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REVIEW ARTICLE

Prospects for using transgenic resistance to insects in crop improvement

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Integrated pest management has historically placed great hopes on host plant resistance. However, conventional host-plant resistance to insects involves quantitative traits at several loci. As a result, the progress has been slow and difficult to achieve. With the advent of genetic transformation techniques, it has become possible to clone and insert genes into the crop plants to confer resistance to insect pests. Resistance to insects has been demonstrated in transgenic plants expressing genes for d-endotoxins from *Bacillus thuringiensis* (Bt), protease inhibitors, enzymes and plant lectins. Most of the plant derived genes produce chronic rather than toxic effects and some insect pests are not sensitive to some of these factors. The potential of plant derived genes can be realised by deploying them in combination with host plant resistance and exotic genes. Genes conferring resistance to insects have been inserted into crop plants such as maize, cotton, potato, tobacco, potatoes, rice, broccoli, lettuce, walnuts, apples, alfalfa and soybean. Genetically transformed crops with Bt genes have been deployed for cultivation in USA, China and Australia. However, very little has been done to use this technology for improving crop production in the harsh environments of the tropics, where the need for increasing food production is most urgent. International agricultural research centres, advanced research institutes and the seed sector should make an effort to use these new tools for increasing food production in poorer regions of the world. There is an urgent need to develop a scientifically sound strategy to deploy exotic and plant derived genes for minimising the extent of losses caused by insect pests. Equally important is the need for following the biosafety regulations, more responsible public debate, social attitude and better presentation of the benefits for a rational deployment of the genetically transformed plants.

There is a continuing need to increase food production, particularly in the developing countries of Asia, Africa and Latin America. And this increase has to come from increased yields from major crops grown on existing cultivable lands. One practical means of achieving greater yields is to minimise the pest associated losses, which are estimated at 14% of the total agricultural production: 52% in wheat, 83% in rice, 59% in maize, 74% in potato, 58% in soybean and 84% in cotton (Oerke et al. 1994). Insects not only cause direct loss to the agricultural produce, but also indirectly due to their role as vectors of various plant pathogens. In addition to direct losses caused by insects, there are additional costs in the form of pesticides applied for pest control, currently valued at US $10 billion annually. In crops such as pearl millet, sorghum, pigeonpea, chickpea and groundnut grown under subsistence farming conditions in the semi-arid tropics, the losses due to various

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biotic and abiotic factors have been estimated to be over US $ 2 billion annually (ICRISAT, 1992). Massive application of pesticides results in adverse effects on the beneficial organisms, leaves pesticide residues in the food and results in environmental pollution. As a result, the chemical control of pests is under increasing pressure. Pesticide use in the world is declining, largely due to major reduction in Europe as a result of regulatory mechanisms, environmental activism and public pressure. This has necessitated the use of target specific compounds with low persistence, and an increase in emphasis on integrated pest management based on host plant resistance to insect pests. Although the benefits to agriculture from the pesticide use to prevent insect associated losses cannot be overlooked, but there is a greater need to develop alternative or additional technologies, which would allow a rational use of pesticides, and provide adequate crop protection for sustainable food, feed and fibber production in the future.

Integrated pest management (IPM) has historically placed great hopes on host plant resistance. However, conventional host-plant resistance to insects involves quantitative traits at several loci, and as a result, the progress has been slow and difficult to achieve. With the advent of genetic transformation techniques based on recombinant DNA technology, it is now possible to insert genes into the plant genome that confer resistance to insects (Bennett, 1994). Genes from bacteria such as *Bacillus thuringiensis* (Bt) and *Bacillus sphaericus* (Gill et al. 1992; Charles et al. 1996) have been the most successful group of organisms identified for use in genetic transformation of crops for pest control on a commercial scale. Protease inhibitors, plant lectins, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins from Bt and related species, and small RNA viruses can also be used alone or in combination with Bt genes to generate transgenic plants for pest control (Hilder and Boulter, 1999). The search for alternatives to Bt has concentrated, with a few exceptions, on genes derived from the plants.

During the course of evolution, plants have developed effective counter measures to withstand the herbivores. Many classes of plant proteins and secondary plant substances have been shown to have toxic or antimetabolic effect on insects and have been proposed as possible candidates for genetic engineering. A common feature of many of these compounds is that they have a chronic rather than an acute toxicity on insects and their effects are less dramatic than those of the synthetic insecticides. Transgenic plants rarely result in 100% control, but tend to retard insect development and growth and development (Estruch et al. 1997). Plant derived genes attack different sites in insects than the synthetic chemicals, and may be deployed in combination with exotic genes and insecticides. Retardation of insect development, slower rate of insect population growth and reduced fitness of the surviving insects would allow a much wider window within which intervention with insecticides can be successfully employed. This will help to generate greater confidence in the IPM by the farmers, who normally prefer complete insect control based on chemical pesticides.

At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), several candidate genes are being evaluated for their biological efficacy against the sorghum shoot fly (*Atherigona soccata)*, spotted stem borer (*Chilo partellus)*, tobacco caterpillar (*Spodoptera litura*) and cotton bollworm or legume pod borer (*Helicoverpa armigera),* which are major crop pests in the semi-arid tropics. The need for genetic transformation of crops to improve crop production in the developing world has been discussed by Ortiz (1998), Sharma and Ortiz (2000) and Ortiz et al. (2000). In this manuscript, information on candidate genes that can be used for genetic transformation of crops has been reviewed to evaluate their potential for genetic improvement of crops for pest management.

Genetic engineering of crop plants

Recombinant DNA technology offers the possibility of developing entirely new biological insecticides that retain the advantages of classical biological control agents, but have fewer of their drawbacks. However, commercial considerations have placed this technology beyond the reach of poorer sections of society, and has generated considerable public debate about its usefulness, effects on the nontarget organisms and the environment, thus preventing the use of an additional tool for increasing the production and productivity of crops. In addition to widening the pool of useful genes, genetic engineering also allows the use of several desirable genes in a single event and reduces the time to introgress novel genes into elite background. Biotechnology has provided several unique opportunities that include:

- access to novel molecules,
- ability to change the level of gene expression,
- capability to change the expression pattern of genes, and
- develop transgenics with different insecticidal genes.

However, transgenics are not a panacea for solving all the pest problems. There are some genuine or perceived concerns. The major limitations of transgenic plants are:

- secondary pests are not controlled in the absence of sprays for the major pests,
- need to control the secondary pests through chemical sprays will kill the natural enemies and thus offset one of the advantages of transgenics,
- cost of producing and deployment of transgenics may be very high,
- proximity to sprayed fields will reduce the benefits of transgenics,
- insect migration may reduce the effectiveness of transgenics, and

- development of resistance in insect populations may limit the usefulness of transgenics.

Therefore, efficient deployment and management of transgenic plants in an effective manner will be an important prerequisite for sustainable use of biotechnology for crop improvement.

