Transgenics, pest management, and the environment

H. C. Sharma* and Rodomiro Ortiz

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, India

Genetic engineering of crop plants to confer resistance to insect pests offers an environmental friendly method of crop protection. Impressive results have been obtained with the expression of *Bacillus thuringiensis* (*Bt*) and other toxin genes in several crops. However, both exotic and plant-derived genes have some performance limitations, and there have been some failures in insect control through transgenic crops. The production and deployment of transgenic crops for pest control need to address the issues related to impact of the transgenic crops on the insect pests, ecological cost of resistance development, effects on the nontarget organisms, availability and distribution of the alternate host plants, and the potential for introgression of genes into the wild relatives of crops. There is a need for a more responsible public debate and better presentation of the benefits for a rational deployment of the genetically-transformed plants for sustainable crop production.

TO keep pace with population growth, there is a continuing need to increase food production, particularly in the developing countries of Asia, Africa and Latin America. Most of the increase in food production has to come from increased yields of major crops grown on existing arable lands. One of the practical means of increasing crop production is to minimize the pest-associated losses, which are currently estimated at 14% of the total agricultural production[1]. In addition to the direct losses, insects also cause indirect losses due to their role as vectors of various plant pathogens, and there are additional costs in the form of pesticides applied for pest control, currently valued at $10 billion annually. Massive application of pesticides not only leaves harmful residues in the food, but also causes adverse effects on non-target organisms and the environment.

With the advent of genetic transformation techniques, it has become possible to insert exotic genes into the plant genome that confer resistance to insects. Amongst these, the bacteria such as *Bacillus thuringiensis* (*Bt*) and *B. sphaericus*[2,3] have been used successfully for pest control through transgenic crops on a commercial scale. Insecticidal genes such as *Bt*, trypsin inhibitors, lectins, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins and small RNA viruses can be used alone or in combination with *Bt* genes in transgenic plants for pest control. Considerable progress has been made in developing transgenic crops with resistance to the target pests over the past two decades[4,5]. Such transgenic plants have shown considerable promise in reducing insect damage, both under laboratory and field conditions, and thus reducing the need to use pesticides for pest management. Genes conferring resistance to insects have been inserted into crop plants such as maize, rice, cotton, potato, tobacco and soybean[6–11]. Transgenic plants with insecticidal genes are set to feature prominently in pest management in both developed and the developing countries in the future[5]. Entomologists, breeders and the molecular biologists need to determine how to deploy this technology for pest management, and at the same time reducing possible environmental hazards. To achieve these objectives, we need to have a proper understanding of the insect biology, behaviour, their 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, and a mechanism to deliver the technology to the resource-poor farmers.

Transgenic crops and pest management

Advantages

Biotechnology has provided 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. Development and deployment of transgenic plants with insecticidal genes will lead to:

- Reduced exposure of farmers, farm labour and non-target organisms to the pesticides;
- Increased activity of natural enemies because of reduction in pesticide sprays;
- Reduced amounts of pesticide residues in the food and food products;

*For correspondence. (e-mail: ICRISAT@cgiar.org)

- A safer environment to live because of reduction in pesticide use.

Host plant resistance (HPR) reduces the need to apply pesticides, and thus is compatible with biological control and other methods of pest management in an integrated pest management (IPM) programme. Pesticides are highly toxic to the natural enemies, pollinators and other non-target organisms and thus result in adverse effects on the environment. Conventional HPR slows down the rate of increase of pest populations and exposes the pests for prolonged periods to the natural enemies[12,13]. The introduction of transgenic plants brings in a new system of HPR into play, which has a potential to influence the tritrophic interactions[14]. The specificity of *Bt* is such that it was expected to have no direct effects on the natural enemies, although indirect effects such as those from the sick and sub-optimal prey might be expected. Synergism has been reported between *Bt* toxins and the HPR for *Trichoplusia ni*[15], and such a situation needs to be exploited for crops and pests of importance in the semi-arid tropics (SAT) to achieve satisfactory control of the target pests, and thus avoiding the need to apply pesticides. Preliminary studies have indicated that there are no adverse effects of transgenic plants on the performance of the natural enemies. However, in-depth studies need to be carried out to characterize their impact on biological control agents in the laboratory involving artificial diets, and then in the field involving transgenic plants. The use of insect prediction models can further help in understanding the impact of transgenic plants on the activity and effectiveness of natural enemies in combination with transgenic crops in IPM.

Sprays based on *Bt* formulations are not generally competitive with chemical insecticides and are unlikely to displace them because of their limited spectrum of activity and lower efficacy compared to synthetic chemicals. In contrast, transgenic plants appear to be sufficiently effective to either displace chemicals or can be used in conjunction with the insecticides or other methods of pest control, making such plants attractive from the standpoint of environmental protection[16]. Simulation models using data from the diamondback moth (*Plutella xylostella*) and the Indian meal moth (*Plodia interpunctella*) have suggested that under some circumstances, transgenic plants bearing only one *Bt* gene may be more effective than the sprays for delaying the development of resistance to *Bt*. A Colorado potato beetle strain that can survive *Bt* sprays and develop to maturity, cannot develop successfully on transgenic plants, not even on plants showing very low levels of *Bt* gene expression. The results suggest that more mechanisms are available to counteract the *Bt* sprays than the *Bt* toxins expressed in transgenic plants. Simulation models have indicated that transgenic plants may be much more durable than sprays of similar efficacy when more than two genes are deployed[17].

Limitations

Transgenics are not a panacea for solving all the pest problems. With the deployment of transgenic crops:

- The secondary pests will no longer be controlled in the absence of sprays for the major pests;
- The need to control the secondary pests through chemical sprays will kill the natural enemies and thus offset one of the major advantages of transgenics;
- The cost of producing and deployment of transgenics may be very high;
- Proximity of transgenic crops to sprayed fields and insect migration may reduce the effectiveness of transgenics;
- Development of resistance in insect populations may limit the usefulness of transgenic crops for pest management.

Ecological impact of transgenic crops on the environment

There are a number of ecological and economic issues that need to be addressed while considering the production and deployment of transgenic crops for insect control. The most important consideration is the immediate reduction in the amount of pesticides applied for pest control. The number of pesticide applications on a crop such as cotton varies from 10 to 40, and most of the sprays are directed against the key pests such as *Heliothis* and *Helicoverpa*. In case the transgenic crops are introduced, the number of pesticide applications are likely to be reduced by two-thirds to half. Reduction in pesticide application would lead to an increase in the activity of the natural enemies, while some of the minor pests may tend to attain higher pest densities in the absence of sprays applied for the control of major pests. The introduction of transgenic crops will have a major impact on the abundance of some insects, and such effects would be negative for some, and positive for others. The magnitude of these impacts would depend on the diversity of insect species for which the crop serves as the main host. The other potential impact of transgenic crops may be from the perspective of farmers.

Efficacy of transgenic crops for controlling non-target pests needs to be determined in each region. Some of the pests maintain high pest densities on alternate hosts, e.g. cereal stem borers on the wild relatives of sorghum. While the expectation is that *Bt* crops would be effective against the lepidopterous pests, their real effects on

different insect species need to be determined. Potential impact of transgenic crops on the beneficial insects will be through reduction in the number of eggs and larvae of the natural hosts, which may also affect the activity of natural enemies. The significance of such effects depends on the importance of the immature stages of the target pest for maintaining the local populations of the natural enemies. This is likely to reduce the numbers of certain natural enemies in the transgenic crops, but their populations may be maintained on the other crops that serve as a host to the target pests. A few of the known predators are specialists on one insect, and hence, the populations of the generalist predators would be maintained on the other insect species[18]. Within field impact may be greater for parasitoids which feed only one insect species. The populations of such natural enemies can only be maintained on the nontransgenic crops or other hosts of the target pest, unless the alternate crops are deleterious to the activity of natural enemies, e.g. *Trichogramma* on *Helicoverpa* eggs laid on pigeonpea and chickpea, where the activity of this parasitoid is impaired by the presence of glandular trichomes. The effect of transgenic crops on the abundance of natural enemies in the transgenic crops should be compared with the nontransgenic fields of the same crop where the natural enemies would be virtually absent because of heavy pesticide application.

