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GENETICALLY MODIFIED ORGANISMS FOR PEST MANAGEMENT: ENVIRONMENTAL IMPACT

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ABSTRACT

Recombinant DNA technology has been exploited to develop genetically modified organisms (GMOs) for pest management that retain the advantages of classical biological control agents, but have fewer or none of their drawbacks. Genes conferring resistance to insects, particularly the δ-endotoxin genes from the bacterium, Bacillus thuringiensis *have been inserted into several crop plants, of which insect-resistant cotton, maize, rice, tomato, and potato have deployed commercially on a large-scale for pest management. Genetic engineering techniques can also be used for producing robust natural enemies, and more stable and virulent strains of bacteria, fungi, viruses, protozoa, and nematodes for use in integrated pest management. Deployment of insect-resistant transgenic plants for pest control will lead to a substantial reduction in insecticide use, reduced exposure of farm labor to insecticides, reduction in harmful effects of insecticides to nontarget organisms, and reduced amounts of insecticide residues in food and food products. Gene introgression through transgenic approach could also be beneficial in the sense that it adds diversity to the genetic pool of the crop plants. However, transgenics are not a panacea for solving all the pest problems, and concerns regarding the biosafety of GMOs to the environment are still inconclusive, and there is a continuous debate regarding their nontarget effects in the environment. There is a concern that large-scale deployment of GMOs for pest management might influence*

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the activity and abundance of nontarget herbivore arthropods, natural enemies, fauna and flora in the rhizosphere and aquatic systems, and toxin flow in the insect fauna through different trophic levels. In addition, the transgene might introgress into closely related wild relatives of crops through pollen resulting in production of more robust weeds, and between plants, bacteria, and viruses resulting in development of resistance to antibiotic/herbicide genes used as markers. However, we should also consider the risk of not using GMOs for pest management and crop production, when the need to increase food production is most urgent. Genetically modified organisms to be deployed for pest management should be commercially viable, environmentally benign, easy to use in diverse agro-ecosystems, and have a wide-spectrum of activity against the target insect pests, but harmless to nontarget organisms. Rapid and cost effective development and adoption of biotechnology-derived products will depend on developing a full understanding of the interaction of genes within their genomic environment, and with the environment in which their conferred phenotype interacts for sustainable crop protection.

INTRODUCTION

There has been a significant progress in handling and introduction of exotic genes into crop plants over the past three decades, and this has provided new opportunities to genetically modify plants to increase crop yields, impart resistance to biotic and abiotic stresses, and improve nutrition. So far, four major traits, *viz.* insect resistance, herbicide tolerance, disease resistance, and virus resistance, either stacked or individually, have been deployed, and are under commercial cultivation in 10 different crops in several countries. Commercial cultivation of genetically engineered crops has increased from 1.7 million ha in 1996 to over 148 million ha in 2010, being grown by over 15.4 million farmers in 16 developing and 9 industrialized countries of the world (James 2010). The soil bacterium, *Bacillus thuringiensis* (*Bt*) (Berliner) has been one of the most important source of genes conferring resistance to insect pests. More than 150 Cry toxins from *Bt* have been cloned and tested for their toxicity to various insect species, especially Lepidoptera, Coleoptera, and Diptera (Crickmore et al. 2011). Vegetative insecticidal proteins (*Vip*) from *Bt*, protease inhibitors from plants and insects, plant lectins, ribosome inactivating proteins, amylase inhibitors, chitinases, secondary plant metabolites, avidins, enzymes, neurotoxins from arthropods, and neurohormones from insects are the other potential genes for engineering insect resistance into crop plants (Liener and Kakade 1969; Green and Ryan 1972; Ryan 1990; Gatehouse et al. 1993; Chrispeels et al. 1998; Gatehouse and Gatehouse 1998; Sharma et al. 2004), and are useful for developing insect-resistant transgenic plants for pest management. In addition to widening the pool of useful genes, genetic engineering also allows the use of several desirable genes in a single event, and thus reducing the time required to introgress novel genes into the elite background.

