Indian Journal of Agricultural Sciences **88** (6): 820–7, June 2018/Review Article

Bio-safety of *Helicoverpa*-resistant transgenic chickpea with *Bacillus thuringiensis* genes in the environment

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Received:1 February 2017; Accepted: 17 April 2018

ABSTRACT

With the advent of recombinant DNA technology, it has become possible to clone and insert genes into the crop plants to confer resistance to insect pests. Genes conferring resistance to insect pests have been inserted into several crops, and efforts are underway to develop transgenic chickpea with resistance to pod borer, *Helicoverpa armigera(Hubner)* – which causes an estimated loss of over $500 million annually. However, concerns have been raised regarding the impact of genetically engineered plants on the non-target organisms, gene flow, and biosafety of the food derived from genetically engineered plants. There is no issue related to gene flow involving transgenic chickpea as most of the studies have indicated little or <1% pollen flow to the neighboring plants. The effects of transgenic plants on the activity and abundance of natural enemies vary across crops, and the insect species involved. In general, there are no major adverse effects of genetically modified insect-resistant crops on the generalist predators such as *Cheilomenes sexmaculatus*, *Coccinella septempunctata*, and *Chrysoperla carnea*, while some adverse effects have been observed on the host specific parasitoids such as *Campoletis chlorideae*, which are largely due to early mortality of the host larvae or poor nutritional quality of the host, rather than direct toxicity of the transgene products to the natural enemies. 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 commonly used for pest management. While no specific studies have been carried out on the biosafety of food derived from transgenic chickpea due to unavailability of events with optimum gene expression for controlling *H. armigera*, there is enough information on the acute and chronic toxicity of *Bt* Cry proteins (Cry1Ab, Cry1Ac, and CryIIa), which have also been deployed in commercial transgenic maize and cotton. The issue of biosafety of the food derived from chickpea could also be addressed based on the principle of nutritional equivalence as and when the transgenic events are available for testing. Insect-resistant transgenic crops would play a significant role in pest management in future, and lead to a major reduction in insecticide sprays. Production and release of transgenic chickpea could be based on the guidelines laid down by the concerned agencies internationally, and the experience gained with other transgenic crops for improving food security and environment conservation.

Key words: Chickpea, *Helicoverpa armigera*, Transgenics, *Bacillus thuringiensis*, Biosafety, Environment

Chickpea (*Cicer arietinum* L.) is the world's third most important food legume, grown in tropical, sub-tropical and temperate regions, and is the premier pulse crop in the Indian subcontinent (Hulse 1991). It is valued for its nutritive seeds with high protein content (25.3 to 28.9%). Chickpea seeds are consumed fresh as a green vegetable, fried, roasted, and boiled as a snack food. The grain after dehulling is largely consumed as split seeds as 'dhal', or the split seeds are ground as flour, which is used to make bread, snacks, and sweets. The straw is used as feed for livestock. It is grown on about 10.38 million ha with a production of 8.57 million tonnes worldwide (FAO 2004). India is the largest producer as well as consumer of chickpea. In India, chickpea is grown on about 6.67 million ha with a production of 5.3 million tonnes (Majumder 2009). Chickpea yields are low (400 to 600 kg/ha), because of several biotic and abiotic constraints, of which the pod borer, *Helicoverpa armigera* (Hubner) is the most important constraint in chickpea production. In addition to chickpea, *H. armigera* also damages several other crops such as cereals, pulses, cotton, vegetables, fruit crops, and forest trees. It causes an estimated loss of US$ 2 billion annually, despite US$ 500 million worth of insecticides used to control this pest worldwide (Sharma 2005).