Several studies have shown that a *Bt* toxin, which is very effective against one insect species, may be weakly active or ineffective against the other insects[19]. Also, if a pest species (e.g. *H. armigera* on cotton) is selected for resistance to *Bt* on one crop, and then moves to another (e.g. pigeonpea), the selection of the pest population for resistance to the *Bt* toxin would continue. In diverse agricultural systems such as those prevailing in the SAT, it would be important to understand the biology and behaviour of the major insect pests in an ecosystem so that informed decisions can be made as to which crops to transform, and the toxins to be deployed. It is also important to consider the resistance management strategies, economic value, and environmental impact of the exotic genes in each crop, and whether a crop serves as a source or sink for the insect pests and their natural enemies. While the developed countries may have the resources to engineer and motivate the development of appropriate strategies for the production and deployment of transgenic plants, the developing countries may be left far behind. Not only is there a lack of understanding as to how much effective the engineered crops would be in providing protection against the target pests, there is also a lack of information on the key pests of the economically important crops. As in the past, the technologies that have been used in the developed countries may not be suitable for the developing countries with complex cropping systems and the species involved. Therefore, there is an urgent need to give a serious thought to these problems and develop appropriate strategies for production and deployment of insect-resistant transgenic crops. Introduction of transgenic crops with insect resistance is expected to reduce the amount and number of pesticide applications. However, greater research effort is needed to identify insecticide molecules that are more effective in combination with the transgenic crops. Introduction of transgenic crops is likely to bring in a qualitative change in our approach to pest control. The issues that need to be addressed while introducing transgenic crops for pest control include: (i) effects on population dynamics of target and non-target insects, (ii) evolution of new insect biotypes, (iii) insect sensitivity, (iv) performance limitations, (v) gene escape into the environment, (vi) secondary pest problems, (vii) environmental influence on gene expression and failure of insect control, (viii) effects on non-target organisms, and (ix) influence on natural enemies.

Effects on population dynamics of target and non-target insects

Transgenics will produce the same effect on the population dynamics of the insects as the plants with conventional host plant resistance[20], e.g. continuous planting of the stem fly-resistant (*Cephus cinctus*) wheat cultivars would completely suppress the stem fly populations below the economic threshold levels within six years. The stem flies can also be kept under check by alternate planting of the resistant and susceptible cultivars. Similar models for the effect of insect-resistant cultivars on insect abundance have also been developed for sorghum shoot fly (*Atherigona soccata*), spotted stem borer (*Chilo partellus*), sorghum midge (*Stenodiplosis sorghicola*), and sorghum head bug (*Calocoris angustatus*)[13]. However, the adoption of insect-resistant cultivars is not as smooth as predicted because the seed of the insect-resistant cultivars has to be purchased much before the population densities of most pests can be predicted, and the insect-resistant cultivars sometimes may not be as high yielding as the commercial varieties and hybrids. Also, the expression of resistance is not the same under different population densities of the target pests and under different environmental conditions. Activity of the natural enemies is density dependent, and this may reduce the parasitoid numbers over time. Such an interaction might result in population densities that are higher in magnitude than those predicted by the simulation models. There is a need therefore to: (i) understand the natural population regulation of the target pest, (ii) assess the field performance of insect-resistant cultivars under diverse environmental conditions, (iii) determine

the long-term effects of the resistant cultivars on insect populations, and (iv) determine the level of adoption of the insect-resistant cultivars.

Evolution of new insect biotypes

Another issue concerning the deployment of transgenic crops is the evolution of new insect biotypes. However, experience from the conventional host plant resistance breeding has shown that there is no direct relationship between the deployment of insect-resistant cultivars and the evolution of new insect biotypes. In case of the Hessian fly (*Myetiola destructor*) in wheat, no direct relationship has been observed between the planting of the resistant cultivars and the population of the Hessian fly. Planting of the Hessian fly-resistant cultivars did not lead to evolution of new biotypes. The time needed for adaptation to antibiosis-resistant genes has been predicted to be 3 to 8 years. However, in case of greenbug (*Schizaphis graminum*), the breeding programmes continue to struggle to keep pace with the evolution of new biotypes in some crops[21,22]. However, there is no relationship between the deployment of greenbug-resistant wheat cultivars and the development of new greenbug biotypes[23]. For sorghum, only 3 of the 11 biotypes of greenbug have shown a correlation between the use of resistant hybrids and the development of new biotypes. Even within the 3 biotypes, no clear cause-and-effect relationship has been established. Based on analysis of these specific insect–plant interactions, future plant resistance efforts should focus on the use of the most effective resistance genes; despite past predictions of what effect these genes may have on insect population genetics.

It is pertinent to know whether the transgenic crops have characteristics that might predispose them to unusually short or long durability. Initially, it was felt that development of resistance to *Bt* may not be an issue since the *Bt* and the pests have co-evolved for millions of years[24,25]. Because of limited exposure and several toxins produced by *Bt*, the rate of development of resistance under natural conditions may not be high. In transgenic plants, the insects are continuously exposed to the exotic genes, and there are distinct possibilities of a faster rate of resistance development in the target pests.

Insect sensitivity

There are many species of insects that are not susceptible to the currently available *Bt* proteins. There is a need to broaden the pool of genes, which can be effective against insects that are not sensitive to the currently available genes. Since first generation transgenics have only one *Bt* toxin gene, lack of control of less sensitive species may present another problem in pest management. This is not the same as development of resistance, which is a progressive decrease in sensitivity to a chemical by a population in response to the use of a product to kill the insects. If there is low or no sensitivity to a chemical in an insect species, it is not resistance. *Spodoptera litura* is less sensitive to toxins from *Bt* var *kurstaki* than *H. armigera*, *Achoea janata*, *P. xylostella* and *Spilosoma obliqua*[19]. The affinity of *d* endotoxins for protease activity in these insect species has shown a negative correlation with the susceptibility of different insect species. The *Km* values (p-moles) for proteases of different species with *d*endotoxin as substrate were 32.84 for *A. janata*, 26.39 for *H. armigera*, 32.78 for *P. xylostella*, 23.06 for *S. obliqua* and 24.15 for *S. litura*. Purified midgut extracts resulted in cleavage of the *d*endotoxin into 11 fragments in *A. janata*, 15 in *H. armigera*, 18 in *P. xylostella*, 12 in *S. obliqua* and 4 in *S. litura*. Thus, protease activity in the midgut seems to influence the insect sensitivity to the *Bt* toxins. *H. virescens* is less sensitive to CryIA(a), CryIC and CryIE, while *S. littoralis* is insensitive to most of the *Bt* toxins. Larvae of *C. partellus* are less sensitive to *Bt* toxins than those of *H. armigera* (H. C. Sharma, ICRISAT, unpublished). CryIC and Cry1E, which are active against *H. virescens*, are ineffective against *H. armigera*[26]. Cry1B is slightly active against *H. armigera*, while it has been reported to be inactive against *H. virescens*[27]. Cry9A, which has a broad spectrum of activity against the lepidopteran insects, has been found to be inactive against *H. armigera*. Thus, there are considerable differences in the sensitivity of different insect species to various *Bt* toxins, and due care has to be taken to deploy *Bt* toxins in different crops or cropping systems.