As a result of advances in genetic transformation and gene expression during the last decade (Bevan et al. 1983; Horsch et al. 1984), there has been a rapid progress in using genetic engineering for crop improvement, of which protection of crops against the insects is a major goal. The potential of this technology has now been widely recognised (Burke and Thomas, 1997). The basic requirements for genetic transformation are:

- a target genome,
- a candidate gene,
- a vector to carry the gene,
- tissue culture and regeneration system,
- modification of the foreign DNA to increase the level of gene expression,
- method to deliver the plasmid DNA into the cell,
- protocols to identify the transformed cell, and
- characterisation of the putative transgenic plants at the molecular and genetic levels.

Once efficient protocols for tissue culture and transformation are developed, the production of transgenic plants with different genes is fairly routine (Sharma and Ortiz, 2000). Such protocols have been reported for several crops in the past. While most of the insect resistant transgenic plants have been developed by using Bt δ-endotoxins, many studies are underway to use non-Bt genes for pest control. A number of such genes interfere with the nutritional requirements of the insects. Such genes include protease inhibitors, chitinases, secondary plant metabolites and lectins. The effective dosages for the plant-derived genes are much higher than those of the Bt toxins.

The first Bt toxin gene was cloned in 1981 and the first transgenic plants were produced by mid-1980s. Since then, several crop species have been genetically engineered to produce Bt toxins to control the target insect pests. Genes conferring resistance to insects have been inserted into crop plants such as maize, cotton, potato, tobacco, rice, broccoli, lettuce, walnuts, apples, alfalfa and soybean (Bennett, 1994; Federici, 1998; Griffiths, 1998). The first transgenic crop was grown in 1994 and large-scale cultivation was taken up in 1996 in USA (McLaren, 1998). Since then, there has been a rapid growth in the area under transgenic crops in USA, Australia and China. Transgenic plants with insecticidal genes are set to feature prominently in pest management in both developed and the developing world in future. Among the developing countries; China, India, Argentina, Mexico, Brazil, Pakistan and South Africa are pursuing the research on transgenic crops vigorously. Entomologists, breeders and the molecular biologists need to determine how to deploy this technology for pest management, and at the same time avoid or reduce possible environmental risks. To achieve these objectives, it is necessary to have an appropriate understanding of the insect biology, behaviour, its response to the insecticidal proteins, temporal and spatial expression of insecticidal proteins in the plants, strategy for resistance management, impact of insecticidal proteins on natural enemies and non-target organisms. Equally important are the issues concerning the transfer of technology to the resource poor farmers. Development and deployment of transgenic plants with insecticidal genes for pest control will lead to:

- reduction in insecticide sprays,
- increased activity of natural enemies, and
- IPM of secondary pests.

Novel genes for genetic transformation of crop plants for resistance to insects

Bacillus thuringiensis toxin genes.

Ishiwata discovered this bacterium in 1901 from diseased silkworm (*Bombyx mori*) larvae. Berliner (1915) isolated it from diseased larvae of *Ephetia kuhniella* and designated it as *Bacillus thuringiensis*. Further research on Bt by Steinhaus (1951) lead to renewed interest in biopesticides, and as a result, the more potent products such as Thuricideâ and Dipelâ were introduced. *Bacillus thuringiensis* is a gram-positive bacterium, which produces proteinaceuos crystalline inclusion bodies during sporulation. There are several subspecies of this bacterium, which are effective against lepidopteran, dipteran and coleopteran insects. Formulations based on Bt occupy the key position, accounting for nearly 90% of the total biopesticide sales (Neale, 1997). It has been used in the field for the past 40 years. *Bacillus thuringiensis* is the most important biological insecticide with annual sales of nearly US $90 million (Lambert and Peferoen, 1992) and there are 67 registered Bt products with more than 450 formulations (Shewry and Gutteridge, 1992). *Bacillus israeliensis* has been used extensively for the control of mosquitoes (Barjac de and Sotherland, 1990).

Bacillus thuringiensis var. *morrisoni* and *B. israelensis* carry four genes that encode mosquito and blackfly toxins Cry IVA, Cry IVB, Cry IVC and Cry IVD (Bechtel and Bulla, 1976). Bt also produces cyto-toxins that synergise the activity of Cry toxins. The identification of the *kurstaki* strain provided a boost for commercialisation of Bt. The HD 1 strain identified by Dulmage (1981) is the most important Bt product in the market. The problems associated with specificity, shelf life, potency and presence of viable spores have been overcome by using modern tools in microbiology and genetic engineering. Genes encoding for the δ-endotoxins have been cloned since 1980s (Schnepf and Whitley, 1981), and expression of first introduced genes in tobacco and tomato provided the first

example of genetically modified plants with resistance to insects (Barton et al. 1987; Vaeck et al. 1987).

Mode of action

Because of the crystalline nature of these proteins, the term Cry is used in gene and protein nomenclature. The toxin genes earlier were classified into four types, based on insect specificity and sequence homology (Hoftey and Whiteley, 1989). Cry I type genes encode proteins of 130 kDa, and are usually specific to lepidopteran larvae, type II genes encode for 70 kDa proteins that are specific to lepidopteran and dipteran larvae, and type III genes encode for 70 kDa proteins specific to coleopteran larvae. Type IV genes are specific to the dipteran larvae. The system was further extended to include type V genes that encode for proteins effective against lepidopteran and coleopteran larvae (Tailor et al. 1992). The Bt δ–endotoxins are now known to constitute a family of related proteins for which 140 genes have been described (Crickmore et al. 1998), with specificities for Lepidoptera, Coleoptera and Diptera. The crystalline proteins get solubilised in midgut at high pH, releasing proteins called δ-endotoxins. The toxin portion is derived from the N-terminal half of the protoxin, while the C-terminal portion is involved in the formation of parasporal inclusion bodies and is usually hydrolysed into small peptides (Choma et al. 1990). The region within the N-terminal toxin domain (amino acids 1 to 279) is composed of α–helices, which are considered important in penetrating the peritrophic membrane. At least six α–helices can be identified in most Cry toxins. Based on the crystal structure of Cry IIA, the other proteins may adopt the same folding scheme with a central hydrophobic helix (helix 5) surrounded by six amphipathic helices (Li et al. 1991). Reducing the hydrophobicity of these regions can reduce the toxicity (Wu and Aronson 1990). The C-terminal region (amino acids 461 – 695) and the highly variable region (amino acids 280 – 460) are considered important in toxin specificity by coding for open β–sheets that bind to glycoprotein receptors in the midgut.