Bacillus thuringiensis (Berliner) (*Bt*) has been used extensively for the management of *H. armigera* in India, China, Philippines, Malaysia, and North America (Gujar

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2005). In addition to the use of *Bt* as a conventional pesticide, biotechnology holds a great promise for developing transgenic chickpea plants which constitutively produce δ-endotoxins from *Bt* for the control of insect pests. Development and deployment of insect-resistant transgenic chickpeas will lead to a drastic reduction in the number of insecticide sprays applied for pest control, increased activity of natural enemies, and reduced amounts of insecticide residues in the food and food products. Transgenic chickpea with *Bt* genes expressing either Cry1Ac or Cry2Aa, or both proteins for resistance to pod borer, *H. armigera* (Sanyal *et al.* 2005, Sharma *et al.* 2005, McPhee *et al.* 2007), and lectin gene expressing *Allium sativum* leaf agglutinin (ASAL), a mannose binding lectin for the control of cowpea aphid, *Aphis craccivora* Koch, another important pest of chickpea (Chakraborti *et al.* 2009), are currently under different stages of development and could become commercially available for resistance to these insects in future. To ensure a sustainable deployment of insect-resistant transgenic chickpea, it is important to study their effects on non-target organisms, and most importantly on the natural enemies of these pests.

Transgenic plants in pest management

Significant progress has been made over the past two decades in handling and introducing of novel genes into crop plants, and has provided opportunities to increase yields, impart resistance to biotic and abiotic stress factors, and improve nutrition. In addition to widening the pool of useful genes, genetic engineering has also allowed the use of several desirable genes in a single event, and thus reducing the time required to introgress novel genes into the elite background. Genes from *Bt* have been used successfully for pest control through transgenic crops on a commercial scale (Hilder and Boulter 1999, Sharma *et al.* 2004). *Bt* toxins are environmentally benign and their use will avoid numerous hazards of synthetic insecticides. Protease inhibitors, plant lectins, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins, and small RNA viruses can also be deployed through the transgenic plants alone or in combination with *Bt* genes for crop protection. The *Bt* toxin gene was cloned in 1981, and transgenic plants with insect resistance were produced in 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 several crops such as maize, cotton, potato, tobacco, rice, broccoli, lettuce, walnuts, apples, alfalfa, and soybean. The first transgenic crop was grown in 1994, and large-scale cultivation was taken up in 1996 in USA (McLaren 1998). The area under commercial cultivation of genetically modified crops has now increased from 1.7 million ha in 1996 to over 179.7 million ha in 2015 by 28 countries across the world (James 2016). So far four major traits, viz. insect resistance, herbicide tolerance, disease resistance, and virus resistance, either stacked or individually, have been commercially deployed, of which transgenic crops with these biotic stresses are grown on more than 76 million ha worldwide (James 2016). However, several other crops such as brinjal, sorghum, chickpea, pigeonpea, cabbage, maize, and rice are at different stages of commercialization in India. The development and deployment of transgenic crops with insect resistance will lead to:

- a major reduction in insecticide sprays,
- reduced exposure to insecticides,
- increased activity of natural enemies,
- reduced amounts of insecticide residues, and
- a safer environment to live.

The benefits to growers will be higher crop yields, lower cost of cultivation, and ease of management. The primary benefit to growers of adopting transgenics for controlling insect pests will be the ability to control the insect species that have become resistant to commonly used insecticides. Pesticide use data has shown a considerable reduction in insecticide applications since the introduction of *Bt* cotton. However, transgenics are not a panacea for solving all the pest problems. The major limitations of using transgenic plants are:

- secondary pests may assume a major pest status,
- control of secondary pests will kill the natural enemies,
- proximity to sprayed fields and insect migration may reduce benefits of transgenics, and
- development of resistance in insect populations may limit the usefulness of transgenics.

