A Review :

Transgenic crops for insect-pests management

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International Journal of Plant Protection, Vol. 4 No. 2 (October, 2011) : 406-410

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SUMMARY

The indiscriminate use of chemical insecticides create many problems *i.e.* environmental pollution, health hazards, insecticides residues found in agriculture products and their commodities as well as killing of beneficial bio-agents and development of resistance in insects. These problems can be solved by the use of transgenic crop plants. The transgenic plants are made through genetic engineering by means of transferring gene not only from the similar plant species but also from different relatives including non plant species. These are derived from different sources *i.e.* microorganisms, plants and insects. The transgenic crop plants are able to reduce the consumption of synthetic chemical pesticides and thus they protect the environment.

Singh, Arun Kumar and Lal, M.N. (2011). Transgenic crops for insect-pests management. *Internat. J. Plant Protec.,* **4**(2): 406-410.

Key words : Transgenic crops, Insect pests, Management, Genetic engineering, Bio-agents

Received : *June, 2011*
Accepted : *September, 2011*

Insects have emerged as a major yield-reducing factor for field and horticulture crops. Losses caused by crop pests are greater in the tropics and semi-tropics, which provide ideal conditions for the multiplication and development of insect pests.

Some insects play an important role in maintaining the balance of nature, some feed on plants and animals. Many of them are scavengers and convert the dead plant and animals' tissue into humus and in enrich the soil. Many species of insects live both as parasites and predators on insect pests of crops and help in suppressing their numbers. They also play an important role in increasing the yield of crops.

The transgenic crops are major tool of pest management and play an important role in maintaining the ecological balance and safer crop production.

Genetic engineering and crop pests:

Genetic engineering is highly specialized branch of biotechnology and usually refers to copying a gene for one living organism (plant, animal or microbe) and transfer to another living organism. Genetic engineering is more powerful than conventional plant breeding. In plant breeding we can transfer gene between close by related species but through genetic engineering, we can transfer the gene not only between the similar plant species but also from different relatives, including non-plant species (Akram *et al.,* 2003).

Sources of transgene preparation for resistant variety against insect:

Microorganism:
- *Bacillus thuringiensis* (Bt. gene)
- Other bacteria

Plant:
- Protein inhibitor (serine, cysteine)
- αAmylase inhibitor
- Plant lecteins

Insect:
- Insect chitinases

Transgene derived from *Bacillus thuringiensis:*

Bt is gram-positive, aerobic, sporulating and soil borne bacterium, which synthesizes crystalline proteins during sporulation. These crystalline proteins are highly insecticidal at very low concentrations (Schnepf *et al.*,1998). These proteins are non-toxic to mammals and other organisms. Bt strains and their insecticidal crystal protein have acquired acceptability as eco-friendly biopesticides all over the world. Bt. strains and insecticidal crystal protein (ICPs) were first found to affect a range of lepidopteran insects, which are recognized worldwide as major agricultural pests on crops. Subsequently discovery of new strain expanded host-range strains are now available which are toxic to coleopterans, dipterans, lice, mites and even nematodes (Kumar *et al.,* 1996). Most families of lepidopatera including species are susceptible to the cry I and cry II crystal protein.

Bt toxins and their classification:

Bt. strains produce two types of toxin. The first type is cry (crystal) toxin encoded by different cry genes. The second type is the cyt (cytolytic) toxin, which can augment the cry toxins enhancing the effectiveness of insect control.

On the basis of homology of the amino acid sequences and spectrum of insecticidal activity, insecticidal crystal protein (ICPs):

Major group of cry gene:

1. Cry I, 2. Cry II, 3. Cry III, 4. Cry IV

Only cyt A which is other ICP cyto toxin is different from other protein of these Cry I and Cry II. Insecticidal crystal protein (ICPs) are effective against certain lepidopteron and 100 dipterans larva, the Cry II insecticidal crystal protein, (ICPs), are effective against certain coleopteron and Cry IV are effective against dipterons, Cry I and Cry II are most common toxins only a few sub species of Bt produce toxin for other insect groups.

Mode of action:

The mechanism of action of the Bt. (ICPs) has been worked out in some details (Kumar *et al.,* 1996). The molecular structure of at least three different insecticidal crystal proteins (ICPs) has been studied (Schnepf *et al.,* 1998). The crystals upon ingestion by the insect larvae are solubilized in the highly alkaline midgut into individual protoxin that binds to the receptor of the mid gut epithelium, gut paralyses and the mid gut disintegrates leading to asmotic imbalance within three hours of ingestion. Larva stops feeding and dies in 1-2 days due to septicemia as spore and gut bacteria proliferate in its blood resulting in stop of first inter larva and thus further feeding damage to the crop is prevented.