THE SUCCESS IN DEVELOPMENT AND DEPLOYMENT OF INSECT-RESISTANT TRANSGENIC CROPS

The *Bt* toxin gene was cloned in 1981, and the first transgenic plants were produced by mid-1980s (Barton et al. 1987; Fischoff et al. 1987; Vaeck et al. 1987). Since then, *Bt* genes conferring resistance to insects have been cloned and inserted into several crop plants such as maize, rice, wheat, sorghum, sugarcane, cotton, potato, tobacco, broccoli, cabbage, chickpea, pigeonpea, cowpea, groundnut, tomato, brinjal, and soybean, (Hilder and Boulter 1999; Sharma 2009; ILSI Research Foundation 2010). The *Bt* genes encoding *cry1Ac, cry2Ab2, cry1Ab, cry1C, cry1A, vip3A(a), cry1F, flcry1Ab, cry9C, cry34Ab1, cry35Ab1, cry3Bb1, cry1A.105,* and *cry3A* have been expressed in several crop plants for conferring resistance to different insect pests, and have been deployed successfully on a commercial scale for pest control (CBD 2010; ILSI Research Foundation 2010). Till date, a total of 14 *Bt* genes have been commercially deployed worldwide through 31 events of genetically modified cotton, 40 events of maize, three events of rice, one event of tomato, and 28 events of potato for the management of lepidopteran and coleopteran insect pests (Table 1), alone or stacked with herbicide resistance genes. The insect-resistant transgenic crops are now grown on more than 45 million ha in several countries of the world (James 2010).

Table 1: Commercially deployed *Bacillus thuringiensis* genes through genetically modified crops for resistance to insect pests.

Crop	No. of events	Insect pests	Gene(s)
Cotton	31	Lepidopteran	*cry1Ac, cry2Ab2, cry1Ab, cry1C, cry1A, vip3A(a), cry1F,* and *flcry1Ab*
Maize	40	Lepidopteran and Coleopteran	*cry1Ab, cry1Ac, cry9C, cry34Ab1, cry35Ab1, cry1F, cry3Bb1, cry1A.105, cry2Ab2, cry3A,* and *vip3Aa20*
Rice	3	Lepidopteran	*cry1Ac* and *cry1Ab*
Tomato	1	Lepidopteran	*cry1Ac*
Potato	28	Coleopteran	*cry3A*

Source: CBD (2010); ILSI Research Foundation (2010).

In India, the major focus of genetic engineering technology in agriculture has been on producing insect-resistant transgenic crops for controlling lepidopteran insects, which cause heavy losses in agriculture. To overcome bollworm problem in cotton, approval was granted for commercial cultivation of *Bt*-transgenic cotton in 2002. The primary target of the first generation (single gene) transgenic cottons in India was to control bollworm complex [*Helicoverpa armigera* (Hubner), *Pectinophora gossypiella* (Saunders), and *Earias* spp.]. To have a broad spectrum of activity against the lepidopteran pests, including insect pests not controlled effectively by a single gene and improve the efficacy against bollworm complex, stacked or dual gene (*cry1Ac,* *cry1Ab*, and *cry2Ab*) transgenic cottons (Bollgard II) were introduced in 2006. At present, both single and dual gene *Bt* cottons are under commercial cultivation in India, wherein over 70% of the transgenic cotton is planted with dual stacked *Bt* genes. India ranks first in cotton acreage (11.1 m ha) occupying about 34% of the global cotton area (35 m ha), of which 10.5 m ha is under *Bt* cotton. So far, five *Bt* genes through six events such as *cry1Ac* through MON531 (Monsanto), truncated *cry1Ac* through Event-1 (JK Agri Genetics Ltd.) and a variety Bikaneri Nerma (UAS Dharwad and ICAR), *cry1Ac* + *cry2Ab* through MON15985 (Monsanto), *cry1Ab* + *cry1Ac* through GFMCry1A (Nath Seeds Ltd.), and *cry1C* through event 9124 (Metahelix) have been approved for cultivation. Based on these six events, a total of 809 cotton hybrids and varieties have been approved for cultivation by the genetic engineering and approval committee between 2002-2010 (IGMORIS 2010). Furthermore, *Bt* cotton with herbicide tolerant gene (Roundup Ready) is also under field testing in India. The *Bt* genes have also been inserted in several other crops such as maize, rice, sorghum, brinjal, tomato, cabbage, cauliflower, sugarcane, chickpea, and pigeonpea for pest management, and are under different stages of testing (Table 2).

ENVIRONMENTAL BENEFITS OF GENETICALLY MODIFIED CROPS

In general, genetically modified organisms (GMOs) have a better predictability of gene expression than the conventional breeding methods, and transgenes are not conceptually different than the use of native genes or organisms modified by conventional technologies. However, the developments in plant biotechnology have both promise and problems. The benefits of growing transgenic crops have been higher yield, lower input costs in terms of pesticide use, reduction in harmful effects of insecticides on nontarget organisms, reduced amounts of insecticide residues in food and food products, and easier crop management (Qaim and Zilberman 2003; Anonymous 2009; Sharma 2009). Adopting transgenic crops also offers the additional advantage of controlling the insect pests that have developed resistance to insecticides. On the other hand, the potential of recombinant technologies to allow a greater modification than is possible with the conventional technologies may have a greater bearing on the environment (Tiedje et al. 1989; Sharma and Ortiz 2000).