Effects of insect-resistant transgenic crops on non-target organisms

The bulk of evidence to date suggests that *Bt* crops are highly selective and the negative effects, if any, are relatively minor in magnitude. The tangible benefits of improved conservation of natural enemy populations in *Bt* crops have been demonstrated in several systems as a result of reduced and selective insecticides, and improvements in other pest management tactics. However, one of the major concerns of transgenic crops is their effects on the non-target organisms; and the efficacy of transgenic crops for controlling the target and the non-target insect pests, and their effects on the natural enemies need to be determined on a regional basis. The significance of such effects would depend on the importance of the immature stages of the target insect for maintaining the populations of the natural enemies. Within field impact may be greater for parasitoids that are monophagous. Finally, the effect of transgenic crops on the abundance of natural enemies should be compared with the non-transgenic fields of the same crop where the natural enemies may be virtually absent because of heavy pesticide application.

The information that use of genetically modified corn may have toxic effects on larvae of the monarch butterfly, *Danaus plexippus* (L.) (Losey *et al.* 1999), has generated a huge amount of publicity, and almost as much misinformation. Wraight *et al.* (2000) reported that there is no relationship between mortality of *Papilio polyxenes* Fab. and pollen deposition from transgenic maize on its host plants. Pollen from the transgenic plants failed to cause any mortality under laboratory conditions. Overall, the studies have revealed that the impact of the current *Bt* maize varieties on monarch butterfly populations is negligible (Gatehouse *et al.* 2002). Then onwards, considerable information has been generated on the relative efficacy of transgenic crops against the target insect pests and their nontarget effects in USA, Australia, and China (Pray *et al.* 2002, Wu *et al.* 2003, Naranjo 2009), but very little information is available till date on the risks of *Bt*-transgenics on loss of susceptibility to *Bt* toxins in target pests, disruption of ecosystem processes, and direct or indirect effects on nontarget organisms and biodiversity on long-term basis in India, and needs a greater attention to harvest the benefits of *Bt* technology (Dhillon and Sharma 2009b). Another serious concern is that the Cry proteins produced by *Bt*-transgenic plants persist in agricultural soils for an extended period of time, and may pose a hazard for non-target soil organisms. However, a soil specific fungal strain, *Chrysosporium* sp. has been identified, which is capable of producing proteases that degrade the Cry1Ac toxin into inactive products in a way that it looses its insecticidal activity against *H. armigera* (Padmaja *et al.* 2008). The risk that transgenic plants pose to the nontarget organisms is a function of feeding behavior, expression of the transgene in the plant, mode of exposure to the insecticidal toxin, and the toxicity of the toxin towards the specific organism, and the major effect of transgenic crops on the activity of non-target organisms have been summarized below.

Predators: Of the nontarget insects, the generalist predators are less exposed to the transgene product as it is likely that not all of the prey will be contaminated. However, it is difficult to assess the effects of transgenic plants on the abundance of generalist predators, whose populations fluctuate in repeat cycles of several generations up to 20 years. Therefore, it is necessary to differentiate them from the host specific parasitoids, which are more likely to be affected by the toxins if the insect host acquires the toxins from the plants. Some observations have suggested that there may be a reduction in the fitness of the predatory chrysopid larvae, *Chrysoperla carnea* (Stephens) directly attributable to *Spodoptera littoralis* (Boisduval) fed on *Bt*-maize (Hilbeck *et al*. 1998, Romeis *et al.* 2004). However, *C. carnea* larvae were not affected when fed on *Bt*-maize reared aphids, or through the *Bt*-maize reared spider mites, *Tetranychus urticae* (Koch), even though the spider mites had much more amounts of Cry1Ab toxin than the lepidopteran larvae (Dutton *et al.* 2002). No adverse effects of *Bt*-transgenic crops have been reported on the development, survival, and reproduction of ladybeetles, *Coleomegilla maculata lengi* Timb., *Hippodamia convergens* (Guérin-Méneville), and *Propylaea japonica* Thunberg through their aphid preys on *Bt*-transgenic crops (Dongan *et al.* 1996, Duan *et al.* 2002, Lundgren *et al.* 2002, Zhu *et al.* 2006). However, poor prey quality and Cry1Ac toxin mediated negative effects have been observed on the predatory beetle, *P. japonica* when fed on young *Spodoptera litura* Fab. larvae fed on *Bt*-transgenic cotton (Zhang *et al.* 2006b). Such negative effects of *Bt* toxins on the coccinellid, *Cheilomenes sexmaculatus* (L.) were also observed when fed on young *H. armigera* larvae reared on *Bt*-ammended artificial diet, or when exposed to Cry1Ab and Cry1Ac *Bt* toxins in 2M sucrose, indicating that these adverse effects might be due to *Bt* toxins processing and poor quality of the insect host (Dhillon and Sharma 2009a). Furthermore, it is also possible that the ladybird, *C. sexmaculatus* might ingest Cry1Ab or Cry1Ac *Bt* toxins expressed in transgenic plants through leaf exudates, honeydew produced by aphids feeding on these plants, and indirectly through herbivores such as *A. craccivora*, *H. armigera*, *Spodoptera litura*, *exigua* (Hub.) or some other lepidopteran larvae, soft bodied insects feeding on transgenic chickpea plants (Dhillon and Sharma 2009a).