The main target for Bt toxins is insect midgut (Knowels, 1994). The crystalline protoxins are inactive, until they are solubilized by the gut proteases (Tojo and Aijawa, 1983; Gill et al. 1992; Milne and Kaplan, 1993), which cleave nearly 500 amino acids from the C-terminus of 130 kDa protoxin and 28 amino acids from the N-terminus, leaving 55 to 65 kDa protease resistant active core comprising the N-terminal half of the protoxin (Hoftey and Whitley, 1989). The Cry 1A toxin is cleaved at the amino terminal R2 arginine residue (Nagamatsu et al. 1984), and the carboxyl terminal K lysine residue (Bietlot et al. 1989). A heterogenous DNA fragment of 20 kilobase is involved in the proteolytic processing of the protoxin (Bietlot, et al. 1993). The 70 kDa Cry II, Cry III and Cry IVD proteins are naturally occurring truncated forms. Brush border membrane vesicles (BBMV) have been identified as the primary binding site for several insect species (Lee et al. 1992). The active toxins bind to the specific receptors located on the apical brush border membrane of the columnar cells. There may be many toxin binding protein receptors, and some have been identified as 12 to 180 kDa glycoproteins (Garczynski et al. 1991; Knowles et al. 1991; Oddou et al. 1991). A 210 kDa membrane protein is the receptor in *Manduca sexta* for Cry 1A(b) toxin (Vadlamudi et al. 1995), and a 120 kDa aminopeptidase N is the receptor for Cry 1A(c) toxin (Knight et al. 1994). Cry 1A(c) binding amino peptidase in *Manduca sexta* has a glycosyl phosphatidylinositol anchor (Garczynski and Adang, 1995). After binding to the receptor, the toxin inserts irreversibly into the plasma membrane of the cell leading to lesion formation. There is a positive correlation between toxin activity and ability to bind BBMV (Gill et al. 1992), and the toxicity is correlated with receptor number rather than receptor affinity (Van Rie et al. 1989). The toxicity of Bt lies in the organisation of α–helices derived from domain I. After binding to the midgut epithelial cells, the α–helices can penetrate the apical membrane to form an ion channel (Knowels and Dow, 1993). The formation of toxin induced pores in the columnar cell apical membrane allows rapid fluxes of ions. The pores are K^+ selective (Sacchi et al. 1986), permeable to cations (Wolfersberger, 1989), permeable to anions (Hendrickx et al. 1989), or permeable to solutes such as sucrose, irrespective of the charge (Schwartz et al. 1991). Carroll and Ellar (1993) observed that midgut permeability in the presence of Cry 1A(c) was altered for cations, anions, neutral solutes and water. Knowles and Dow (1993) suggested that Bt toxins lead to cessation of K^+ pump that leads to swelling of columnar cells and osmotic lysis. The disruption of gut integrity leads to death of the insect through starvation or septicaemia. These pores possess both selective (only K^+ passes through) and nonselective (Na^+ and anions pass through) properties depending on the pH (Schwartz et al. 1993). The lepidopteran insect midgut is alkaline and the pores probably permit K^+ leakage. Formation of this cation selective channel destroys the membrane potentials (English and Slatin, 1992) resulting in midgut necrosis, degeneration of peritrophic membrane and epithelium and ultimately bacterial septicemia, which occurs after larval death due to toxins (Sneh and Schuster, 1981; Salama and Sharaby, 1985).

Differences in the extent of solubilization of different toxins may explain the differences in the toxicity of various proteins (Meenakshisundaram and Gujar, 1998). Decreased solubility could be one potential mechanism for insect resistance to Bt proteins (McGaughey and Whalon, 1992). In cotton bollworm (*Helicoverpa zea*), Cry IIA is less soluble than Cry 1A(c) and fails to bind to a saturable binding component in the midgut brush border membrane (English et al. 1994). The unique mode of action of Cry IIA may provide a useful tool for management of resistance to Bt toxins. Although binding of the Cry toxins to the receptors determines the species sensitivity to various toxins, there are distinct exceptions, e.g., Cry 1A(c) binds

to the ligand bands of beet armyworm (*Spodoptera exigua*) brush border membrane proteins, but there is very little toxicity to the insect (Garczynski et al. 1991). Cry 1A(b) is more toxic to the gypsy moth than Cry 1A(c), but does not bind well with the receptors in the brush border membrane (Wolfersberger, 1990).

Transgenic crops with Bt gene

A number of vectors have been developed for transferring the genes of interest into crop plants. The system involves a marker gene for resistance to antibiotics or herbicides (phosphoincithrin), a replication site, and a multiple cloning site (MCS) with several restriction sites for DNA insertion. Foreign DNA can be inserted into the vector using restriction enzymes that recognise a specific DNA sequence. Insertion of foreign DNA interrupts gene expression of an identifiable protein product to indicate DNA incorporation. Construction of DNA sequence for incorporation into vectors consists of several components, e.g., in case of Bt, the gene should be first converted from AT-rich (typical of bacteria) to GC-rich (typical of higher plants) to increase toxin expression. Most changes are made to the third codon thereby minimising changes in the amino acid sequence and increasing the expression of Bt toxin by 10 to 100 fold (Perlak et al. 1991). For expression of the Bt gene in the higher plants, a recognisable promoter and a terminator sequence must bracket the Bt gene. Popular constitutive promoters include cauliflower mosaic virus (CaMV35S) and ubiquitin. Tissue specific promoters include PEPC (phosphoenolpyruvate carboxylase) (for green tissue) and maize pollen specific promoter (Koziel et al. 1993). The size of the vectors ranges from 5000 to 11000 bp depending on the Bt gene and the promoter incorporated into the vector (Koziel et al. 1993). Delivery of the vectors into the nucleus has been achieved by using *Agrobacterium* mediated transformation and biolistic methods (Raineri et al. 1990; Koziel et al. 1993).

The first transgenic tobacco plants with Bt were produced in 1987 (Barton et al. 1987; Fischoff et al. 1987; Vaeck et al. 1987). These plants expressed full length or truncated Bt toxin genes (Cry 1A) under the control of constitutive promoters. The expression was quite low in tobacco plants, resulting in only 20% mortality of tobacco hornworm (*Manduca sexta*) larvae. Truncated Cry1A genes encoding for the toxic N-terminal fragment provided better protection to tobacco and tomato plants. Plants transformed with truncated gene expressed about 0.02% of total leaf soluble protein. Gene truncation, use of different promoters, enhancer sequences and fusion proteins resulted in only a marginal improvement in gene expression (Barton et al. 1987; Vaeck et al. 1987; Perlak et al. 1990; Carozzi et al. 1992). Cotton cultivar Coker 312 was first transformed by using modified PM Cry1A(c) gene under the control of CaMV35S promoter containing a duplicated enhancer region. The transformed plants showed total protection against *Trichoplusia ni*, *Spodoptera exigua* and *Heliothis zea*. The maximum level of toxin protein was 0.1% of the total soluble protein. By placing the FM Cry 1A(c) gene under the control of *Arabidopsis thaliana* small subunit promoter with its chloroplast transit peptide sequence, Wong et al. (1992) produced tobacco plants with 10 to 20 fold increase in Cry 1A(c) mRNA and protein compared to gene constructs with CaMV35S promoter with duplicated enhancer region. This increased the Bt toxin production to nearly 1% of the total leaf protein.

Tissue specific regulation of Bt Cry1A(b) gene has been utilised to achieve high and regulated expression in the leaves and pollen grains. The promoter derived from PEPC controls the expression of Cry1A(b) in green tissue (Hudspeth and Grula, 1989), while the promoter derived from calcium dependent protein kinase (CDPK) gene is pollen specific (Estruch et al. 1994). Combination of green tissue specific PEPC and pollen specific CDPK tissue promoters provides high Cry A(b) gene expression in leaves and pollen, where it is most effective in controlling the European corn borer (*Ostrinia nubilalis)*. The intron 9 of maize PEPC is located between Cry1A(b) structural gene and the 35S terminator, and its presence increased the expression of Bt gene (Hudspeth and Grula, 1989). Three untranslated termination sequences from CaMV35S are present adjacent to the PEPC intron 9 and provides the polyadenylation site (Rothstein et al. 1987). The catalytic activity of mature CDPK protein in maize is affected by calcium channels. Fusion of this sequence to Cry1A(b) does not manifest any changes in the calcium requirements of the maize plant.