Advances in various pest management technologies through genetic engineering have reduced the use and harmful environmental effects of insecticides (Brookes and Barfoot 2006), and most prominent amongst these has been the use of *Bt* genes to enhance protection against some of the most serious insect pests such as cotton bollworms and cereal stem borers. The use of genetically modified crops, which have biological activity against selective insect pests, qualifies as one of the most effective components of pest management. One of the most obvious effects in production practices with the introduction of *Bt* crops has been the change in insecticide use

Table 2: Bt-transgenic crops under different stages of deployment in India in 2010.

Crop	Trait (s)	Gene(s)	Event(s)	Status	Institution
Maize	IR	*cry1Ab*	Event *Bt11*	BRL-1	Syngenta Biosciences Pvt. Ltd.
	IR	*cry1F*	Event TC 1507	BRL-1	Dow AgroSciences India Pvt. Ltd.
	IR andHR	*cry2Ab2* + *cry1A.105* and *CP4EPSPS*	MON 89034 and NK603	BRL-1	Monsanto India Ltd.
	IR and HR	*cry1F* & *PAT* and *CP4EPS PS* genes	(TC1507 x NK603) and (DAS-01507-1 x MON-00603-6)	BRL-1	Pioneer Overseas Corporation Ltd.
Rice	IR	*cry1Ab, cry1Ca* and *bar c*	56 events	ES	Bayer Biosci. Pvt. Ltd.
		ry1Ac and *cry1Ab*	566 events	ES	Metahelix Life Sci Pvt. Ltd.
		Constructs RPD5, RPD8, RPD10, and RPD11; and AT-CDKB1	139 events	ES	BASF India Ltd.
Tomato	IR	*cry1Ac*	8 events	ES	ICAR
Cabbage	IR	*cry1Ba, cry1Ca* and *bar*	3 events	ES	Nunhems India Pvt Ltd
Cauliflower	IR	*cry1Ba, cry1Ca* and *bar*	3 events	ES	Nunhems India Pvt Ltd
Sugarcane	IR	*cry1Ab*	10 events	ES	ICAR
Brinjal	IR	*cry1Ac*	-	ES	ICAR
Cotton	IR	*cry1Ac* and *cry1F*	Event 3006-210-23 and Event 281-24-236	BRL-1	Dow AgroSciences India Pvt. Ltd.
		cry1Ac and *cry1EC*	Event-1 and Event-24	BRL-1	JK Agri Genetics Ltd.
		cry1Ac	ILK-*Bt* 77-1 to 7 and Anjali-Ac*Bt*-1 to 3	ES	ICAR
		cry1F	Anjali-*FBt*-1 and 2	ES	ICAR
		cry1Ac	G-822-*Bt* and PA255-*Bt*	ES	ICAR

IR = Insect resistance, HR = Herbicide resistance, BRL = Biosafety research level, ES = Event selection. *Source*: http://igmoris.nic.in/field_trials.asp 2010.

patterns, where India experienced <4% reduction in the Environmental Impact Quotient (EIQ), while Australia (22%), USA (23%), and China (28%) realized a greater reduction in EIQ (Naranjo 2009). In China, *Bt* cotton growers have reduced pesticide spraying for cotton bollworm control from 20 to 6 times per year, and there is a concomitant reduction in cost of cultivation over the non-transgenic cotton by 28% (Huang et al. 2002). Similar benefits of *Bt* cotton have also been observed in India, Australia, Mexico, Argentina, South Africa, and Indonesia. Adoption of transgenic crops also offers the additional advantage of controlling insect pests that have become resistant to commonly used insecticides. Total pesticide market in India in 1998 was valued at US$770 million, of which 30% was used in cotton only (Indian Chemical Industry 2007). Subsequent to the introduction of *Bt* cotton, the sharpest decline in insecticides use occurred in cotton. The cost of insecticides used in cotton declined from US$147 million in 1998 to US$65 million in 2006, showing a 56% decrease in pesticide use (9,000 tons of active ingredient equivalent to a saving of US$82 million) (Anonymous 2009). India ranks first in the world in acreage under *Bt*-cotton, covering 10.5 million ha out of the total cotton area of about 11.1 million ha (representing >90% of total area under cotton in 2010), with a record production of about 34 million bales of seed cotton. Cotton production has almost doubled with the introduction of *Bt*-transgenic cotton in India (Gujar et al. 2010). On an average, the farmer's using single gene *Bt*-cotton earned Rs 8,669 (US$ 222), while the farmers using BGII (with stacked genes) gained Rs 10,009 (US$256) as additional income per acre as compared to the conventional cotton farmers (IMRB International 2008). At the national level, *Bt* cotton farmers gained US$288 million (Rs 1,127 crores) from reduced pesticide usage, and contributed US$ 3.23 billion (Rs 12,608 crores) as additional income to the Indian economy in 2007 (IMRB International 2008). The field trials with *Bt*-rice in China have indicated that *cry1Ab / Ac* rice could reduce insecticide application by 60% as compared to the non-*Bt* rice (Huang et al. 2005; Huang and Hu 2007; Huang et al. 2008; Wang et al. 2010), and this trend is similar to that reported for *Bt*-cotton and *Bt*-corn (Brookes and Barfoot 2009).