Direct negative effects of plant lectins and protease inhibitors have also been reviewed for arthropods belonging to the orders of Coleoptera, Lepidoptera, Acaridae, Orthoptera and Neuroptera (Dhillon *et al.* 2008). Birch *et al.* (1999) reported that the fecundity, egg viability and longevity of ladybirds significantly decreased when feeding on aphids reared on GNA-transgenic potatoes. GNA appears to bind to mid-gut epithelial cells of ladybird larvae, which might cause irreversible damage. Laboratory studies have also shown direct toxic effects of plant lectins, GNA and avidin on the insect predators - *C. carnea*, *Adalia bipunctata* (L.), and *Coccinella septempunctata* (L.) (Hogervorst *et al.* 2006, Lawo and Romeis 2008, Dhillon *et al.* 2008), suggesting that additional tests under more realistic exposure conditions need to be conducted for the safe deployment of lectin-transgenic plants. Although, no major differences have been observed in the abundance of predators, viz. coccinellids, chrysopids, spider, etc., in the fields with *Bt*-transgenic and non-transgenic cotton under Indian conditions (Sharma and Pampapathy 2006, Sharma *et al.* 2007, Dhillon *et al.* 2009b), but the effects of transgenic plants on the activity of predators vary across crops, type and nature of gene expressed in the transgenic plants, and the insect species involved. Therefore, long term field studies need to be conducted to characterize the unintended effects of transgenic chickpea plants as and when available for commercial cultivation.