Fujimoto et al. (1993) used a similar approach to transform rice plants as discussed above for maize. The transformed plants had nearly 0.05% toxin of the total soluble leaf protein, and showed resistance to the rice leaf folder *(Cnaphalocrosis medinalis)* and yellow stem borer (*Chilo suppressalis)*. Synthetic Cry III genes have also been expressed in tobacco and potato plants for the control of Colorado potato beetle (*Leptinotarsa decemlineata*) (Perlak et al. 1993). Tobacco and tomato plants expressing Cry1A(b) and Cry1A(c) genes have also been developed (Van der Salm et al. 1994) to control lepidopteran insects. The sequence motiffs that affect mRNA stability in plant cells were removed from the Bt genes. The expression of Cry1A(b) - Cry1A(c) genes provided protection against *S. exigua*, *M. sexta* and *H. virescens.* Hence, it is feasible to increase the levels of resistance in transgenic plants and also employ different genes for managing development of resistance to Bt in insect populations**.** Evolving levels of insect resistance to Bt can be dramatically reduced through the genetic engineering of chloroplasts in plants or by inserting more than one gene in the same plant. Due to maternal inheritance of chloroplasts, this approach seems to be environmental friendly by preventing the spread of chloroplast based genes into the environment. Svab and Maliga (1993) expressed Cry1A gene in tobacco chloroplasts using chloroplast transformation vectors and particle bombardment. The transplastomic plants expressed the Bt toxin at high levels and achieved complete control of

lepidopteran insects (McBride et al. 1995). Transgenic tobacco leaves expressing the Cry2Aa2 protoxin in chloroplasts when fed to susceptible, Cry1A-resistant (20 000 to 40 000-fold resistant) and Cry2Aa2-resistant (330- to 393-fold resistant) insects (*H. virescens*, *H. zea* and S. *exigua*), 100% mortality was observed in all insect species and strains (Kota et al. 1999).

These results suggested that plants expressing high levels of a non-homologous Bt protein might be able to overcome or significantly delay the development of broad-spectrum resistance to Bt in the field. This technique does not need modification of the Bt gene because the chloroplast transcriptional and translational apparatus are prokaryotic and it is possible to have many copies of the Bt gene in the cells. Expression level is high if driven by promoters such as *rbcl* and *cab* and there is no risk of transfer of the Bt genes through pollen to the related plants. Twelve plasmids have been inserted into soybean through the particle bombardment gun (Hadi et al. 1996). Marker free transformation system has been developed recently (Yoder and Goldsborough, 1994). Isopentyl transferase gene has also been used to develop marker free plants (Ebinuma et al. 1997). The advances in genetic transformation over the past two decades are now being successfully employed to develop crop plants with durable resistance to insects.

Considerable progress has been made in developing transgenic crops with resistance to the target pests over the past decade (Hilder and Boulter, 1999). Such transgenic plants have shown good promise in reducing insect damage, both in laboratory and field conditions. Successful control of pink bollworm (*Pectinophora gossypiella*) has been achieved through transgenic cotton (Wilson et al. 1992). In transgenic cotton BTK, the mean percent injury has been observed to be 2.3 in flowers and 1.1 in capsules compared to 23 and 12% in Coker 213, respectively (Benedict et al. 1996). The cottonseed yield being 1050 kg per ha in Coker 312 compared to 1460 kg per ha in BTK. Significant variation in insecticidal activity has been observed in transgenic plants at different growth stages during the season, and in different parts of the cotton plant (Zhao et al. 1998a). The efficacy of Bt in leaf and squares was high during the second generation of the insect, but declined in the third and fourth generations in North China. The surviving third and fourth generation larvae, after feeding on flowers of Bt cotton, fed on the bolls until pupation, which could cause selection in the field populations of *H. armigera*. The increase in resistance was 7.1-fold after 17 generations of selection in the laboratory, with an average mortality of 67.2% for each generation. The resistance grade of Bt cotton declined from high resistance against a non-selected population to medium levels of resistance against the selected population, indicating a potential problem of development of resistance in insects to Bt cotton. Adamczyk et al. (1998) observed no differences in survival in fall armywrom (*Spodoptera frugiperda*) larvae between the normal and CryIA(c) transformed cotton cultivars and in the number of larvae that pupated and eclosed as adults. Field trials of transgenic maize with Cry type toxins have shown that they are highly effective against the European corn borer *and* can withstand up to 50 larvae per plant at the whorl leaf stage and about 300 larvae at the anthesis stage (Armstrong et al. 1995). Leaf feeding is restricted to pin holes and the stem tunnelling to 0.2 tunnels per transgenic plant compared to 9 tunnels per plant in the nontransgenic control plants. With 2400 larvae at the mid-whorl stage and 1200 larvae at the anthesis stage, the leaf damage rating in the transgenic plants was 1.6 compared to 7.2 in the controls (damage evaluated on a 1 – 10 scale) and stem tunnelling was 1.7 cm in the transgenic plants transformed with CryIA(b) gene compared to 59 cm in the control plants (Koziel et al. 1993). Maize plants with CryIA(b) gene were resistant to the sugarcane borers (*Diatraea grandiosella* and *Diatraea saccharalis)* (damage rating 2.4 – 2.6 compared to 10.0 in the susceptible control with 50 larvae per plant at the 6-leaf stage). However, only a slight reduction in damage was recorded due to the fall (leaf damage rating 8.0 – 8.7 compared to 9.5 – 10.0 in the controls) (Bergvinson et al. 1997). Bt transformed plants also showed better resistance to *D. grandiosella* than those derived from the conventional host plant resistance-breeding program. Larvae collected 25 days after infestation were fewer and smaller on the transgenic hybrid CML 139 x CML 167 than the larvae collected from the control hybrid. The effectiveness of the transgenic hybrid was less pronounced against the sugarcane borer (*Diatraea saccharalis)*. The level of resistance was comparable to the conventional host plant resistance. Transgenic maize expressing Cry 9C, an insecticidal crystal protein from *Bacillus thuringiensis* subsp. *tolworthi*, effectively controlled both generations of the European corn borer (Jansens et al. 1997). CBH 351 tested in plots containing only Cry 9C transgenic plants had 0.14 and 0.09 cm tunnelling per stalk compared with more than 30 and 23 cm tunnelling per stalk for the negative controls in the field trials conducted in Belgium and Iowa, respectively. Williams et al. (1997) observed that transgenic hybrids sustained significantly less leaf feeding damage than the resistant check by the fall armyworm and Southwestern corn borer *(Diabrotica undecimpuncta howardi*). The high levels of resistance to fall armyworm and near immunity to south-western maize borer of transgenic maize hybrids provided the highest levels of resistance documented for both pests.

