target insect exoskeleton. Expression of an insect chitinase in transgenic tobacco enhances resistance to some Lepidopterans.

Transgenic crops expressing insecticidal plant genes:

Transgenic crop	**Transgene(s)**	**Origin of Transgene**	**Target insect pest**
Cotton	CpTi Manduca serpin	Cowpea M. sexta	*H. armigera*
Maize	Avidin WGA	Chicken Avidin Wheat	Storage Insect Pest *Ostrinianubilalis, Diabrotica spp.*
Potato	CpTi GNA BCH OC-1 Kti3, C-ii, PI-IV	Cowpea Snowdrop Bean Chitinase Rice Soybean	*Lacanobia oleracea* *L. oleracea, Myzus persicae, Aulacorthum solani* *M. persicae, A. solani* *Leptinotarsa decemlineata* *Spodoptera littoralis*

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Rice	CpTi Pot PI-II Pin 2 GNA	Cowpea Potato Potato Snowdrop	*Chilo suppressalis, Sesamia inferens* *C. suppressalis, S. inference* *C. Suppressalis* *Nilaparvata lugens, Nephotettix virescens*
Wheat	*CMe* *GNA*	*Barley* *Snowdrop*	*Sitotroga cerealella* *Sitobion avenae*

In spite of all these advantages, biotechnology does face uncertainties:

Gene manipulation is a gray area and needs to be addressed systematically with a pragmatic approach, especially in developing countries, where most of the farmers are either poor or marginal. The resistance against delta-endotoxin is not ruled out, though it may be slow as compared to insecticides because of continuous exposure of the insect to the toxin. Transgenic plants that are resistant to pests have a selective advantage that may lead them to become weeds. It has been claimed that there are a number of risks to humans associated with eating transgenic food crops (like reduced nutritional quality leading to dietary deficiencies or health problems. If Lepidopteran herbivores were removed from plant species, other insects might experience competitive release and become more common.

Conclusions:

Biotechnology has provided new avenues for management of insect pests and it holds great potential to be included in IPM system. The low toxicity of proteinase inhibitors and Bt-delta endotoxin as compared to conventional insecticides would reduce the selection pressure and may slow down the development of resistance. Transgenic plants would also provide protection to those plant parts, which are difficult to be treated with pesticides. Thus, transgenics may prove useful for controlling bollworms and borers, which are difficult to control by means of insecticides. The cost of application in the form of equipment and labor will be nil or negative. There would be no problem of contamination in the form of drift and groundwater contamination or risk to a field worker. By using transgenic technology, the toxin is delivered directly to the target organism. The fact that the plant delivers the toxin and not a person also serves as a means to reduce operator exposure to pesticides. Insecticidal activity would be restricted to those insects which actually attack the plants. Transgenic plants would be safe to non-target species and human beings. Transgenic plants already have inbuilt resistance to various insects replacing some of the pesticides usage with protection which is intrinsically biodegradable, thus reducing the use of chemical insecticides and minimizing the problem of environmental pollution.