Performance limitations

Efficacy of transgenics cannot be compared with the synthetic pesticides or the expectation that no additional protective intervention is involved once the transgenic crops are deployed. Even the best transgenics cannot be compared with the synthetic chemical insecticides. However, enough information has not been generated involving transgenics in a genuine IPM system. Such trials can demonstrate long-term benefits of the transgenic crops, especially if the factors such as environmental and human health hazards are taken into account. Currently deployed transgenic crops produce only one *Bt* toxin protein, while the *Bt* strains used for commercial formulations produce several toxins in addition to other factors that increase insect mortality. Avoidance may be one strategy that the insects employ against the transgenic plants. In choice tests, *H. vires-*

cens larvae avoid the diet containing *Bt*[28]. Similar behaviour has been observed in case of sorghum shoot fly (*A. soccata*) and spotted stem borer (*C. partellus*) (H. C. Sharma, ICRISAT, unpublished). The current CryIA(b) construct employs phosphoenolpyruvate carboxylase (*PEPC*) promoter, which enables the expression in the green tissue, and as a result, the expression is greater in the young plants. Some insects such as stem borers and shoot fly migrate to the plant whorl or stem tissue with incomplete chlorophyll formation. If the toxin is expressed in insufficient amounts in such a tissue, the insects can develop mechanisms to withstand low levels of toxins in the transgenic plants. Behavioural avoidance of the tissue expressing the toxin gene can be another component in insect resistance to the transgenic plants. Therefore, care should be taken to express the toxins in sufficient amounts at the site of damage or feeding by the insects.

Gene escape into the environment

Incorporation of genes encoding for *d*-endotoxin proteins into crop plants has provided appreciable levels of resistance to the target pests, resulting in tremendous excitement in crop protection. However, serious concerns have been raised regarding gene escape into the wild relatives of crop plants[10,29]. Escape of resistance genes into the wild relatives may lead to faster development of resistance in insect populations. More challenging is the escape of herbicide-resistance genes into the wild relatives of crop plants, which can become totally resistant and impossible to control with the available chemicals. Assessment of realistic risk for gene transfer through pollen is available for many regions[30], and agriculturally sound procedures need to be developed for different regions[31]. However, herbicide application is quite low in the developing countries, and resistance to herbicides may not be a serious problem in the near future.

Pollen dispersal from transgenic cotton is low, but increases with an increase in the size of the source plot[32]. The results have shown that a 20 m buffer zone would limit the dispersal of transgenic pollen from small-scale field tests in cotton. The risks of transgenic maize in relation to teosinte (*Zea diploperennis*) are considered smaller than the dangers presented by urbanization[33]. The risk of hybridization with teosinte increases progressively towards the south of Mexico, with increasing use of teosinte. In a resistant strain of Colorado potato beetle, ingestion of Cry3A toxin significantly increased flight activity, indicating that physiological resistance was probably reinforced by the behavioural escape from toxic environments[34]. Behavioural differences between resistant and susceptible beetles may affect gene flow between transgenic and the adjacent nontransgenic crops.

CryIA(b) protein as a component of post-harvest transgenic maize plants dissipates readily on the surface of, or cultivated into, soil[35] and has not been detected in silage prepared from transgenic plants[36]. Under laboratory conditions, plasmid transfer[37] between *Bt* subsp. *tenebrionis* and *Bt* subsp. *kurstaki* HD 1 (resistant to streptomycin) strains occurs at a frequency of 10^{-2}. However, no plasmid transfer has been observed in soil release experiments and in insects on leaf discs. The *Bt* toxins were detectable on the clay-particle-size fraction of non-sterile soil after 40 days. When the toxins bind on clay minerals, they become resistant to utilization by micro-organisms. Binding of the *Bt* toxins to humic acids reduced their potential for microbial biodegradation[38]. These results indicate that *Bt* toxins in transgenic plants and microbes could persist, accumulate and remain insecticidal in soil as a result of binding to humic acids. The effect of such *Bt* toxins on the non-target organisms needs to be investigated.

Secondary pest problems

Most crops are not attacked by a single pest species, but a complex of insect pests. In the absence of competition from the major pests, secondary pests may assume a major pest status. The *Bt* toxins may be ineffective against such pests, e.g. leaf hoppers, mirid bugs, root feeders, and mites. This will offset some of the advantages expected of the cultivation of transgenic crops. Management of phytophagous stink bugs is necessary in transgenic *Bt* cotton[39]. Insecticide application for the control of stink bugs is necessary if more than 20% of the bolls are damaged in mid- to late-season. In another study, no differences were observed between transgenic and nontransgenic cultivars in boll weevil or aphid damage, beneficial arthropods or fiber characteristics[40]. Effective and timely control measures should be adopted for the control of secondary pests on transgenic crops. There is a need to identify genes that could be deployed to control pests not susceptible to *Bt*. While there is a trend to develop target-specific compounds for chemical control, it will be desirable to have genes with a broad spectrum of activity for use in genetic transformation of crops, provided this does not influence the beneficial organisms.

Environmental influence on gene expression and failure of insect control

There have been some failures of the transgenic crops being unable to provide adequate level of pest control. In Texas, *H. zea* populations destroyed *Bt* cottons due to

high tolerance to *Bt* toxin, CryIA(c)[41]. Similarly, *H. armigera* and *H. punctigera*, which are also quite tolerant to *Bt* toxins, destroyed the cotton crop in the second half of the growing season in Australia because of reduced production of *Bt* toxins in the transgenic crops[4]. Possible causes for the failure have been suggested to be: inadequate expression, effect of environment on expression of *Bt* genes, locally resistant insect populations, and development of resistance due to inadequate management.

In general, the toxin expression within plants is fairly uniform. However, environmental conditions may influence the production of *Bt* toxins in transgenic plants. Cotton crop flooded with 3 to 4 cm deep water for 12 days lost resistance to insects significantly compared with the control plants irrigated normally. Similar reaction has been observed in *Bt* cotton under overcast and rainy weather continuously for 21 days. When the waterlogging was over, the cotton plants recovered gradually and their insect resistance increased again to some extent. Under flooded conditions, the activity of superoxide dismutase increased considerably in *Bt* cotton plants at first, and then dropped continuously[42]. Epistatic and environmental effects on foreign gene expression could influence the breeding, stability and in the case of pest resistance, efficacy and durability of the foreign gene[43]. *CryIA* gene expression is variable and is influenced by genetic and environmental factors. The *CryIA* phenotype segregated as a simple, dominant Mendelian trait. However, non-Mendelian segregation occurred in some lines derived from MON 249. Expression of *CryIA* genes in cotton lines is influenced by one or more of the following: site of gene insertion, gene construct, background genotype, epistasis, somaclonal mutations, and the physical environment. Appropriate evaluation and selection procedures should be used in a breeding programme to develop crop varieties with pest-resistant traits conferred by foreign genes.

Effects on non-target organisms

One of the major concerns of transgenic crops is their effects on the non-target organisms, about which little is known at the moment. The *Bt* proteins are rapidly degraded by the stomach juices of the vertebrates. Most *Bt* toxins are specific to insects as they are activated in the alkaline medium of the insect gut. However, *Bt* proteins can have harmful effects on the beneficial insects. Although such effects are much less severe than those of the broad-spectrum insecticides. Therefore, there is a need for information on long-term chronic effects of *Bt* genes on human beings and other non-target organisms.

Genetically modified oilseed rape, expressing genes conferring resistance to insects and fungi (cowpea trypsin inhibitor (CpTI) for insects, chitinase for fungi, and beta-1,3-glucanase) has been assessed for its impact on the environment[44]. Chitinase did not affect learning performance of honeybees, beta-1,3-glucanase affected the level of conditioned responses, with the extinction process occurring more rapidly as the concentration increased and CpTI induced marked effects in both conditioning and testing phases, especially at high concentrations. The decrease in learning performance induced by CpTI observed at the individual level has been confirmed at the colony level.