Parasitoids: Parasitic wasps are sensitive to changes in the nutritional quality of their hosts, as host-parasitoid relationships are usually quite intricate. It is thus not surprising that the parasitoid activity is affected when their hosts are affected by the *Bt* protein. Sub-lethal effects of *Bt* proteins on the host larvae may reduce their nutritional quality for the parasitoid, and poor nutritional quality of the host results in detrimental effects on their development and survival. Considerable information is available on the host-mediated effects of *Bt*-transgene/transgenic crops on the parasitoids (Romeis *et al.* 2006, Sharma *et al.* 2007, Sharma *et al.* 2008, Dhillon and Sharma 2010). Sub-lethal doses of *Bt* toxins have shown negative effects on the development and survival of the parasitoids as a result of poor nutritional quality of the insect host and early mortality of the insect host, rather than direct *Bt* toxicity (Sharma *et* *al.* 2008, Dhillon and Sharma 2010). Such negative indirect effects of continuous exposure of host lepidopteran larvae to *Bt*-transgenic cotton under field conditions have earlier been reported on larval parasitoids, *Cotesia marginiventris* (Cresson), *Copidosoma floridanum* (Ashmead) (Baur and Boethel 2003), and *C. chlorideae* (Liu *et al.* 2005, Sharma *et al.* 2007). Zhang *et al.* (2006) observed low effects of *Bt* on *C. chlorideae* attacking Cry1Ac-resistant *H. armigera* larvae treated with the HD-73 strain of *Bt* containing only 44% of Cry1Ac. This might be because of sub-lethal dose of the *Bt* toxin on which more number of *Bt*-resistant *H. armigera* larvae survived resulting in low effects on survival of the parasitoid. Furthermore, transgenics may reduce the number of certain natural enemies in areas planted with transgenic crops, but their populations may be maintained on the other crops that serve as a host to the target pests (Dhillon and Sharma 2007). Different studies have shown that aphids reared on a GNA-containing artificial diet have a detrimental effect on aphid parasitoids such as *Aphelinus abdominalis* (Dalman) and *Aphidius ervi* Haliday (Couty *et al.* 2001a, b). A reduced longevity and fecundity was reported for the aphid parasitoids, *Aphidius colemani* Viereck and the caterpillar parasitoid, *Eulophus pennicornis* (Nees) when feeding a GNA-sucrose solution (Romeis *et al.* 2003, Bell *et al.* 2004). The *Allium sativum* leaf lectin (ASAL) proteins have also shown some adverse effects on the fitness and survival of *C. chlorideae*, but these effects were again indirect through host insect, rather than direct effects (Arora *et al.* 2007). Although, *Bt*-transgenic chickpea has been found compatible with entomopathogenic fungus, *Metarhizium anisopliae* for the management of *H. armigera* (Lawo *et al.* 2008), compatibility of *Bt*-transgenic chickpea with *H. armigera* larval parasitoid, *C. chlorideae*, and ASAL-transgenic chickpea with *A. craccivora* predator, *C. sexmaculatus* have not been tested yet, which might influence its activity and abundance in the chickpea ecosystem, and need to be tested in more realistic conditions as and when these transgenic plants are available.

Fauna and flora in the rhizosphere: Potential effects of genetically transformed crops on non-target species are not restricted only to the environment above ground, but also on those inhabiting the soil rhizosphere (Jepson *et al*. 1994). Some genetically engineered crops affect soil ecosystems (Griffiths *et al.* 2000), but the long-term significance of any of these changes is unclear. This may decrease the rate of plant decomposition and of carbon and nitrogen levels thus affecting soil fertility. Toxins from the transgenic plants are introduced into the soil primarily through incorporation of the crop residues into the soil after crop harvest or through the root exudates (Saxena *et al*. 1999). No effects have been detected in culturable bacteria, fungi, protozoa, and nematodes from the *Bt*-maize fields (Saxena and Stotzky 2001). 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. It is unlikely that expression of *Bt* and any other genes in transgenic plants would have an adverse effect on the soil microflora. There are no significant differences in mortality or weight or earthworms (*Lumbricus terrestris*) after 40 days in soil planted with *Bt* maize or after 45 days in soil amended with *Bt* maize. Toxin has been detected in the gut and casts of earthworms, but is cleared in 2 to 3 days after being placed in fresh soil.

