



Genes conferring insect resistance in crop plants

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Tools of molecular biology and genetic engineering have provided humankind with unprecedented power to manipulate and develop novel crop genotypes towards a safe and sustainable agriculture in the 21st century. The use of gene transfer technology to introduce insect-resistance genes into crop plants provides an economical and environmentally sustainable alternative to the extensive use of chemicals for the control of insect pests. Transgenic crops expressing individual *Bacillus thuringiensis* (Bt) delta -endotoxin genes have been commercially available for several years, while other insecticidal genes, notably those encoding protease inhibitors and lectins, are undergoing perimental trials.

Toxin genes from *Bacillus thuringiensis* :

Bt toxins: Bt is a gram-positive soil bacterium distinguished from other bacilli by its production of insecticidal proteins. Because of their insecticidal activity, a large number of Bt strains have been isolated and characterized. These isolates are classified into some 30 subspecies differentiated by the antigenic properties of their flagellae as well as a variety of biochemical tests. During sporulation, Bt synthesizes massive quantities of one (or) more insecticidal proteins that are organized as a crystal (or) crystals in the sporangium. These crystals are eventually released into the environment as the spores mature and the cells lyse.

Limitations :

- Lack of stability failure to penetrate too narrow specificity.
- Production is relatively expensive.
- It's application requires agricultural machinery.
- Requires repeated applications.
- Insects are exposed for limited time.

Vegetative insecticidal proteins (Vips): These proteins are produced during vegetative growth of cells and are secreted into the growth medium sequences encoding for Vip have been cloned sequenced and the protein has been expressed in *E. coli*. It has structural dissimilarity with Bt toxin structure so it has divergent insecticidal mechanism. These toxins together with Bt toxins make them ideal candidate for deployment in insect management programmes.

Toxins from predators and neuropeptides : Genes encoding neurotoxins from predatory mites and scorpion have been deployed in recombinant baculoviruses to

increase their biological activity. Insect-specific neurotoxin *AaIT* from the venom of the scorpion, *Androctonus australis* Hector has shown insecticidal activity against *H. armigera* while insecticidal peptide from spider expressed in tobacco plants has shown resistance to *H. armigera*.

Insecticidal viruses: There are many viruses pathogenic to insect pests. Genomes of small viruses can be introduced into crop plants, which will synthesize the viral particles and acquire entomocidal property. Eg. *H. armigera* stunt virus (HaSv) is a tetravirus specific to lepidopteran insects. It is harmless to beneficial insects and the environment and its deployment in transgenic plants would not pose any risks.

Genes from bacterial other than Bt: *Photorhabdus liminescens* dwells inside the gut of *entomophagous* nematodes belong to family Heterorhabditidae. These nematodes invade the insect haemocoel and release the bacteria from other gut. The bacteria proliferate and kill the host within 24-48 hr. The nematodes feed on the bacteria and host cadaver.

Insect chitinases : Chitin is insoluble structural polysaccharide that occurs in the exoskeletal and gut lining of insects which protect the insect against water loss and abrasive agents. Eg. Expression of cDNA for chitinase obtained from the tobacco hornworm, *Manduca sexta*, in tobacco plants offered partial protection against *Heliothis virescens*. The larvae feeding on transgenic plants exhibited several growth aberrations and died prematurely.

Plant originated toxic genes:

Secondary plant metabolites : Many secondary plant metabolites such as alkaloids, flavonoids, terpenoids, saponins, and non protein amino acids confer resistance to insect pests, and some of these secondary metabolites are produced in response to insect feeding (Sharma and Norris, 1991). Exogenous application of jasmonate and abscisic acid induces the production of proteinase inhibitors and results in increased resistance to insects.

Enzymes : Several enzymes expressed in transgenic plants have shown resistance to lepidopteran insects. Cholesterol oxidase from *Streptomyces* is highly toxic to cotton boll weevil (*Anthonomus grandis* Boh.) (Cho *et al.*, 1995), while bacterial isopenentenyl transferase (*ipt*) (involved in cytokinin biosynthesis) fused with proteinase inhibitor II

(*PI-IK* gene) in *Nicotiana plumbaginifolia* reduces feeding by *M. sexta* (Smigocki *et al.*, 1993) and retards the development of peach potato aphid, *M. persicae*.