Trypsin inhibitor and wheat germ agglutinin (WGA) did not show acute toxicity to honeybees. *In vivo*, trypsin inhibitor caused a decrease in the amount of trypsin activity and did not have a significant effect on esterase activity[45]. *In vitro*, trypsin inhibitor inhibited about 80% of non-specific protease activity and 100% of trypsin activity. *In vivo*, WGA at high concentration in food (1 mg ml^{-1}) elicited a large decrease in trypsin activity and did not have a significant effect on esterase activity. *In vitro*, WGA did not show any significant effect on trypsin and non-specific protease activities, but slightly activated esterase activity. Serine proteinase inhibitor (PI) (CII from soybean), cysteine PI (OCI from rice), chicken egg white cystatin, and Bowman-Birk soybean inhibitor do not produce harmful effects on honeybees at the concentrations expressed in transgenic plants[46–48]. Consumption of high doses of PI induces proteinase overproduction[47,49]. Trypsin endopeptidase inhibitors, bovine pancreatic trypsin inhibitor (BPTI), and soybean trypsin inhibitor (SBTI) have been found to be toxic to adult honeybees at 1% weight: volume in sugar solution[50]. Activity of three major midgut endopeptidases of bees (chymotrypsin-like, *N*-succinyl-L-ala-L-ala-L-pro-L-leu-*p*-nitroanilide [SAAPLpNA]-hydrolysing and trypsin-like) and an exopeptidase, leucine amino peptidase was measured in bees fed on these two inhibitors. *In vitro* tests using control bee midgut extracts showed that BPTI had high binding affinity for trypsin and less for both chymotrypsin and SAAPLpNA-hydrolysing activity, and SBTI had high affinity for SAAPLpNA-hydrolysing activity and trypsin, and less affinity for chymotrypsin.

Transgenic rape does not appear to have harmful effects on the lifespan and behaviour of honeybees, but further tests may be necessary[46]. Oilseed rape expressing the PI under the control of CaMV35S promoter does not threaten the honeybees, since the transgene is not expressed in pollen and nectar.

Influence on natural enemies

The incidence and dynamics of natural enemies in *Bt* and non-*Bt* fields has been observed to be almost the same[51]. Transgenic tobacco did not show a significant effect on natural infestations of predacious insects[52].

Risks to non-lepidopteran insects due to CryIA(c) protein expressed in transgenic cotton have been found to be negligible[53].

Predators. Recent observations have suggested that there may be a reduction in the fitness of the predatory chrysopid larvae directly attributable to caterpillars fed on *Bt*-maize[52,54]. However, any direct effects of *Bt* through transgenics would still be much lower than those of the synthetic insecticides. Laboratory studies on *Leptinotarsa decemlineata* and its predator *Coleomegilla maculata* have shown no adverse effects of the *Bt*-based insecticide on the predator[55]. However, under choice conditions, the predator showed a distinct preference for the untreated eggs than those treated with *Bt*[48]. Its activity was not affected by pure transgenic and mixed seed potato fields[56]. Cry3A-intoxicated *L. decemlineata* can be eaten by *C. maculata* without any observable adverse effects on their survival or predation potential[57]. Its predatory activity can also decrease the rate at which *L. decemlineata* adapted to the *Bt* toxins if mixed plantings are used[58]. No statistically significant effects on survival, aphid consumption, development or reproduction have been observed in *Hippodamia convergens* fed on *Myzus persicae*, reared on potatoes expressing δ-endotoxin of *Bt* subsp. *tenebrionis*[59].

When adult two-spotted ladybirds (*Adalia bipunctata*) were fed for 12 days on peach-potato aphids (*M. persicae*) colonizing transgenic potatoes expressing lectin from *Galanthus nivalis* (GNA) in leaves, the ladybird fecundity, egg viability and longevity decreased over the following 2 to 3 weeks[60]. No acute toxicity to the ladybird beetles due to the transgenic plants was observed, although female longevity was reduced by up to 51%. Adverse effects on ladybird reproduction caused by eating peach-potato aphids from transgenic potatoes were reversed after switching the ladybirds to pea aphids from non-transgenic bean plants. The results suggested that expression of a lectin gene for insect resistance in a transgenic potato can cause adverse effects to predatory ladybird beetles via aphids in their food chain. In another study, no adverse effects were observed on pre-imaginal development or mortality of *Chrysoperla carnea* when reared on *Rhopalosiphum padi* that had fed on *Bt*-maize[61]. However, abundance of *Labia grandis* was lower in pure and mixed plants of transgenic potatoes than in pure nontransgenic potato plants[82]. Daily feeding of *Prophylea japonica*, *Coccinella septempunctata*, and *Erigonidium graminicola* on insects that had fed on *Bt* cotton produced Holling type II functional response[62]. The abundance of the predator *P. japonica* increased by 11.8 and 45.5%, respectively, in natural and integrated control plots; while that of *E. graminicola* [*Hylyphantes graminicola*] decreased by 3.6% in both. The activity of *Chrysopa* sp. decreased by 20.0% and increased by 38.7%, respectively, and that of *Orius minutus* decreased by 30.4 and 9.0%, respectively[63].

Parasites: Parasitism levels by *Campoletis sonorensis* were greater on the transgenic than on the nontransgenic plants, which may be due to fewer larvae on the transgenic plants. *C. sonorensis* and toxic plants each decreased survival of larvae during the first six days on transgenic plants. *C. sonorensis* and toxic plants acted synergistically in combination, decreasing larval survival beyond the level expected for an additive interaction[64]. Synergistic increases in mortality and parasitism have been detected in two trials when development rates on toxic plants and control plants were equal, indicating existence of another mechanism[64]. *Bt* toxin-mediated partial resistance is compatible with natural enemies for the control of *H. virescens*. However, a simulation using a theoretical population genetic model suggested that synergism of the level measured in this study could accelerate pest adaptation to resistant plants.

Cardiochiles nigriceps did not significantly reduce 6-day survival of host larvae and did not interact with plant toxicity[65,66]. Egg parasitism of third-generation noctuids in the field of *Bt*-transgenic cotton has been observed to be lower than in the conventional cottons[51]. In natural and integrated control plots, the parasitoids *Campoletis chlorideae* and *Microplitis* sp. abundance decreased by 79.2 and 87.5, and 88.9 and 90.7%, respectively, and the activity of *Lysiphlebia japonica* increased by 85.1 and 90.2%, respectively[63]. Percentage of parasitism by the parasitoid *Diadegma insulare* was not significantly different between the mixed and non-mixed plots of transgenic crop[67]. There was no effect of transgenic corn on the parasitization of *O. nubilalis* by *Eriborus tenebrans* and *Macrocentrus grandii*[68]. These data suggest that intra-field mixtures could serve to decrease density of a target pest such as the diamondback moth, while not adversely affecting the activity of natural enemies. The effects of transgenic crops on the natural enemies vary across crops and the cropping systems. Some of the variation in extent of parasitization may be due to differences in pest abundance between the transgenic and the non-transgenic crops, since the abundance of natural enemies is influenced by the density of their prey. Wherever the transgenic crops have shown adverse effects on the natural enemies, these effects may still be far lower than those of the broad-spectrum pesticides.

Microflora: Under field conditions, the microflora of *Bt* transgenic potato plants has been observed to be minimally different from that of chemically and microbially treated commercial potato plants[69]. It is unlikely that expression of *Bt* and any other genes in transgenic

plants would have an adverse effect on the soil microflora.

Transgenic crops and public health

In general, no adverse effects of *Bt* proteins have been observed in higher animals, including mammals. There are no specific receptors for CryIA(b) protein present in the gastrointestinal tract of mammals, including man[70]. Slight histopathological effects have been observed in the gut mucosa. No other signs of systemic adverse effects have been noted in mice and rabbits following oral administration. Chemical analysis of tomato fruits has shown that there were no major changes in composition of the transgenic tomatoes. Oral exposure to transgenic *Bt* tomatoes poses no additional risk to human and animal health. However, a number of aspects concerning the safety assessment of transgenic *Bt* tomatoes would require further study[71]. No statistically significant differences in survival or body weight have been observed in broilers reared on meshed or pelleted diets prepared with *Bt* transgenic maize and similar diets prepared using control maize[72]. Broilers raised on diets prepared from transgenic maize exhibited significantly better-feed conversion ratios and improved yield of the pectoralis minor breast muscle. However, it was not clear whether this enhanced performance was attributable to the transgenic maize *per se* or due to possible slight differences in overall composition of the formulated diets. Transgenic maize showed no deleterious effects on the broilers.