DEVELOPMENT OF RESISTANCE AND RESISTANCE MANAGEMENT

Insect pest populations have shown a remarkable capacity to develop resistance to chemical pesticides, and over 500 species of insects have developed resistance to insecticides. Extensive and intensive exposure of pests to *Bt* toxins through transgenic crop plants may lead to development of resistance to *Bt* toxins. Laboratory screening has resulted in the development of *Bt* resistant populations in Lepidoptera, Coleoptera, and Diptera (Tabashnik 1994). This highlights the fact that possibilities for resistance development are real. In India, small farm holdings may not necessarily follow refuge norms. Adherence to refugia strategy in India is generally poor and Manjunath (2005) and Dhillon and Sharma (2007) argue that, much like the situation in China, there are sufficient alternate bollworm/pod borer hosts such as chickpea, pigeonpea, sorghum, tomato, etc, are present to fulfill the refuge requirement without active participation by growers. However, developmental asynchrony of bollworms on *Bt* transgenic and *Bt* sprayed crops due to variation in toxin expression, weather, and overlapping generations favor assortative mating among resistant moths from *Bt* plants (Perez *et al.* 1997, Liu *et al.* 1999), and help development of *Bt* resistance in target insect pests. Furthermore, if more host crops of *H. armigera* with *Bt*-transgene are commercialized, the chances of development of resistance to *Bt* will increase, and need careful attention and resistance management policy, including refugia strategy. In addition to use of refuge and other tactics for pre-empting resistance, India has active program to monitor for the development of resistance in target pests to *Bt* toxins and/or have developed baseline toxicity to various Cry proteins (Kranthi *et al.* 2005a, Mahon *et al.* 2007). Baseline susceptibility studies and the ability of *H. armigera* to develop resistance to *Bt* to some extent have justified the fears of likelihood of development of resistance under field conditions. However, monitoring of insects in regions with high adoption of *Bt* crops has not yet led to detection of resistance in field populations of target insect pests, except for evolution of resistance in pink bollworm to *Bt*-cotton expressing *cry1Ac*, in four districts of Gujarat, viz. Amerli, Bhavnagar, Junagarh, and Rajkot in Gujarat, India (Hindustan Times 6 March 2010). There is significant variation in tolerance of *H. armigera* to different Cry toxins, which may be attributed to the differences in genetic make up of the populations from different locations and host crops, temperature, and agroclimatic conditions (Gujar *et al.* 2010). Although, increases in the frequency of resistance alleles caused by exposure to *Bt* crops in field have not yet been documented, there is a need to take critical look at the potential for development of resistance to *Bt* transgenic crops and develop strategies to deploy different *Bt* toxins alone or in combination with other novel genes and plant traits associated with resistance to insect pests in different crops.

A number of conceptual strategies have been developed for resistance management, and the high dose-refuse strategy is the currently recommended resistance management strategy, which is based on high level of toxin expression in the *Bt* plant, recessive inheritance of *Bt* resistance trait, and low occurrence of resistance allele frequency in the wild field population of the target insect pests (Gould 1998, Christou *et al.* 2006, Kranthi *et al.* 2006, Mahon *et al.* 2007, Gujar *et al.* 2008, 2010). In India, permission for environmental release of *Bt* cotton was on the recommendation that there should be 20% of the *Bt* crop or five rows of non-*Bt* cotton as a refuge should be grow on the edge of the *Bt* field. In case same toxin genes are deployed in different crops (cotton, maize, chickpea, and pigeonpea), it is important to implement planting of refuge crops by the farmers. At the same time, researchers must consider deploying different genes or combinations of different genes in different crops to minimize the risk of development of resistance in pest populations, mainly the major pest, *H. armigera*. In addition, it will be important to deploy *Bt* genes in combination with resistance genes from the cultivated germplasm and the wild relatives of chickpea (Sharma 2009).

Gene flow

One of the risks of growing transgenic plants for pest management is the potential spread of the transgene beyond the target area (Chevre *et al.* 1997). Genes from unrelated sources may change the fitness and population dynamics of hybrids between native plants and the wild species. Plant breeding efforts, in general, have tended to decrease rather than increase the toxic substances, as a result, making the improved varieties more susceptible to insect pests. However, there is a feeling that genes introduced from outside the range of sexual compatibility might present new risks to the environment and humans, and lead to development of resistance to herbicides in weeds, and to antibiotics. While some of these concerns may be real, the others seem to be highly exaggerated. There are no records of a plant becoming a weed as a result of plant breeding (Cook 2000). This may be because of low risk of crop plants to the environment, extensive testing of the crop varieties before release and adequate management practices to mitigate risks inherent in crop plants.