DEVELOPMENT AND EFFECTIVENESS OF GENETICALLY MODIFIED PLANTS FOR PEST MANAGEMENT

The *Bt*-transgenic crops are quite effective in reducing the numbers of, and damage by the target insect pests. Current commercial insect-resistant GM plants mainly rely on the production of toxin proteins derived from the *Bt*, and are effective for controlling a limited number of insect pests. The *Bt* maize confers resistance against European corn borer, *Ostrinia nubilalis* (Hubner); corn earworm, *Helicoverpa zea* (Boddie); south-western corn borer, *Diatraea grandiosella* Dyar; fall armyworm, *Spodoptera frugiperda* J.E. Smith; pink maize borer, *Sesamia calamistis* Hampson; common stalk borer, *Papaipema nebris* Guenee; corn rootworm complex, *Diabrotica* spp.; and

Chilo partellus (Swinhoe) (Orr and Landis 1997; Lynch et al. 1999; Archer et al. 2000; Storer et al. 2001; Binning and Rice 2002; Horner et al. 2003 ; Castro et al. 2004; Huang et al. 2006; Van den Berg and Van Wyk 2007; Obonyo et al. 2008); *Bt*-potato against potato tuber moth, *Phthorimaea opercullela* (Zeller) and Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Lecardonnel et al. 1999; Arpaia et al. 2000); *Bt*-cotton against bollworm complex [tobacco budworm, *Heliothis virescens* (F.); cotton bollworm, *H. armigera;* spotted bollworm, *Earias* spp.; and pink bollworm, *P. gossypiella*] (Wilson et al. 1992; Benedict et al. 1996; Guo et al. 1999; Mohan and Manjunath 2002; Sharma and Pampapathy 2006); and *Bt*-tomato against the tomato fruit borer, *H. armigera*; tobacco hornworm, *Manduca sexta* L.; tomato fruitworm, *H. zea;* and tomato pinworm, *Keiferia lycopersicella* (Walsingham) (Delannay 1989; Kumar 2004). The *Bt* toxin genes (*cry1Ac* and *cry2A*) have also been introduced into rice to protect against lepidopteran pests, particularly the yellow stem borer, *Scirpophaga incertulas* (Walker); striped stem borer, *Chilo suppressalis* (Walker); and rice leaf folder, *Cnaphalocrocis medinalis* (Guenée) (Ye et al. 2001, 2003; Khanna and Raina 2002; High et al. 2004; Chen et al. 2011). China's Ministry of Agriculture has approved commercial production of *Bt*-rice lines Huahui No. 1 and *Bt* Shanyou 63 (expressing *cry1Ab/Ac* fusion gene) with resistance to stem borers in Hubei Province in 2009. China has become the first nation in the world to commercialize insect-resistant genetically modified rice, which might result in a positive influence on global acceptance of *Bt*-rice, and speed up the adoption of biotech food and feed crops internationally (James 2009; Chen et al. 2011). New *cry* genes (*cry4Cc1, cry30Ga1,* and *cry56Aa1*) with insecticidal activity against stem borers have also been identified (Li et al. 2009; Zheng et al. 2009). Toxins from *B. thuringiensis* var *morrisoni* have shown biological activity against the sorghum shoot fly, *Atherigona soccata* (Rondani), and Cry1Ac, Cry1C, Cry1E and Cry2A are moderately effective against the spotted stem borer, *C. partellus* (Sharma et al. 2004). Sorghum plants having the *cry1Ac* gene have been developed under the control of a wound inducible promoter from a maize protease inhibitor gene (*mpi*) for resistance to *C. partellus* (Seetharama et al. 2001; Harshavardhan et al. 2002; Girijashankar et al. 2005).