Arencibia et al. (1997) used a truncated CryIA(b) gene in transgenic sugarcane plants under the control of the CaMV35S promoter. Transgenic sugarcane plants showed significant larvicidal activity against neonate larvae of sugarcane borer (*D. saccharalis*) despite low expression of CryIA(b).

Transformation of high-quality rices of group V is a feasible alternative to sexual hybridisation (Ghareyazie et al. 1997). Truncated CryIA(b) gene has been introduced into several cultivars of rice (*indica* and *japonica*) by microprojectile bombardment and protoplast systems (Datta et al. 1998). The expression was driven by two constitutive

promoters (35S from CaMV and Actin-1 from rice) and two tissue-specific promoters (pith tissue and PEPC for green tissue from maize). Eighty-one transgenic plants caused 100% mortality of the yellow stem borer (*Scirpophaga incertulas*). The transgene, Cry IA(b), driven by different promoters showed a wide range of expression (low to high) of Bt proteins stably inherited in a number of rice cultivars with enhanced yellow stem borer resistance. Maqbool et al. (1998) transformed the rice cultivars Basmati 370 and M7 by using Cry 2A insecticidal gene against the yellow rice stem borer and the rice leaf folder. Nayak et al. (1997) reported that two rice lines transformed with synthetic Cry IA(c) were highly toxic to yellow stem borer larvae and reduced the insect feeding. Rice plants expressing Cry IA(b) and Cry IA(c) genes were highly toxic to striped stem borer (*Chilo suppressalis*) and yellow stem borer (*Scirpophaga incertulas*), with mortalities of 97 to 100% within 5 days after infestation. Bt genes have also been inserted into an elite maintainer line, R68899B with the Cry IA(b) gene (Alam et al. 1999). Insect bioassays showed enhanced resistance to yellow stem borer.

Successful expression of Bt genes has also been obtained in tomato (Delannay et al. 1989). Transgenic potato plants containing Cry IA(b) gene Bt 884 and a truncated gene Cry IA(b)6 against potato tuber moth (*Pthorimaea operculella*) resulted in less damage to the leaves. However, the size of the leaf tunnels increased over time in plants containing only the Bt 884 gene, while there was no increase in those containing Cry IA(b)6 (Jansens et al. 1995). The later also resulted in 100% mortality of the insects in tubers stored up to six months. Transformed brinjal plants have shown a significant insecticidal activity of transgenic brinjal fruits against the larvae of fruit borer (*Leucinodes orbonalis*) (Kumar et al. 1998. A modified gene of *Bacillus thuringiensis* var. *tolworthi*, encoding a coleopteran insect-specific Cry IIIB toxin has been used to transform the female parent of the eggplant commercial hybrid Rimina (Arpaia et al. 1997). Twenty-three out of 44 plants showed significant insecticidal activity toward neonate larvae of Colorado potato beetle. Brinjal cultivar Picentia and the wild species *Solanum integrifolium* have also been transformed with both a wild type (wt) and four mutagenized versions of Bt 43 belonging to the Cry 3 class (Innacone et al. 1997). Transgenic plants obtained with the more modified versions, BtH and BtI were fully resistant to Colorado potato beetle (*Leptinotarsa decemlineata*) first- and third- instar larvae, while Bt 43 wt, BtE and BtF genotypes did not cause mortality and did not affect larval development. Synthetic Cry 1C gene introduced into broccoli (*Brassica oleracea* ssp. *italica*) provided protection not only from susceptible diamond back moth (*Plutella xylostella*) larvae, but also from diamond back moth selected for moderate levels of resistance to Cry 1C. Cry1C containing transgenic broccoli was also resistant to the cabbage looper (*Trichoplusia ni*) cabbage butterfly (*Pieris rapae*). Selvapandian et al. (1998) transformed tobacco plants using Cry 1Ia5 insecticidal toxin from Bt strain from India, which provided complete protection against *H. armigera*. The effectiveness of this toxin was comparable to Cry 1A (b) or Cry 1A(c) genes.

A commercial formulation of Bt (Biolep ®) has been found to be effective against the sorghum shoot fly. Toxins from *B. thuringiensis* var *morrisoni* have shown appreciable biological activity against the shoot fly larvae. CryIA CryIC, CryIE and CryIIA are active against the spotted stem borer (*Chilo suppressalis*) larvae, while Cry IA is most effective against *H. armigera* (Sharma et al. 1999). Although there are no reports of Bt transformed sorghums, Rensburg et al. (1999) reported that transformed maize was effective against the spotted stem borer and the maize talk borer (*Busseola fusca*), which are the two most important pests of sorghum in Asia and Africa. Spotted stem borer was more susceptible than the maize stalk borer to the same events. Effectiveness of Bt toxins has also been demonstrated in maize against the stem borers infesting sorghum in Latin America (Vergvinson et al. 1997). Efforts are in progress to transform sorghum involving Bt genes at ICRISAT and at the University of Queensland (Brisbane, Australia) (I. Goodwin; personal communication).

A codon-modified Cry IA(c) gene has been introduced into groundnut (Singsit et al. 1997). Feeding bioassay indicated various levels of resistance to lesser corn stalkborer (*Elasmopalpus lignosellus*), from complete larval mortality to a 66% reduction in larval weight. Chickpea cultivars ICCV 1 and ICCV 6 have been transformed with Cry IA(c) gene and a plasmid containing *nptII* gene as the selection marker (Kar et al. 1997). Insect feeding assays indicated that the expression level of the Cry IA(c) gene was inhibitory to the development and feeding by *H. armigera*. Efforts are also in progress at ICRISAT to insert Bt genes into pigeonpea and chickpea to control the pod borer (Ortiz et al. 2000).

Enzyme inhibitors

Protease inhibitors

Disruption of amino acid metabolism by inhibition of protein digestion has been a key target for use in insect control (Johnson et al. 1989; Hilder et al. 1992). 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 been cloned and introduced into transgenic plants. Insects also produce SPIs, which are active against and presumably involved in regulating their digestive proteases. These can also be used against the insects by expressing them in transgenic plants (Wasmann et al. 1994; Thomas et al. 1995ab). Other species of insects rely on thiol proteases (cysteine proteases) as their primary digestive protease. These can be targeted with thiol protease inhibitors (TPIs). TPIs have been reported to be effective for controlling the maize rootworm (*Diabrotica* sp.), against which there are no effective Bt proteins (Edmonds et al. 1996). Transgenic

tobacco plants expressing trypsin inhibitor gene at nearly 1% (derived from cowpea via CaMV35S constitutive promoter) have resulted in increased mortality, reduced insect growth and reduced plant damage by *H. virescens* (Hilder et al. 1987). Similar results have also been shown *H. zea* (Hoffman et al. 1992). Transgenic tobacco has also been shown to enhance protection against *Spodoptera littoralis* and *M. sexta*. Thomas et al. (1994) reported that a cDNA encoding the anti-elastase protease inhibitor (PI) from *M. sexta* reduced the onset of thrip predation, suggesting that this methodology can establish insect resistance within this agronomically important legume. Sweet potato cultivar Tainong 57 trypsin inhibitor gene introduced into tobacco cultivar W38 retarded larval growth of *S. litura* as compared to control plants (Yeh et al. 1997).