Expression of cry genes in plant cell:

The levels of cry proteins expression in the plant tissues were not very high. The modified cry genes (cry IAb. and cry IAc) for better expression in plant cells (Perlak *et al.,* 1991) the codan usage of prokaryotic genes of Bt was altered to resemble that of higher plants in addition many features like presence of putative poly a type signals and splice sites which destabilize. Bt. mRNAs in plant cell were removed without altering the amino acid sequence of the insecticide crystal protein (ICPs).

Some crop plants like rice, maize, peanut, soybean, tomato and cabbage were transformed with various modified cry genes (De Maagad *et al.,* 1999). The native gene (Cry IIA5) expression resulting in significant resistance to *H. armigera* in transgenic tobacco (Selvapandiyan *et al.,* 1998)

Cry I Ac gene into the chloroplast genome of tobacco which expressed the cry protein to a very high level (3-5% of leaf soluble protein) (Mc Bride *et. al.,* 1995). Their range of activity can be further extended by protein engineering (Fang *et al.*, 2007). Commercial transgenic

Table 1 : Cry gene resistant against some insects

Crops	Gene	Target pests
Cotton	Cry 1Ab/Cry1Ac	Bollworms
Corn	Cry1Ab	European corn borer
Potato	Cry IIIa	Colorado potato beetle
Rice	Cry 1Ab/Cry1Ac	Stem borers and leaf folders
Tomato	Cry 1Ac	Fruit borers
Potato	Cry1Ab	Tuber moth
Egg plant	Cry1Ab/Cry1B	Shoot and fruit borer
Anola	Cry1Ac	Diamond back moth
Soyabean	Cry1Ac	Soyabean looper
Corn	Cry1H/Cry9c	European corn borer

Table 2 : Transgence derived from plant sources for development against same insect resistant variety

Crops	Gene	Target pests
Tobacco	Cowpea serine P1	Tobacco bud worm
Tobacco	Potato serine P1	Tobacco horn worm
Rice	Cowpea serine P1	Stemborer
Potato	Cowpea serine P1	Lacanobia
Potato	Oryzacystain	Potato beetle
Tobacco	Hornworm P1	Whitefly
Pea	Bean α-AI	Bruchids
Potato	Showdrop lectin	Potato aphid
Rice	Snowdrop lectin	Brown plant hopper

Insect Chitinase

plants expressing these proteins are under development (Christou *et al*., 2006).

Chloroplast transformation besides providing highly foreign proteine expression also ensure maternal transmission of the foreign and therefore avoiding the spread of transgene through pollen as of now more than 30 plant species have been transformed with Bt. cry genes (Schuler *et al.,* 1998), the cryAc gene in rice (1R.64) for resistant to yellow stem borer (Nayak *et al.*, 1997). Vegetable crops such as brinjal and tomato were transformed by synthetic/modified cryIAb and CryIAc gene, respectively to confer resistance against fruit borers (Kumar *et al.,* 1998).

Transgene derived from plant:

Protein inhibiters:

Plants have a wide array of defiance protein including protinaceus protinase inhibitor and lactin induced in response to insect attack (Ryan *et al.,* 1990). They were first shown as plant defence protein in 1972 when the induction of PIs in potato and tomato was observed due to wounding and insect herbivory (Ryan *et al.,* 1990).

Protein inhibitors are divided into two groups:

Serine proteas inhibitors:

The first gene of plant origin to be transferred successfully to another plant spp. resulting in enhanced insect resistance was isolated from cowpea encoding a double-headed trypsin inhibiter search for resistant genotype of cowpea bruchid beetle.

Gatehouse *et al.* (1979) established that resistance to *C.macultatus* in TVu 2027 was associated with higher level of brown-brik type of protease inhibitors called cowpea trypsine inhibitors which showed that a complex proteolytic system consisting of serine protiniases and leucine amino peptides in the insect gut. Confers a high level of resistance to oryzacysta I and brown brik inhibitor in beetle larva.

Mode of action:

Mode of action of Cpti is to inhibit essential digestive protease resulting in abnormal development and death due to deficiency of essential amino acids. Although Cpti inhibits mammalian trypsin which is not toxic to mammals. Cpti is degraded by pepsin in the acidic conditions of stomach before it incounters the serine protease of the small intestine in the mammalian gut.

Cystein protease inhibitors:

Two types of proteins in cereal grain inhibit insect digestive enzymes and may play roles in preventing insect and microbial attack in cereal grain. A cysteine protease inhibit oryzacystain from rice has been isolated that inhibitors nearly all the proteolytic activity in the rice weevil and beetle midgut. A cc gene (corncystatine) was introduced into protoplast of rice and the cystatine activity of the transgenic rice plant was assessed against a crude midgut proteinase fraction from *Sitophilus zeamais.*

Plant lectins:

Lectins are a group of plant proteins that bind to carbohydrates. Including chitin. Some of these have shown to provide protection against insect attack on seed of common bean (*P. vulgaris)* contain a carbohydrate-binding lectine proteine called phytohemagglutine (PHA).