The quality of produce from the transgenic plants is in general similar to that from the nontransgenic plants of the same cultivar. The levels of the antinutrients gossypol, cyclopropenoid fatty acids, and aflatoxin in the seed from the transgenic cotton are similar to or lower than the levels present in the parental variety and other commercial varieties. The seed from the *Bt* transformed cotton lines is compositionally equivalent to, and as nutritious as the seed from the parental and other commercial cotton varieties[73]. The *Bt* toxins may be expressed or remain in plant parts to be consumed by human beings or dairy cattle, e.g. the raw seed of line 81 [with *CryIA(b)* gene] showed 14.00 μg per g active protein, and line 531 [with *CryIA(c)* gene] contained 2.22 μg per g of active protein by ELISA method. Processing removed in excess of 97% of the active proteins in the transgenic cotton seed[74].

Some of the protein families that contribute to the defence mechanisms of food plants have members which are allergens or putative allergens, and some of these proteins have a potential for use in molecular approaches to increase resistance to insect pests. These include α-amylase and trypsin inhibitors, lectins and pathogenesis-related proteins[75]. Several self-defence substances made by plants are highly toxic to mammals, including humans. In such cases, the source of the transgene is of no relevance in assessing the toxicological aspects of foods from transgenic plants. This may result in a trade-off situation between nature's pesticides produced by transgenic plants or varieties from traditional breeding programmes, synthetic pesticides, mycotoxins or other poisonous products of pests.

Trypsin inhibitors and plant lectins, which contribute to a plant's defence mechanism in nature, have a potential for use in developing transgenic crops with resistance to insects. However, these compounds have shown some adverse effects in nutritional studies involving rats. However, no crop plants expressing these genes have been deployed for commercial cultivation. Rats fed on purified cowpea trypsin (EC 3.4.21.4) inhibitor in a semi-synthetic diet based on lactoalbumin (10 g inhibitor kg^{-1}) for 10 days showed a moderate reduction in weight gain in comparison with controls, despite an identical food intake[76]. The reduction in the growth rate was about 20% on a live weight basis. The corresponding value for the dry weight of the carcasses was about 7%, because of different water content of the body in the two groups of rats. Although most of the CpTI was rapidly broken down in the digestive tract, its inclusion in the diet led to a slight, though significant, increase in the nitrogen content of faeces but not of urine. Accordingly, the net protein utilization of rats fed on inhibitor-containing diets was also slightly lower while their energy expenditure was elevated. The slight anti-nutritional effects of CpTI were probably due to the stimulation of growth and metabolism of the pancreas. Thus, the nutritional penalty for increased insect-resistance after the transfer of the *CpTI* gene into food plants is quite low in the short term. At a level that provides insecticidal protection for plants, but does not reduce the growth of young rats, GNA had a negligible effect on weight and length of the small intestine, even though there was a slight, but significant hypertrophy of this tissue[77]. However, the activities of brush border enzymes were affected, sucrase-isomaltase activity was nearly halved, and those of alkaline phosphatase and aminopeptidase increased significantly. Most of the changes in gut metabolism caused by the incorporation of GNA in the diet were less extensive than those found with toxic phytohaemagglutinin. Long-term animal studies are needed to establish whether it is safe to use GNA in transgenic plants destined for human consumption. Incorporation of *N*-acetylglucosamine-specific agglutinins from WGA, thorn apple (*Datura stramonium*) or nettle (*Urtica dioica*) rhizomes in the diet at the level of 7 g kg^{-1} reduced the apparent digestibility and utilization of dietary proteins and the growth of rats, with WGA being the most damaging[78]. As a result

of their binding and endocytosis by the epithelial cells of the small intestine, all three lectins interfered with its metabolism and function to varying degrees. WGA induced extensive polyamine-dependent hyperplastic and hypertrophic growth of the small bowel by increasing protein content, RNA and DNA. Furthermore, an appreciable portion of the endocytosed WGA was transported across the gut wall into the systemic circulation, where it was deposited in the walls of the blood and lymphatic vessels. WGA also induced the hypertrophic growth of the pancreas and caused thymus atrophy. Transfer of *WGA* genes into crop plants has been advocated to increase their insect resistance. However, the presence of this lectin in the diet may harm higher animals at concentrations required to be effective against most insect pests.

Development of resistance and strategies for resistance management

Development of resistance

Insect pest populations have shown a remarkable capacity to develop resistance to chemical pesticides. Over 500 species of insects have developed resistance to insecticides[79]. Most of the transgenic *Bt* crops express only one toxin gene and lack the complexity of the commercial *Bt* formulations. In addition, the plants continuously produce the toxins, and the insects are exposed to the *Bt* toxins throughout the feeding cycle or season, and this places the insect population under continuous and heavy selection pressure. With the development of resistance to *Bt* toxins, the value of microbial insecticides based on *Bt* proteins will diminish greatly due to lower sensitiveness of the target pest to the *Bt* formulations. One of the consequences of such a development will be that the farmers have to return to broad-spectrum insecticides, which will lead to environmental hazards associated with the use of synthetic insecticides. The potential of development of resistance to *Bt* proteins is not only of concern to the farmers, but to the scientists, extension agencies, and the transgenic plant industry. The investment made in the past would be turned useless unless this issue is addressed on an urgent basis. Most of the transgenic plants produced so far have *Bt* genes under the control of cauliflower mosaic virus (CaMV35S) constitutive promoter, and this system may lead to development of resistance in the target insects as the toxins are expressed in all parts of the plant[80]. Toxin production may also decrease over the crop-growing season. Decreasing levels of toxin production may lead to development of resistance to the toxin used, and to other related *Bt* toxins to which the insect populations may initially be quite sensitive. Low doses of the toxins eliminate the most sensitive individuals of a population, leaving a population, in which resistance can develop much faster.

The ability of insects to overcome host plant resistance is always a grave risk, and ways to delay the onset of resistance in insect populations will be an ongoing debate as transgenic crops are deployed. There are several reports on the development of resistance to *Bt* in different insect species. Diamondback moth, (*P. xylostella*) populations in several parts of the world have developed resistance to *Bt* formulations. Laboratory screening has resulted in the development of *Bt*-resistant populations in Lepidoptera (*H. virescens*, *Spodoptera exigua*, *S. littorallis*, *Trichoplusia ni*, *P. xylostella*, *Ephestia kuehniella*, *Cadara cautella*, *Homoeosoma electellum*, *Plodia interpunctella* and *Christoneura fumiferana*), Coleoptera (*Chrysomella scripta* and *Leptinotarsa decemlineata*) and Diptera (*Aedes aegypti*, *Culex quinquefasciatus*, *Drosophila melanogaster* and *Musca domestica*)[25]. This study indicated that the possibilities for resistance development are real. With the transgenic plants now being produced in both public and private sectors, the real challenge is to develop a strategy for deployment of transgenic plants for sustained protection of crops from insect pests. Different insect species react to *Bt* toxins differently. The resistance can develop quickly in *Diatraea saccharalis*, as considerable proportion of larvae have been observed to survive up to 8 days on transgenic maize[14]. However, *D. grandiosella* has shown a considerably lower frequency of surviving individuals.