Several protease inhibitor gene constructs have been introduced into different transgenic crops. However, the observed effects have not been considered to be sufficiently convincing to lead to a serious attempt at commercialising these genes. Some plants produce high levels of natural SPIs, and introduction of an additional heterologous inhibitor increases the level of resistance, e.g., in sweet potato to *Euceps postfaciatus* (Golmirizaie et al. 1997). Insects in general are adapted to counter the defensive measures of their natural hosts, but may still be susceptible to a related defence mechanism from the non-host plants. Jongsma et al. (1995) observed that only 18% of the gut proteinase activity from caterpillars raised on transgenic plants expressing potato inhibitor II was sensitive to inhibitor II, compared to 78% in caterpillars raised on control plants. Increased activity of inhibitor II insensitive protease almost compensated for the decline in inhibitor II sensitive activity.

The ability of some species to compensate for protease inhibition by switching on to an alternative proteolytic activity or over-producing the existing activity would limit the application of protease inhibitors in such species. Deployment of protease inhibitors for insect control requires a detailed analysis of the particular crop-insect interactions. The range of dissociation constants (Kd) for different PIs (protease inhibitors) with specific proteases is large, and this can be used to select the most effective inhibitor for gene transfer in a particular situation (Christeller and Shaw, 1989), e.g., transgenic tobacco expressing high levels of Kunitz type of trypsin inhibitor from soybean (SBTI) performs better than the tobacco plants expressing CpTI-tobacco against *H. virescens.* Proteolysis by gut extracts is 40-fold more susceptible to inhibition by SBTI than to CpTI (Gatehouse et al. 1993). However, CpTI is considered to be more useful for transfer, because unlike many SPIs, it is not deleterious to mammals (Puzstai et al. 1992). Also, many SPIs are toxic to beneficial insects such as honeybees (Malone et al. 1995; Burgess et al. 1996), but CpTI is not. Alpteter et al. (1999) observed increased resistance in wheat expressing trypsin inhibitor to Angoumois grain moth (*Sitotroga cereallela*). The cowpea trypsin inhibitor (CpTI) containing transgenic cotton lines have been found to be highly resistant to cotton bollworm (Li et al. 1998). Expression of potato trypsin inhibitor gene confers resistance to insects in rice (Duan et al. 1996). Accumulation of soybean Kunitz trypsin inhibitor (SKTI) in rice confers resistance to brown planthopper (*Nilaparvata lugens*) (Lee et al. 1999). A gene encoding a cowpea trypsin inhibitor (CpTI) in transgenic rice significantly increased resistance to *C. suppressalis* and *Sesamia inferens* (Xu et al. 1996). The results suggested that the cowpea trypsin inhibitor might be useful for the control of rice insect pests. Cowpea trypsin inhibitor gene CpTI has also been introduced into *Brassica oleracea* var. *capitata* cultivars Yingchun and Jingfeng (Fang et al. 1997). The transformed plants showed resistance to *P. rapae* in laboratory tests. Vain et al. (1998) transformed 25 clones with genes coding for an engineered cysteine proteinase inhibitor (oryzacystatin-I delta D86, OC-I). Adults of *Psylliodes chrysocephala* fed identically on leaf discs from control or transformed plants of oilseed rape expressing constitutively the cysteine proteinase inhibitor oryzacystatin I (OCI) (Girard et al. 1998). When larvae were reared on transgenic plants expressing OCI, they showed an increase in weight compared to those reared on control plants. Larvae fed on transgenic plants also exhibited a 2-fold increase in both cysteine and serine proteolytic activity in response to the presence of OCI. There is a considerable plasticity in insect digestive physiology and feeding behaviour in response to Pis. Transformed poplar cultivar Jean Pourtet did not affect larval mortality, growth and pupal weights of *Lymantria dispar* and *Clostera anastomosis* (Confalonieri et al. 1998).

Alpha amylase inhibitors

Carbohydrate metabolism in insects has been targeted through the use of α–amylase inhibitors. Amylase inhibitors from wheat (WAAI) and common bean (BAAI) have been characterised. Transgenic tobacco expressing WAAI gene has been reported to increase mortality of the lepidopteran larvae between 30 to 40% (Carbonero et al. 1993). However, the results from other experiments have not been consistent. Genes encoding for BAAI have been expressed in pea by the *pha*1 gene promoter to direct high levels of expression in seeds to increase the levels of resistance to *Collasobruchus* spp. (Shade et al. 1994; Schroeder et al. 1995). Enhanced levels of resistance to the bruchids have also been obtained in seeds of transgenic adzuki beans (Ishimoto et al. 1996).

Plant lectins

Plant lectins are a heterogenous group of sugar binding proteins, which have a protective function against a range of organisms. Plant lectins are classified mainly on the basis of their sugar binding properties. Lectins from snowdrop, pea, wheat, rice, castor, soybean, mungbean, garlic, sweet potato, tobacco, chickpea and groundnut have been isolated and characterised. Lectins produce chronic

effects on survival and development of insect pests elonging to different insect orders (Shukle and Murdock, 1983; Czapla and Lang, 1990; Habibi et al. 1992; Powell et al. 1993; Gatehouse et al. 1995; Powell et al. 1995). Transgenic tobacco expressing pea lectin has shown adverse effects against *H. virescens* (Boulter et al. 1990). Unlike wheat germ agglutinin (WGA) and phaetohaemogluttinin (PHA), lectins have low mammalian toxicity. Greater insecticidal activity has been observed in chitin binding lectins from wheatgerm and common bean. Transgenic maize expressing WGA has shown moderate activity against *O. nubilalis* and *Diabrotica* sp. (Maddock et al. 1991). However, mammalian toxicity of this lectin is high and it may not be therefore a good candidate for use in genetic transformation of crops.

Sap sucking Hemiptera can be controlled by certain lectins (Hilder et al. 1995). A gene encoding the mannose specific lectin from snowdrop expressed in tobacco showed enhanced resistance to peach potato aphid (*Myzus persicae*). Transgenic potato expressing GNA has also been shown to be less susceptible to peach potato aphid (Gatehouse et al. 1996). However, the viability of the coccinellids was reduced when fed on aphids reared on transgenic plants.

Rice cystanin I in transgenic potato caused up to 53% mortality in larvae reared on transgenic leaves, compared with < 17% mortality in the control (Lecardonnel et al. 1999). Fitches et al. (1997) observed that at 21 days after hatch, mean larval biomass of the tomato moth (*Lacanobia oleracea*) was reduced by 32 and 23%, in the artificial diet containing GNA and excised leaves of transgenic tomato plants, respectively. In glasshouse trials, a 48% reduction in insect biomass was observed after 35 days. However, prolonged compensatory feeding by the larvae on the excised leaves of transgenic plants resulted in 15% more leaf feeding than the control plants. Adaptation to low levels of GNA, in terms of biomass recovery and compensatory feeding, was observed within one generation in the detached leaf assay. No significant effects of GNA on larval survival were observed in the artificial diet and detached leaf bioassays, whereas survival decreased by nearly 40% in the glasshouse bioassay.