Enzyme inhibitors:

Protease inhibitors: Protease inhibitors of plants are involved in plant defense against insect and nematode attack. Transgenic tobacco plants expressing soybean trypsin inhibitor (*SBTI*) result in increased mortality, reduced growth, and reduced plant damage by *H. virescens*, *H. zea*, *Spodoptera littoralis* (Biosd.) and *M. sexta* and *H. armigera* (Charity *et al.*, 1999).

Alpha-amylase inhibitors: Transgenic tobacco plants expressing alpha-amylase inhibitors from wheat (*WAAI*) increased the mortality of the lepidopteran larvae by 30 to 40 per cent and those from bean (*BAAI*) are resistant to *Colloasobruchus* spp.

Lectins: Plant lectins are a heterogenous group of sugar-binding proteins, which have a protective function against a range of organisms. A gene encoding pea lectin in tobacco plant has shown enhanced resistance to *H. virescens*. Larvae of cotton budworm fed on cotton transformed with a lectin gene have a reduced weight, but there is no effect on larval survival.

GNA-transgenic lectin crops : The insecticidal effects of GNA-tobacco were evaluated against *M. persicae*, a serious polyphagous pest worldwide and a vector of >100 disease-causing viruses. GNA-transgenic rice was developed and evaluated against the brown planthopper, *N. lugens*. Plant lectins are particularly effective against the sap sucking Hemiptera.

Plant chitinases : Effects of plant chitinases on insects seen marginal at best (eg. Slight deleterious effects of bean chitinase on aphids when fed in artificial diet) transgenic tobacco plants expressing chitinase from tobacco hornworm were protected from feeding by larvae of tobacco budworm, which decreases larval biomass and survival.

Plant metabolic enzymes : Tryptophan decarboxylase from periwinkle was expressed in tobacco where in it induced synthesis of tryptamine and tryptamine – based alkaloids. Mechanism by which tryptamine interferes with insects eg. Pupal emergence of whitefly decreased as a result of feeding on such plants.

Novel genes of plant origin : Cloning of genes from higher plants resistant to insect pests is feasible by a molecular breeding approach. Eg. Cloning of *Mi-1* gene from wild tomato has given an opportunity to control root-knot nematode and potato aphid simultaneously.

Oxidative enzymes: Polyphenol oxidase and peroxidase enzymes have the potential to oxidize components present in plant tissues into potentially toxic compounds; polyphenol

oxidase can significantly reduce the protein quality of an insect diet and thus, reduce the growth of larvae feeding on the diet.

Lipid oxidases: Two enzymes oxidize lipids, lipoxygenase and cholesterol oxidase are potential insecticidal proteins for expression in transgenic plants. Lipoxygenase oxidizes long chain unsaturated fatty acids to their peroxides and is responsible for the development of rancidity in legume flours. Lipoxygenase has been shown to be toxic to lepidopteran larval when fed in artificial diet, also toxic to BPH.

Manipulation of secondary metabolism: Secondary metabolites of plants act as most significant factors in determining host resistance, reliance on defensive proteins noted in plant genetic engineering for insect resistance. Nevertheless, genetic manipulation of secondary metabolism remains a goal for plant genetic engineers, and its exploitation to increase insect resistance in transgenic crops is inevitable.

Fusion proteins : Proteins with known insecticidal properties may be fused either to proteins that will increase their binding activity or fused to other insecticidal proteins. *Gna* (*Galanthus nivalis*) gene has been fused to a gene encoding insect hormone elatostain and to a gene encoding spider venom. Expression of single hybrid δ -endotoxin gene and SN 19 gene in potato confer resistance to CPB (Colorado Potato Beetle) and PTM (Potato Tuber Moth).

Future needs:

- Harmless to natural enemies and non-target organism
- Target the sites in insects that have developed resistance to conventional insecticides
- Very little work done to increase the food production in harsh environment of semi arid tropics.
- Use these technology to cereals, legumes and oil seed crops
- Follow biosafety regulation for food, feed and environment
- Available to farmers who cannot afford high cost of seeds and pesticides

References:

- Charity, J. A., Anderson, M. A., Bittisnich, D.J., Whitecross M. and Higgins, T.J.V. (1999).** Transgenic tobacco and peas expressing a protienase inhibitor from *Nicotiana alata* have increased resistance. *Molecular Breed.*, **5**: 357-365.
- Smigocki, A., Neal, J. W. Jr, McCanna, I. and Douglass, L. (1993).** Cytokinin mediated insect resistance in *Nicotiana* plants transformed with the *ipt* gene. *Plant Molecular Biol.*, **23**:325-335.