Survival of susceptible *P. xylostella* second instars on *Cry1Ac*-expressing broccoli declined from 99.1 to 19.2%, at 24 and 72 h, respectively, while the survival of resistant larvae was 98.6 and 90.8%, respectively[81]. The rapid response to laboratory selection shows genetic variation in populations of diamondback moth in their susceptibility to *Bt* and suggests that intense selection may produce much higher levels of resistance than those previously reported from the field[82]. Field populations of diamondback moth have developed resistance to a commercial formulation containing a mixture of *Bt* toxins, an event that raises doubts about the ability of mixtures to retard resistance development. Extensive and intensive exposure of pests to *Bt* toxins through transgenic crop plants or other tactics may lead to widespread pest resistance to Bt. However, in some insect species, the probability of development of resistance may be very low, e.g. *Ostrinia nubilalis* has been observed to develop some tolerance to low levels of CryIA(b) in the diet, but it has not been possible to initiate or sustain the insect colonies at concentrations in the diet closer to the actual levels expressed in the transgenic maize plants[83]. After 13 generations of selection pressure, no colony survived on transgenic *Bt*

maize hybrids in the greenhouse. Similarly, field strains of soybean looper collected from soybean and *Bt*-cotton were less susceptible to *Bt* formulation Condor XL in dosage-mortality and discriminating concentration bioassays than the reference strain, and *Bt*-cotton strains were least susceptible to Condor XL[84]. These data indicated reduced susceptibility of field soybean looper strains compared to the reference strain exposed to Condor XL.

Mechanisms of resistance: Reduced binding of *Bt* toxins to midgut epithelium is one of the mechanisms of resistance in *P. xylostella*[85]. With midgut proteases being similar in resistant and susceptible populations, the proteolytic processing may not be a mechanism of resistance[86]. Population of *H. virescens* selected for resistance to CryIA(c), has also shown resistance to CryIB, CryIC, and CryIIA[87]. This suggests that there is a broad-based mechanism of resistance to *Bt*. Complete degradation of *Bt* toxins by the proteolytic enzymes is the principal mechanism of resistance in *S. frugiperda*[88]. Development of resistance may be due to changes in insecticidal crystal protein (ICP) receptors, and alterations in ICP receptors are a general mechanism by which insects can adapt to *Bt*[89]. The absence of cross-resistance to ICPs other than those present in the selecting agent, and the finding that these ICPs bind to distinct receptors indicate that the use of ICP mixtures or multiple ICPs expressed in transgenic plants may be a valuable resistance management tactic. Resistant strain of *H. virescens* processes the active toxin more quickly than the susceptible strain[90]. The observed quantitative and qualitative differences in degradation of *d*-endotoxin by larval gut proteases that occur during larval maturation may account for the difference in susceptibility to the *d*-endotoxin[88]. This finding should be taken into consideration when designing strategies for the development of transgenic crops expressing *d*-endotoxins as potent insecticidal proteins.

P. xylostella populations resistant to Cry1A(b) have a single binding site for CryIA(b). Heterologous competition showed that CryIA(c) competed as effectively as CryIA(b) for the CryIA(b) binding site, whereas CryIA(a) competed less effectively. The lack of cross-resistance suggests that CryIA(a) and CryIA(c) possess other binding sites than those recognized by CryIA(b). It has been suggested that this specific resistance could correspond to a biotype present in the Philippines[91]. Linkage group 9 (marker locus *MPI*) contributed as much as 80% of the total resistance to Cry1A growth inhibition in *YHD2* strain[92]. Recombination between the resistance locus or loci and the marker locus used to identify linkage group 9 occurred only when the informative hybrid parent was used as a male parent, which was expected because crossing-over does not occur in *H. virescens* females. Linkage group 11 (marker locus *GDA*) made a smaller contribution to resistance that was only detectable when the effect of linkage group 9 was removed. In addition to the effects of these two linkage groups, slight but significant differences between families suggested that additional unlinked loci have minor effects on resistance. Measurements of the resistance levels conferred by a small number of genes with the largest effects may be useful in predicting the selection response of *H. virescens* in the field.

Induction of proteinase activity may represent the mechanism by which insects that feed on plants overcome plant proteinase inhibitors (PIs)[93]. Herbivorous insects can overcome the activity of PIs by secreting inhibitor-resistant enzymes[94]. The insect's midgut contains a number of different proteins with trypsin-like activity. Some of these trypsin(s) are susceptible to inhibition by PI, while other trypsin(s) are not susceptible to inhibition. When inhibitor-resistant insects ingest PI, the level of activity of inhibitor-resistant trypsin(s) is enhanced in the midgut, thus allowing the insect to digest dietary protein in the presence of PI. This information suggests that a suite of PIs may be required to inhibit the majority of proteolytic activity in the midgut of the target organism, and thus reduce insect growth and development. Once the PIs have been identified, their genes can be transgenically inserted into plants to enhance phytochemical resistance against herbivorous insects. An adaptive mechanism in *Helicoverpa* elevates the levels of other classes of proteinases to compensate for the trypsin activity inhibited by dietary PI[95]. Partial compensation of cysteine proteinase activity inhibition by increasing serine proteinase activity allowed the larvae to overcome the effects of oryzacystatin consumption in *Baris coerulescens*[96]. This illustrates the problems that could arise when we try to achieve high levels of protection for plants against the insects possessing a complex digestive proteinase pool.

Inheritance of resistance: Resistance traits in *H. virescens*[87] could be present at a frequency of 10^{-3}. Field frequency[97] of alleles for resistance to *Bt* has been estimated to be 1.5×10^{-3}. This high initial frequency underscores the need for caution in deploying transgenic crops to control insect pests. Single-pair mating technique greatly increases the efficiency of detecting recessive resistance alleles, because alleles that decrease the target site sensitivity to *Bt* toxins and other insecticides are often recessive. This technique could be useful in estimating resistance allele frequencies in other insects exposed to transgenic insecticidal crops or conventional insecticides. Frequency of *Bt* resistance alleles[98] from a wild Minnesota population of European corn borer is below 0.013.

The inheritance of resistance in insect populations to *Bt* toxins is recessive, and is due to one or a few major loci[24,99,100]. In *Plodia interpunctella*, resistance is autosomal and recessive or partially recessive[101]. In *H. virescens*, resistance to *Bt* var *kurstaki* strain HD 1 is autosomal, incompletely dominant, and controlled by several genetic factors. However, in another study, the resistance in *H. virescens* was found to be partially recessive[102], and is thought to be inherited as an additive trait involving more than one loci, but in another strain, resistance is recessive[87]. Resistance in European corn borer (*O. nibulalis*) to the commercial formulation of *Bt* is inherited as an incompletely dominant autosomal gene[103]. Thus, development of resistance in insect populations to *Bt* can be slower compared to conventional insecticides depending on the nature of gene action.

In *P. xylostella*, an autosomal recessive gene conferred high levels of resistance to four *Bt* toxins (Cry1A(a), Cry1A(b), Cry1A(c), and Cry1F). Nearly 21% of the individuals from a susceptible strain were heterozygous for the multiple-toxin resistance gene. The resistance allele frequency was 10 times higher than the most widely cited estimate of the upper limit for the initial frequency of resistance alleles in susceptible populations. Therefore, insects may evolve resistance to some groups of toxins much faster than previously expected[104]. However, it has been observed that resistance to *Bt* in diamondback moth was an incompletely recessive, autosomal trait probably controlled by a single allele that did not confer detectable levels of reduced fitness in the absence of exposure to *Bt*[105]. As one of the few studies to demonstrate stable resistance to *Bt* subsp. *kurstaki* from insects that were collected from the field and not subjected to further selection in the laboratory, these results clearly indicate the need to develop specific resistance management strategies for *Bt* before there is a widespread evolution of resistance. In seven strains of *P. xylostella*, resistance to *Bt* declined when exposure to insecticide ceased (mean $R = -0.19$). In four other pests *(H. virescens, L. decemlineata, M. domestica* and *P. interpunctella*), resistance to *Bt* declined slowly or not at all (mean $R = -0.02$) in the absence of exposure of *Bt*. Reduced biotic fitness associated with resistance is the most likely cause of instability of resistance in *P. xylostella*[106].

Strategies for resistance management

Deployment of transgenic plants should be based on the overall philosophy of IPM, and consider not only gene construct, but alternate mortality factors, reduction of selection pressure, and monitor populations for resistance development to design more effective management strategies. This approach is particularly important when considering food security in the SAT. To increase the effectiveness and usefulness of transgenic plants, it is important to develop a strategy to minimize the rate of development of resistance in insect populations to the target genes through: (i) use of resistance management strategies from the beginning, (ii) gene pyramiding, (iii) gene deployment, (iv) regulation of gene expression, (v) development of synthetics, (vi) refugia, (vii) destruction of carryover population, (viii) control of alternate hosts, (ix) use of planting window and (x) use of economic thresholds and IPM.