Concanavalin A inhibits development of tomato and peach-potato aphid when expressed in transgenic potato plants (Cao et al. 1999). Larvae of tomato moth fed on concanavalinA (ConA) in artificial diet exhibited decreased survival, but had only a small effect on larval weight (Gatehouse et al. 1999). When fed to peach-potato aphids in liquid artificial diet, ConA reduced aphid size by up to 30%, retarded development and reduced the fecundity by >35%, but had little effect on survival. With both insects, there was a poor correlation between lectin dose and quantitative effect. Constitutive expression of ConA in transgenic potato retarded larval development and decreased the larval weight by >45%, but showed no significant effect on survival. In cotton, larvae of cotton budworm fed on transformed tissues exhibited a reduction in weight, but the lectin did not result in larval mortality (Satyendra et al. 1998). A 35% reduction in larval growth was observed following feeding on F_2 plants transformed with lectin A gene. Plants bearing lectin B reduced larval weight and plants segregating for lectin C retarded larval growth.

Transgenic sugarcane plants engineered to express either the potato proteinase inhibitor II or the snowdrop lectin gene showed increased antibiosis to larvae of sugarcane grubs (*Antitrogus consanguineus*) in glasshouse trials (Nutt et al. 1999). Canegrubs feeding on transgenic line UP 87, transformed with the potato gene, gained as little as 4.2% as compared to those fed untransformed control plants.

Similarly, larvae feeding on the roots of transgenic line G 87, transformed with the snowdrop gene, gained only 20.6% of the weight of grubs feeding on the non-transgenic control plants. Plants transformed with a proteinase inhibitor from ornamental tobacco did not affect the weight gain of grubs. Snowdrop lectin at levels greater than 0.04% of total soluble protein decreased the fecundity, but not the survival of grain aphids (Stoger et al. 1999). It is suggested that transgenic approaches using insecticidal genes such as GNA in combination with integrated pest management present promising opportunities for the control of wheat pests. Transgenic haploid rice shoots with GNA have shown resistance to brown plant hopper (BPH) (Yang-Chang et al. 1998). GNA lectin expressed in the transgenic rice plants decreased survival and overall fecundity (production of offspring) of the insects, retarded insect development and had a deterrent effect on BPH feeding (Rao et al. 1998).

Enzymes

Transgenic expression of certain enzymes has been suggested as another alternative to Bt genes. One of the most important enzyme is chitinase, which is an important component of insect integument. Transgenic tobacco plants expressing chitinase have shown increased resistance to lepidopteran insects (Ding et al. 1998). Similar effects have been observed with tobacco plants expressing bean chitinase (Gatehouse, 1995). A chitinase from *Serratia marcesens* has been shown to act synergistically with Bt toxin against *S. littoralis* (Rigev et al. 1996). Corbin et al. (1994) reported the expression of cholesterol oxidase gene in bacteria and plant protoplasts. Cholesterol oxidase from *Streptomyces* is highly toxic to cotton boll weevil (*Anthonomus grandis*) (Purcell et al. 1993; Cho et al. 1995). Lipoxigenase has also been shown to exhibit toxic effects (Shukle and Murdock, 1983) and has been expressed in transgenic plants, but resistance to insects has not been demonstrated.

Polyphenol oxidases and peroxidases increase the inhibitory effect of 5CQA (5-Caffeoyl quinic acid) and cholorogenic acid by oxidising the dihydroxy groups to

ubiquinones that covalently bind to nucleophilic (-SH2 and NH2) groups of proteins, peptides and amino acids. Use of bacterial isopentenyl transferase (ipt) gene, involved in cytokinin biosynthesis, has been fused with a promoter from the proteinase inhibitor II (PI-IIK) gene and introduced into *Nicotiana plumbaginifolia* (Smigocki et al. 1993). *Manduca sexta* larvae consumed up to 70% less of the PI-II-ipt leaf material on flowering plants than the larvae feeding on controls. Development of *M. persicae* nymphs was also delayed. Approximately half as many nymphs reached adulthood on PI-II-ipt leaves than on controls.

Zeatin and zeatinriboside levels in leaves remaining on PI-II-ipt plants after hornworm feeding were elevated by about 70-fold and the chlorophyll a/b content was twice to that of controls. Exogenous applications of zeatin to the PI-II-ipt leaves enhanced the level of resistance to the tobacco hornworm and almost completely inhibited normal development of the green peach aphid. Transcript levels of an acidic chitinase gene were low and minimally inducible in PI-II-ipt leaves. The mode of action of the cytokinin gene product on enhanced insect resistance is not clear, but may involve the products of secondary metabolic pathways. *Manduca sexta* larvae feeding on tomato plants, constitutively expressing a prosystemin antisense gene, had approximately 3 times higher growth rates than the larvae feeding on nontransformed control plants (Orozco-Cardenas, 1993).

The levels of proteinase inhibitor I and inhibitor II proteins in leaves of tomato plants expressing the antisense prosystemin gene remained at undetectable levels until the sixth day of larval feeding and then increased throughout the plants (100 to 125 µg per g of leaf tissue after 14 days). In control plants, levels of proteinase inhibitor I and II proteins increased rapidly from the second day of larval feeding, and by the eighth day contained levels of 225 and 275 µg per g of leaf tissue, respectively, and then increased slowly thereafter. Prosystemin mRNA levels in antisense and control plants after 6 and 12 days of larval feeding correlated with levels of inhibitor I and II protein levels. These experiments demonstrate that resistance of plants toward an insect pest can be modulated by genetically engineering a gene encoding a component of the inducible systemic signalling system regulating a plant defensive response.

Vegetative insecticidal proteins

Supernatant of vegetative *Bacillus cereus* culture have two compounds; VIP 1 and VIP 2, which have been shown to possess toxic effects toward insects (Estruch et al. 1997). VIP 3 has been isolated from *B. thuringiensis* supernatants, which is highly toxic to *Agrotis* and *Spodoptera* (Estruch et al. 1996). The activity of these proteins is similar to δ-endotoxins. 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. Efforts are underway to use these proteins for inducing resistance to insect pests.

Toxins from predators

Insect predators such as spiders and scorpions produce peptides, which are powerful neurotoxins that have been expressed in transgenic plants. However, expression of scorpion toxin in transgenic plants results in toxicity to insects fed on them (Barton and Miller, 1991). Genes encoding neurotoxins from predatory mites (Tomalski and Miller, 1991) and scorpion (Stewart et al. 1991) have been deployed in recombinant baculoviruses to increase their biological activity.