Chickpea pollen is disseminated by insects. The flowers are visited by butterflies, honeybees, solitary bees, and bumblebees (Malhotra and Singh 1986, Free 1993, Tayyar *et al.* 1996). Chickpea pollen formed at low temperature is usually sterile, and most current cultivars will not set pods if the average daily temperature is below 15ºC (Savithri *et al.* 1980, Srinivasan *et al.* 1998, 1999, Croser *et al.* 2003). There is no information on the longevity of chickpea pollen. There is no published information on distance of pollen flow in chickpea. However, several experiments have been conducted to estimate outcrossing rates between adjacent plants, but the cross-fertilization in chickpea is below 1%. The highest outcrossing rates reported were between 1.25% (Toker *et al.* 2006) to 1.92% (Gowda 1981). The isolation distance for chickpea seed production is 3 m in the United States (CCIA 2009). In OECD countries, chickpea varieties for seed production should be isolated from other crops by a definite barrier or a space sufficient to prevent mixture during harvest (OECD 2008). Cultivated chickpeas does not exhibit seed dormancy, but some wild *Cicer* species do (Singh and Ocampo 1997). Chickpeas are not known to occur in the wild, but volunteer plants may appear as weeds in subsequent cropping cycles, but does colonize successfully without human intervention.

Bio-safety of food from transgenic chickpea

Genetically modified plants have been released in different countries, but the regulations governing the use of transgenic plants vary considerably. Existing regulations have been applied to the production and release of genetically modified plants, but have not been adequate to address the potential environmental effects from the transgenic plants. The biosafety issues related to the deployment of transgenic plants include risks for animal and human health such as toxicity and food quality/safety, allergies, and resistance to antibiotics. The need and extent of safety evaluation may be based on the comparison of the new food and the analogous food, if any, and the interaction of the transgene with the environment (Cook 2000). Foods derived from genetically modified plants are now appearing in the market and many more are likely to emerge in the future. The safety, regulation, and labeling of these foods are still contentious issues in most countries (MacCormick *et al.* 1998). Several studies have focused on understanding the intended alterations in composition of food crops that may occur as a result of the genetic modification (Kuiper *et al.* 2003). The *Bt* maize and its constituent products are nutritionally equivalent to the conventional non-transgenic crops, as it does not include detectable protein or DNA derived from the genetically modified plant (Berberich *et al.* 1996, Zdunczyk 2001, George *et al.* 2004). The relationship between quantity of genetically modified foods and DNA is linear if genetically modified DNA is nuclear, but there is no such correlation between the quantity of genetically modified food and the RNA or protein. These comparisons, together with the history of the safe use of corn as a common component of animal feed and human food support the conclusion that *Bt* corn is compositionally equivalent to, and as safe and nutritious as, conventional corn hybrids grown commercially. Therefore, results available to date are reassuring and have revealed no significant differences in the safety and nutritional value of food derived from the 1st generation of genetically modified plants in comparison with non-transgenic varieties (Flachowsky *et al*. 2005). In order to implement the regulations governing the production and marketing of food derived from genetically modified organisms, there is a need to develop and standardize technology to detect such foods (Holst-Jensen 2006).

Food labeling

It is important to ensure food safety at production and testing stages, as it is difficult to address this issue in the unorganized sector. In addition, a requirement to label approved genetically modified food would necessitate a constant monitoring system, and the cost of such monitoring system will be formidable for many developing countries.

Conclusions

The application of biotechnological tools holds a great potential for alleviating some of the major constraints to crop productivity in developing countries. Natural enemies play an important role in suppressing pest populations. To ensure a sustainable deployment of transgenic insect-resistant plants, it is important that they are compatible with other control methods, including biol-ogical control. Therefore, there is a need to understand the effects of transgenic crops on the target and non-target insect pests, and the beneficial natural enemies under laboratory and field conditions. Such information will be useful for understanding the influence of transgenic plants on the non-target organisms, and sustainable deployment of transgenic crops for crop protection.

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