Use of resistance management strategies from the beginning: To increase the usefulness and effectiveness of the transgenic plants, it is important to implement the resistance management strategies from the beginning. The increased possibility of development of resistance to *Bt* has prompted the development of a number of conceptual strategies for resistance management[17,25,99]. Most of these strategies are based on mixtures of toxins to be deployed for insect control, tissue specific production and induced toxin production. In mixing genes within a plant (gene pyramiding or gene stacking), genes of two or more insecticidal proteins or different genes need to be introduced into the same plant. In tissue-specific production, the plants are engineered so that the toxin is produced only in the tissues where the insect feeds. In induced toxin production, the plant is engineered in such a way that it produces the toxin when the insect starts feeding.

Individual farmers may have limited incentive to adopt resistance management technologies for *Bt* endotoxins, and that the greatest incentive lies with the *Bt* industry[107]. However, the implementation of a coordinated, industry-wide *Bt* resistance management effort is likely to be constrained by competition among segments of the *Bt* industry interested in different technologies (sprays vs transgenic plants), and among producers of *Bt* products using the same technology. A number of studies using prediction models have indicated that expression of toxins at very high levels could slow down pest adaptation to a toxin if the ecology and genetics of the pest and cropping system fit specific assumptions[108]. These assumptions relate to: (i) inheritance of resistance factors, (ii) ecological cost of resistance development, (iii) behavioural response of larvae and adults to the toxins, (iv) plant-to-plant movement of larvae, (v) adult dispersal and mating behaviour, and (vi) distribution of host plants that do and do not produce the toxin(s).

A deterministic population dynamics model has been modified to include single-locus, and two-allele genetics, and used to simulate strategies for delaying resistance in the European corn borer to transgenic maize[109]. Using the hypothesis of partial dominance of the resistant gene[110], this model suggested that only a high level

of migration (very likely, in most agricultural areas) or a sensible reduction of the fitness of resistant Colorado potato beetles, associated with the change in their genome, can guarantee a long-lasting efficacy of the transgenic cross[111]. Likewise, through the use of a stochastic, spatially explicit simulation model, factors that may influence the regional development of resistance in *H. virescens* to a *Bt* endotoxin in transgenic cotton have been explored. Spring movement of emerging adults onto wild hosts delayed the development of resistance if the movement is far enough from the field in which the pupae overwintered. Increase in the summer migration rate and the distance moved delayed resistance development up to a point at which higher rates did delay the development of resistance. A susceptible sunflower moth population and a laboratory-selected *Bt*-resistant population did not differ in mortality, developmental periods, pupal weight, sex ratios or fecundity when *Bt* was not present. *Bt* sunflower might not lead to the development of a *Bt*-resistant sunflower moth population[112]. Thus, the strategies for resistance management would depend on the number and nature of gene action, insect behaviour and insect–genotype–environment interaction.

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. Several genes such as trypsin inhibitors, secondary plant metabolites, vegetative insecticidal proteins, plant lectins, and enzymes that are selectively toxic to insects can be deployed along with the *Bt* genes to increase the durability of resistance. The durability of transgenic crops can be increased through multigene, multi-mechanistic resistance[113]. Considerable advances have been made in biotechnology for introducing and expressing multiple transgenes in crops[114,115]. It has been suggested that Cry1A(c) and Cry1F can be expressed together in transgenic plants for effective control of *H. armigera*[26], to increase the durability of resistance. Activity of *Bt* in transgenic plants can be enhanced by serine protease inhibitors[116]. Activity of *Bt* can also be increased in combination with tannic acid[15]. Transgenic poplars expressing proteinase inhibitor and *CryIIIA* genes exhibited reduced larval growth, altered development and increased mortality compared to the control[117].

The codon-modified *CryV-Bt* gene (*CryV-Bt*) from *Bt* 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*[118]. All *CryV-Bt/PVYocp*-transgenic lines were more resistant to potato tuber moth and PVYo infection than the non-transgenic Spunta. Insecticidal action of the transgenic plants expressing *Bt* and *CpTI* genes was significantly higher than that of the plants expressing the *Bt* gene alone[119]. 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.

Gene deployment: There is need to develop appropriate strategies for gene deployment in different crops or regions depending on the pest spectrum, their sensitivity to the insecticidal genes, and interaction with the environment. The deployment of different genes and their level of expression should be based on insect sensitivity and level of resistance development. High levels of Cry1C production can protect transgenic broccoli not only from susceptible or Cry1A(R) diamondback moth larvae, but also from those selected for moderate levels of resistance of Cry1C[120]. The Cry1C-transgenic broccoli is also resistant to two other lepidopteran pests of crucifers (cabbage looper and imported cabbage worm).

Regulation of gene expression: Regulation of expression of transgenes by the use of appropriate promoters is most important for durability and specificity of resistance. In most cases, resistance genes have been inserted with the constitutive promoters such as *CaMV35S*, maize ubiquitin or rice Actin 1, which direct expression in most plant tissues. Limiting the time and place of gene expression by tissue-specific promoters such as phenylalanine ammonia lyase (PHA-L) for seed-specific expression, RsS1 for phloem-specific expression or inducible promoters such as potato *pin*2 wound-induced promoter might contribute to the management of resistance development, and unfavourable interactions with the beneficial insects. For efficient pest control, it is important that effective levels of insect control proteins are expressed in the site where the insects feed. Greater risk of resistance build-up would arise from prolonged exposure to sublethal levels of the transgene product. Restricted expression in tissues may contribute to minimizing the yield penalty associated with the transgene expression[121,122]. There are specific situations where specific promoters would have a clear advantage such as root feeding insects.

Evolving levels of resistance in insects can also be dramatically reduced through the genetic engineering of chloroplasts in plants[123]. Transformed tobacco leaves

expressing *Cry2Aa2* protoxin at levels between 2 and 3% of total soluble protein, 20- to 30-fold higher than the current commercial nuclear transgenic plants, are effective against the resistant populations of *H. zea*, *H. virescens*, and *S. exigua*. Expressing high levels of a nonhomologous *Bt* protein should be able to overcome or at the very least, significantly delay broad-spectrum *Bt*-resistance development in the field.

Development of synthetics: One targetted deployment strategy is the development of synthetics. By incorporating various constitutively expressed Cry toxins into lines adapted for specific environments, synthetics can be formed quickly, which are effective against the pest complex, and are compatible with the natural enemies[14]. Once released to the farmers, the synthetics can be maintained as narrow-based populations at the farm level by removing the plants showing insect damage. Lines with resistance through the conventional HPR can also be included as a component in developing the synthetics to increase the durability of resistant germplasm. Pyramiding CryIA(b) insecticidal protein with high-terpenoid content should increase resistance to *H. virescens* and improve the durability of the *CryIA(b)* trait in commercial cotton[124]. Potato cultivars Russet and L235-4 were susceptible to *P. operculella*, while 54% mortality was observed when the larvae were fed on USDA8380-1 (HPR) leaves[125]. High levels of expression of CryV in the leaves of USDA8380 resulted in 96% mortality of *P. operculella*. These transgenic lines provide a germplasm base to combine insect resistance mechanisms and novel genes as a means to achieve durable host plant resistance.