Secondary plant metabolites

Many secondary plant metabolites such as alkaloids, steroids, foliar phenolic esters (rutin, cholorogenic acid, etc.) terpenoids, cyanogenic glycosides, glucosinolates, saponins, flavonoids, pyrethrins and nonprotein amino acids act as potent protective chemicals. Some of the secondary plant metabolites are produced in response to insect feeding, infection by pathogens, and abiotic stress factors. These compounds are called phytoalexins (Sharma and Agarwal, 1983; Ebel, 1986; Sharma and Norris, 1991). Peptide hormones are supposed to induce production of proteinase inhibitors. Systemically induced responses are modified through synthesis and action of jasmonic acid via its lipid precursor, e.g., linoleic acid in tomato. Application of exogenous jasmonate induces the production of proteinase inhibitors. *Arabidopsis* mutants deficient in linoleic acid cannot synthesise jasmonate and are susceptible to the fungal gnat (*Bradasia impatiens*). Xu et al. (1993) observed enhanced resistance in rice by wounding methyl jasmonate and abscisic acid in transgenic plants. Effective manipulation of metabolic pathways involved in the production of plant secondary metabolites by introduction (or elimination by antisense RNA technology) of enzyme encoding sequences is quite difficult (Hallahan et al. 1992; McCaskill and Croteau, 1998). Increased production of many of these chemicals imposes a measurable cost in yield on the host plants (Vrieling et al. 1991). There is evidence that such cost is not involved in natural protection mechanisms based directly on protective proteins (Brown, 1988). Expression of relatively large amounts of a foreign protein such as CpTI does not impose a cost in yield in transgenics (Hilder and Gatehouse, 1991), and most transgenics are described as phenotypically normal. Much of the cost to plants of non-protein secondary compounds appears to be associated with less synthesis than with sequestration or detoxification of these compounds. Cost in yield could be another constraint in utilisation of this approach.

Gene pyramiding

Many of the candidate genes, that have been used in genetic transformation of crops, are either too specific or are only mildly effective against the target insect pests. Some insect species are also insensitive to some of these genes.
Therefore, to convert transgenics into an effective weapon in pest control, e.g., by delaying the evolution of insect populations resistant to the target genes, it is important to deploy genes with different modes of action in the same plant.

Activity of Bt in transgenic plants can be enhanced by serine protease inhibitors (MacIntosh et al. 1990). Activity of Bt can also be increased in combination with tannic acid (Gibson et al. 1995). Cornu et al. (1996) reported that transgenic poplars expressing proteinase inhibitor and CRY IIIA genes exhibited reduced larval growth, altered development and increased mortality as compared to the control. Hoffmann et al. (1992) evaluated tobacco plants expressing *Bacillus thuringiensis* var. *kurstaki* HD-73 delta-endotoxin or cowpea trypsin inhibitor (CpTI) for their efficacy against *Helicoverpa zea* in the field. Mortality of *H. zea* larvae was significantly higher and leaf damage significantly lower for the genotypes containing *Bacillus thuringiensis* gene compared with nontransgenic control.

The codon-modified CryV-Bt gene (CryV-Bt) from *Bacillus thuringiensis* subsp. *kurstaki*, which is specifically toxic to Lepidoptera and Coleoptera and a potato Y potyvirus Yo coat protein gene (PVYocp), in which the aphid transmission site was inactivated, have been inserted into potato cultivar Spunta using *Agrobacterium tumefaciens* (Li et al. 1999). All CryV-Bt/PVYocp-transgenic lines were more resistant to potato tuber moth and PVYo infection than nontransgenic Spunta. Four CryV-Bt/PVYocp transgenic lines were equal in potato tuber moth mortality to a CryV-Bt transgenic line.

Two transgenic lines, 6a-3 and 6a-5 showed greater resistance to potato tuber moth and PVYo than the other cryV Bt/PVYocp transgenic lines. This study indicated that multiple genes, conferring insect pest resistance and virus resistance, could be engineered into and expressed simultaneously in a potato cultivar. Zhao et al. (1998b) compared transgenic tobacco plants expressing the *Bacillus thuringiensis* (Bt) gene CryIA and the cowpea trypsin inhibitor gene CpTI in transgenic plants containing the Bt gene alone and to non-transgenic plants for their insecticidal activity against *H. armigera* larvae. Insecticidal action of the transgenic plants expressing both genes was significantly higher than that of the plants expressing the Bt gene alone. Only fifth-instar larvae could survive until pupation when fed the Bt + CpTI diet. After 11 generations of selection in *H. armigera* for resistance to CryIA + Bt proteins, there was significantly less resistance to the insecticidal proteins than in larvae selected on Bt plants or artificial diet.

Herrera et al. (1997) introduced the CryIA(c) gene into an isolate of *Pseudomonas fluorescens* capable of colonising sugarcane, on two broad host range plasmids, pDER405 and pKT240, carrying 13 and 28 copies, respectively. Bioassays on *Eldana* larvae showed that the strain carrying the gene integrated into the chromosome was as toxic as the one carrying it on pKT240. Glasshouse trials indicated that sugarcane treated with *Pseudomonas fluorescens* 14:Omegon-Km-cry were more resistant to *Eldana* damage than untreated sugarcane.

Concluding remarks

The ideal transgenic technology should be commercially feasible, environmentally benign (biodegradable), and easy to use in diverse agroecosystems as well as show a wide-spectrum of activity against the crop pests. It should also be harmless to the natural enemies, target the sites in insects that have developed resistance to the conventional pesticides, flexible enough to allow ready deployment of alternatives (if and when the resistance is developed by the pest), and preferably produce acute rather than chronic effects on the target insects.

The value of chronic effects in IPM need to be emphasised. Transgenic crops have many of these requirements. Some of the criteria can be achieved by exploiting genes that are based on antibody technology (Hilder and Boulter, 1999). Single chain antibodies (ScFvs) can be used to block the function of essential pest proteins. The potential of plant expressed antibodies or antibody fragments to serve as insect control agents against nematodes, pathogens and viruses has also been described (Atkinson, 1993; van Engelen et al. 1994; Rosso et al. 1996). This approach of controlling insects would offer the advantage of allowing some degree of selection for specificity effects, so that pests, but not the beneficial organisms, are targeted. The development of a delivery system from transgenic plants to the insect haemolymph will remove a key constraint in the transgenic approach to crop protection.

Incorporation of Bt genes will have a tremendous effect on pest management. We need to pursue the management strategy that reflects the pest biology, insect plant interactions and their influence on the natural enemies to prolong the life span of the transgenics. Refugia can play an important role in resistance management and should take into account the pest complex, the insect hosts and the environment. Expression of more than one gene (gene pyramiding) and single chain antibody genes, which would be compatible with the likely trends in pesticide discovery using biology derived target based methods. Emphasis should be placed on combining exotic genes with conventional host plant resistance, and also with traits conferring resistance to other insect pests and diseases of importance in the target region. Several genes conferring resistance to insects can also be deployed as multilines or synthetics.

While several crops with commercial viability have been transformed in the developed world, very little has been

done to use this technology to increase food production in the harsh environments of the tropics. Some attempts are being made in transforming crops such as sorghum, pigeonpea, cowpea, chickpea and groundnut with Bt genes for resistance to insect pests. There is a need to use these tools for providing resistance to insects in cereals, legumes and oil seed crops that are a source of sustenance for poorer sections of the society. Equally important is the need to follow the biosafety regulations and make this technology available to farmers, who cannot afford the high cost of seeds marketed by the private sector. International research centres, advanced research institutions in the developed world and the national agricultural research systems can play a major role in promoting biotechnology for food, feed and fiber production in the developing world.

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