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DIFFERENT COMPONANTS USED IN GENE TRANSFER IN INSECT

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Objective

- To introduce a foreign gene into a plant requires a variety of plasmid DNA vectors and specific techniques to deliver the naked DNA into cells.
- Most common technique is Agrobacterium-mediated transfer that is a soil bacteria that infects plants.
- Agrobacterium has a Ti plasmid that contains a T-DNA that is transferred from the bacteria to the host plant cell.
- The plasmid also contains many virulence genes, which are required for the infection process.

Vegetative Insecticidal Proteins (VIPs)

- Supernatant of vegetative *Bacillus cereus* culture have two compounds; *VIP **1*** and *VIP 2,* which are toxic to insects.
- *VIP 3* is highly toxic to *Agrotis* and *Spodoptera.*
- The activity of these proteins is similar to δ-endotoxins of *Bt*. The acute toxicity of vegetative insecticidal proteins is in the same range as that of the δ-endotoxins from *Bt*.
- They induce gut paralysis, followed by complete lysis of the gut epithelium cells, resulting in larval mortality.
- Several transgenic events of cotton and corn are under field-testing to be deployed for pest management in the near future.

***Photorhabdus* Toxin Proteins**

- *Photorhabdus* toxins *(TcdA* gene) represent one of the alternatives to *Bt* toxins for deployment in transgenic crops.

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- Increased activity of the toxin *TcdAl* requires potentiation by either of two pairs of *TcdBl* and *TccCl* or TcdB2 and TccC3.
- The Tc toxins are complex proteins, and both toxin genes and their potentiator pairs need to be expressed to reconstitute full activity in insect-resistant transgenic plants.

Protease Inhibitors

- Many insects, particularly Lepidoptera, depend on serine proteases (trypsin, chymotrypsin, and elastase endopoteases) as their primary protein digestive enzymes.
- Genes encoding members of various serine protease inhibitors (SPIs) have adverse effects on insects, and have been cloned and introduced into transgenic plants.
- Insects also produce SPIs, which are active against, and presumably involved in regulating digestive proteases.
- Transgenic tobacco, cotton and chickpea plants expressing *SBTI, CpTi,* and *Allocasia* GTPI resulted in reduced growth of insects.

α-Amylase Inhibitors

- Insect α-amylase (α-l,4-glucan-4-glucanohydrolases, EC 3.2.1.1) constitutes a family of endo-amylases that catalyze the hydrolysis of α-D-(l α 4)-glucan linkages in starch components, glycogen, and other carbohydrates.
- Amylases play a key role in carbohydrate metabolism of microorganisms, plants, and animals.
- Transgenic tobacco plants expressing α-amylase inhibitors from wheat (WAAI) increased the mortality of the lepidopteran larvae by 30 to 40%.
- Pea expressing α-amylase inhibitor genes from *Phaseolus vulgaris* (AI1) are resistant to the bruchids.

Enzymes

- Transgenic expression of certain enzymes has been suggested as another alternative to *Bt* genes.
- Transgenic tobacco plants expressing chitinase have shown increased resistance to lepidopteran insects.

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- Polyphenol oxidases and peroxidases increase the inhibitory effect of 5CQA (5-caffeoyl quinic acid) and cholorogenic acid.
- Cholesterol oxidase and isopentyl transferase expressed in transgenic plants have also shown resistance to lepidopteran insects.

Plant Lectins

- Plant lectins are a heterogenous group of sugar binding proteins, and produce chronic effects on survival and development of insect pests.
- Lectins from snowdrop, pea, soybean, mungbean and garlic have been isolated and bio-assayed against insects.
- Greater insecticidal activity has been observed in chitin binding lectins and the lectin genes of wheat germ and common bean.
- Transgenic tobacco plants expressing pea lectin have shown adverse effects against *Heliothis virescens*.

Neurotoxins

- Spiders and scorpions produce powerful neurotoxins that have, been expressed in transgenic organisms.
- Gene expressing the venom of scorpion, ants, and the spider, *Androctonus australis* has been closed.
- Transgenic plants of tobacco have been obtained containing an insecticidal spider peptide gene and some of these plants have shown resistance to *Helicoverpa armigera*.
- The role of neurotoxins from insects and spiders needs to be studied in greater detail before they are deployed in other organisms and plants because of their possible toxicity to mammals.

Baculoviruses

- Enhancin genes from *Trichoplusia ni* or *H. armigera* baculoviruses introduced into tobacco plants resulted in slower development and increased larval mortality on some transgenic lines.
- A recombinant *Autographa californica* AcNPV with the enhancin gene from *T. ni* granulovirus, *AcEnh26* expressed in tobacco plants showed a 10-fold increase in AcNPV infection.

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- Long-term feeding of transgenic tobacco material with the enhancin gene resulted in adverse effects on the larvae of *Mythimna separata* and *Spodoptera exigua*, and has potential for use in pest management.

Neuropeptides and Peptidic Hormones

- Neuropeptides and small peptidic hormones regulate several physiological processes in insects and are active at very low concentrations.
- There are possibilities for deploying these molecules through transgenic plants to disrupt physiological processes of insects.
- Tobacco budworm, *H. virescens* larvae fed on leaves from the transgenic plants showed a reduced growth rate compared to those fed with control plants.

Biotin-binding Proteins

- Biotin-binding proteins (BBPs), such as avidin and streptavidin represent potent insect control compounds, which could be delivered via transgenic plants.
- Black field cricket nymphs, *Telcogryltus commodus* had significantly reduced growth and survival when fed on lettuce leaves painted with purified avidin.
- Transgenic tobacco and apple plants expressing avidin and streptavidin conferred a high level of insect resistance in transformed tobacco plants to potato tuber moth, *Phthorimoea operculetla*, and in apple plants to light brown apple moth, *Epiphyas postuittana*
- Efficacy and stability of transgene
- Development of resistance and resistance management.