Refugia: One of the main strategies to manage the deployment of resistance to *Bt* toxins is using high dose and production of refugia, in which certain percentage of the crop consists of non-*Bt* plants (4–20% in maize, and 20–40% in cotton). The non-*Bt* plants produce the susceptible insects, which have a probability of mating with those emerging from the *Bt* crops nearby, and thus dilute the frequency of the resistant individuals. The growers have a contractual obligation to grow the non-*Bt* crops. The refuges can be sprayed or unsprayed. In the latter case, the area under non-*Bt* crop has to be much larger than that under unsprayed conditions. Refugia can improve the durability of transgenic plants. The optimal spatial and temporal scale of refugia is likely to be unique for each insect–plant interaction. For refugia to be effective, they should be closer to the transgenic plants so that the moths produced in the transgenic plants have the opportunity to mate with the insects produced on the transgenic plants. The refugia in addition to diluting the frequency of resistance genes will also enhance the capacity of biological control agents. For polyphagous pests such as *H. armigera*, which feeds on several field crops and alternate hosts in the wild, there may not be any need to maintain the refugia in the SAT.

Separate refuges are superior to seed mixtures for delaying resistance. If a high toxin dose cannot be achieved, and a small fraction of homozygous susceptible and heterozygous European corn borer neonates survive on transgenic maize, then resistance can develop in 10 to 33% of the time required under the assumption of a successful high dose that kills all heterozygous neonates. The time to resistance development in general is significantly longer in regions where the same fields were used as a refuge year after year, compared with regions where the refuge fields are changed randomly from year to year. Larval movement between *Bt* and non-*Bt* plants may increase the rate at which resistance developed, but this may be ameliorated with increasing mortality costs associated with larval movement. Movement of *H. zea* larvae from nontransgenic to the transgenic plants may result in an increase in damage and reduce the yield in mixed stands of *Bt* and non-*Bt* plants[126]. In cotton, the number of eggs in terminals did not differ between mixtures and pure stands of transgenic or nontransgenic plants[127,128]. Planting two-row strips may be as good as separate refuges in delaying resistance, but their adoption carries greater risk because of the uncertainty surrounding the movement and survival of neonates[109]. Transgenic and nontransgenic plants could be grown in separate rows with a wider row spacing (strip planting) to minimize the rate of resistance development[129]. As the proportion of nontransgenic plants increased, the number of larvae and amount of injury increased.

Destruction of carryover population: Destruction of pupae or the carryover population (that has been exposed to *Bt* crops in the previous generations) from one season to another is an important component of resistance management. Ploughing the fields immediately after the crop harvest will expose the pupae of insects such as *Heliothis/Helicoverpa*, and *Spodoptera* to biotic and abiotic factors[130]. Destruction of stems or burning of stubbles of cereal crops will help in reducing the carryover of stem borer larvae. Therefore, appropriate agricultural practices need to be followed that reduce the carryover of pests from one season to another, including appropriate crop rotations, and observing a 'close-season'.

Control of alternate hosts: Removal of alternate hosts is required in case alternate hosts play an important role in pest population build-up[130]. Efforts should be made to remove the alternate hosts of the pests from the vicinity of the crop. This practice will help in reducing the

pests' density, and low to moderate levels of pest abundance can be effectively controlled by the transformed crops.

Use of planting window: Following a planting window, when the crop can escape pest damage or avoid peak periods of insect abundance, can also be useful in maximizing the benefits from transgenic crops or prolong the life of transgenic crops[130]. Observing a close season and planting the crop with first monsoon rains have been effective in controlling the damage by sorghum shoot fly (*Atherigona soccata*) and sorghum midge (*Stenodiplosis sorghicola*). Similar strategies can be employed to prolong the effectiveness of transgenic crops.

Use of economic thresholds and IPM: Crop growth and pest incidence should be monitored carefully so that appropriate control measures can be initiated in time. Care should be taken to use control options such as natural enemies, nuclear polyhedrosis virus (NPV), neem or entomopathogenic nematodes and fungi, which do not disturb the natural control agents. Use of pesticide formulations such as soil application of granular systemic insecticides and spraying soft insecticides such as endosulfan may be considered to suppress populations in the beginning of the season. Broad-spectrum and most toxic insecticides may be used only during the peak activity periods of the target pest. Efforts should be made to rotate pesticides with different modes of action, and avoid repetition of insecticides belonging to the same group or the insecticides that fail to give effective control of the pests.

Trasngenic crops are compatible with other methods of pest control. The number of injured flower buds, bolls and terminals, and the number of larvae in the pure stand of nontransgenic cotton did not differ following spraying for Lepidoptera control. A greenhouse test with transgenic cotton (NuCotn 33) containing the insect-resistant Bollgard R gene showed that the insect polyhedrosis virus AcNPV-Aalt acted additively with NuCotn 33 in reducing the bollworm damage, whereas, the transgenic cotton itself was sufficient to control tobacco budworm infestations[131]. Insects such as *H. virescens*, *H. zea*, *T. ni* and *S. exigua* are many times more sensitive to a subsequent Karate spray treatment when they have survived a prior exposure to *Bt*[132]. The enhanced insecticidal activity enables a more practical resistance management strategy for transgenic crops. The effect of toxic plants on the relative fitness of toxin-adapted and non-adapted larvae of *H. virescens* was not mediated by the fungus, *Nomuraea rileyi*[133]. Thus, transgenic crop can be used in conjunction with other methods of pest control without any detrimental or antagonistic effect.

Public attitude and economic viability

Farmers' perception of the genetically engineered plants is quite favourable because of reduced exposure of the farmers, farm workers and the environment to pesticides, which is considered as a major advantage of the transgenics[134]. However, there is a considerable unease in the general public about the transgenic crops. Most of the public concerns are about the safety of the genetically engineered crops, and possible adverse effects on the environment. The antibiotic gene used as a marker to select for gene transfer may lead to resistance in pathogens infecting human beings. However, the general scientific view is that the risk of compromising the therapeutic value of antibiotics is almost negligible. Methods have been developed for removing selectable marker genes after selection of the transgenics[135,136]. The new technologies should be tested rigorously for potential allergenic, toxic and antimetabolic effects in a transparent manner[137]. There is a need for introducing the technology through the peer-reviewed press than through the general media, balanced presentation of the technology to the general public, and an understanding of the trade-offs. The role of transgenics in reducing the load of pesticides in the environment needs to be considered seriously.

Public opinion is sharply divided on the benefits and uses of transgenic technology. There is a need to disseminate the information about potential uses and limitations of transgenic plants in crop production and their effects on the environment. Transgenic maize with *Bt* genes has been banned in European Union due to presence of bacterial promoter/antibiotic (ampicillin) resistance gene. The end result is a serious setback to the public acceptability of transgenic crops. Since many companies are involved in producing the synthetic insecticides, some of them may retard the process of using agricultural biotechnology, and may be partly responsible for adverse reaction of the public to the transgenic crops. There is a continued need for the governments and the NGOs to actively peruse this area of research and extension.

The benefits in terms of increasing the production and productivity of crops through the deployment of transgenic crops for pest management are very high. However, there is a potential gap between academic expectations and commercial reality in some cases, e.g. no commercial cultivars have been released by using *CpTI* genes, although its feasibility has been established long ago[4,138]. The chronic effects on insects and inconsistency in gene expression have meant that such a level of activity is not sufficient to lend confidence for comercialization. The chronic rather than acute effects of genes such as trypsin inhibitors and plant lectins means that such genes will find application at best in IPM pro-

rammes or be deployed in conjunction with *Bt* genes and insecticides.

Future prospects

The use of crop protection traits through transgenics will continue to expand in future and gene stacking will become very common, which may be related to transformation with two or more genes against the same trait or different traits. There will be considerable emphasis on agronomic traits such as fertilizer use efficiency, stress tolerance, photosynthetic efficiency, and grain yield and quality. 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 targetted. The development of a delivery system for insecticidal proteins from transgenic plants to the insect haemolymph will remove a key constraint in the transgenic approach to crop protection[4]. We need to pursue the management strategies that reflect the pest biology, insect–plant interactions and their effect on the natural enemies, to prolong the usefulness 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. Therefore, careful monitoring of population dynamics of the target pests and their natural enemies is essential for programmes aimed at limiting the exposure of the target pests to the transgenic plants, with the aim of continuously decreasing the abundance of the target pests.

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