A lectin from snowdrop (*Galanthus nivalis*) when expressed in transgenic tobacco and potato has been found to be toxic to aphid (Hilder, 1995) and the tomato moth (*Lacanobia oleracea*) Gatehouse *et al.* (1997). Foissac *et al.* (2000) have expressed the showdrop lactin in transgenic rice. Transgenic plant showed resistance against brown plant hopper (*Nephatettix virescens*). Wheat germ agglutinin lea lactin jacaline and rice lactin have been expressed in plant like tobacco, maize and potato mainly against aphid (Schuler *et al.,* 1998).

Mode of action:

The lectin binds to glycoproteins in the peritrophic matrix lining the insect midgut to disrupt digestive processes and nutrient assimilation which causes insect death.

aAmylase inhibitors:

The common bean (*Phaseolus vulgaris*) contains seed protein called phytohemagglutinine, arceline and a amylase inhibitors. The a amylase inhibitors form a complex with certain insect amylases and is supposed to play a role in plant defence against insect.

The introduction and expression of the bean a amylase inhibitors gene in pea confers resistance to the bruchid beetles (Shade *et al.,* 1994)

Transgenic Azuki bean carrying a AI gene was resistant to three species for Bruchids Ishimoto *et al.* (1996) introduced a AI gene in an Indian genotype of chickpea (C-235) and derived significant protection against Bruchids.

Insect chitinase:

Chitin is an insoluble structural polysaccharide that occurs in the exoskeletan and gut lining of insect. It is believed to protect the insect against water loss and abrasive agents because of critical function of chitine. It has been considered as a potential target for insecticidal proteins, expression of proteins which will interface with chitin metabolism likely to have a serious effect on the growth of moulting insects. In this aspect chitinase produced by insect, themselves has been used as on insecticidal protein.

Expression of cDNA for chitinase has been obtained from the tobacco hornwom. *Manduca sexta* in tobacco plants offered partial protection against *Heliothes virescens* (Frutos *et al.,* 1999). The larva feeding on the aberration prematurely.

Plant metabolic enzymes:

Tryptophane decarboxylase from periwinkle was expressed in tobacco where it induced synthesis of Tryptamine and Trytamine-based alkaloids (Schuler *et al.,*1998) Pupal emergence of white fly decreased as a result of feeding on such plant and other enzyme such no ployphenol oxidase and lipoxygenase have been shown to be toxic to insects. (Schuler *et al.,* 1998)

Insecticidal viruses:

Genome of small viruses can be introduced into crop plant which will synthesize the viral particles acquire entomocidal property for instance, *H armigera* stunt virus *(HaSv)* is a tetravirus specific to lepidoptera.

Insect is very remotely related to virus of plant and animals. *HaSv* is harmless to beneficial insects and the environment and its deployment in transgenic plants would not pose any risk (Gordon *et al.,* 1995).

Advantage of transgenic plants in IPM:

Transgenic crop has provided new avenues for management of insect pests and it holds great potential to be include in IPM system.

– The low toxicity of proteinase inhibitors and Bt-¶-endotoxin as compared to conventional insecticides would reduce the selection pressure and may slow down the development of resistance.

– Since all plant parts including growing points would remain covered with toxins, depend on weather for efficacy of the sprays would be eliminated

– Since toxins will always be there, so there will be no need of continuous monitoring of pests.

– 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 labour will be negative.

– The development cost is only a fraction of the cost of development of a conventional pesticides.

– There would be no problem of contamination in the form of drift and ground water contamination or risk to the field workers.

– 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 eliminate the problems of shell life and field stability faced by pesticide by formulations as they provide on site biosynthesis of the toxins.

– Transgenic plants will have inbuilt resistance to various insects replacing some of the current pesticide usage with protection which is intrinsically biodegradable, thus reducing the use of chemical inspections and minimizing the problem of environmental pollution.

Draw backs of transgenic plants:

– The resistance against ∂-endotoxin is not ruled out, though it may be slow as compared to insecticides because of continuous exposure or the insect to the toxin.

– Some weeds may introgress an insect resistance gene from related transgenic plants and make them less susceptible to their usual herbivores, thus exhibiting greater reproductive success and may create a weed problem.

– If lepidopteran herbivores were removed from plant species, other insects might experience competitive release and become more common.

– The use of transgenic plants possessing resistance factor in one locality may affect the insect population dynamics in other areas.

– Although Lepidoptera are herbivores as larvae, many are important pollinators of wild species as adults. Therefore, if larval mortality is high, plant communities might also be disrupted by decreasing availability of pollinators.

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