Pigeonpea plants transformed with *cryIE-C* gene from *Bt* under the control of *CaMV35S* promotor and *nptII* as a selection marker have shown resistance to the larvae of tobacco caterpillar, *Spodoptera litura* (Fab.) (Surekha et al. 2005). A codon-modified *cry1Ac* gene has been introduced into groundnut by using micro-projectile bombardment (Singsit et al. 1997). The immunoassay of plants selected with hygromycin has shown the expression of Cry1Ac protein up to 0.16% of the total soluble protein. Complete mortality or up to 66% reduction in larval weight has been recorded in the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). There was a negative correlation between larval survival and larval weight of the lesser cornstalk borer with the amount of *Bt* protein. Parrott et al. (1994) reported the successful expression of a truncated synthetic *cry1Ab* gene from *B. thuringiensis* subsp. *kurstaki* HD-1 in soybean, however, the protein expression was undetectable in T_1 plants, and showed a similar level of resistance to velvet bean caterpillar, *Anticarsia gemmatalis* (Hubner) to that of a conventionally bred resistant line (Beach and Todd 1987, 1988). Subsequent attempts to obtain improved expression of a *Bt* toxin gene in *Bt*-transgenic soybean plants (Jack-*Bt*) showed 3 to 5 times less defoliation by corn earworm, *H. zea*, 8 to 9 times less damage from velvet bean caterpillar, *A. gemmatalis*, and 4-times greater resistance to soybean looper, *Pseudoplusia includens* (Walker) compared to untransformed Jack plants in detached leaf feeding and cage bioassays (Stewart et al. 1996; Walker et al. 2000). However, the recent advances in transformation of grain legumes such as chickpea (Sharma et al. 2006a), pigeonpea (Sharma et al. 2006b), cowpea (Obembe 2008), lentil (Gulati et al. 2002), and *Phaseolus* spp. (De Clercq et al. 2002) have sparked a new hope to manage the pests associated losses in grain legumes.

ENVIRONMENTAL IMPACT OF GENETICALLY MODIFIED CROPS

Effect of genetically modified plants on population dynamics of the target insect pests

The Cry1Ab toxin expressed in the *Bt* corn usually suppresses *H. zea* growth and development, but only partially prevents kernel damage by *H. zea* in the corn ears (Buntin et al. 2001). However, Daly and Buntin (2005) reported that the only aerial insect arthropods, whose numbers were strongly affected by the *Bt* corn was the corn earworm, *H. zea*, and the kernel damage caused by *H. zea* was less in *Bt* corn, which presumably made *Bt* corn ears less attractive to other insects. The European corn borer, *O. nubilalis* females did not show any oviposition preference towards non-*Bt* or *Bt*-maize, and no significant differences were observed in numbers of eggs laid by *O. nubilalis* on *Bt* and non-*Bt* maize (Orr and Landis 1997; Hellmich et al. 1999; Pilcher and Rice 2001). *Sesamia calamistis* and *C. partellus* moths do not discriminate between *Bt* and non-*Bt* maize for egg laying under no-choice and choice conditions (Van den Berg and Van Wyk 2007; Obonyo et al. 2008). The stem borers, *Busseola fusca* (Fuller), *S. calamistis*, and *C. partellus* were effectively controlled by *Bt* maize expressing the Cry1Ab insecticidal protein, however, *Bt* maize did not affect the survival of the first-instar larvae, development and survival of fourth-instar larvae, or moth longevity of the cutworm, *Agrotis segetum* (Denis and Schiffermüller), which is the most common and injurious pest of maize seedlings in South Africa, indicating that *Bt* maize will most likely not have any significant effect on the control of *A. segetum* under field conditions (Erasmus et al. 2010). The numbers of eggs laid by diamond back moth, *Plutella xylostella* L. females did not differ between *Bt* and non-*Bt* canola (Ramachandran et al. 1998), broccoli (Tang et al. 1999), and cabbage (Kumar 2004). Fang-fang et al. (2005) observed a significant decline in food consumption and growth of the older rice leaf folder, *C. medinalis* larvae fed on the cut-leaves of transgenic rice expressing a synthetic *cry1Ab* gene than those fed on Xiushui 11, indicating that the younger leaf folder larvae are more sensitive to *Bt*-rice than older ones. However, LanZhi et al. (2007) reported that the rice leaf folder, *C. medinalis* didn't show any significant differences in egg density between transgenic rice expressing *cry1Ac* and *cpTI* genes and the control lines during early days of infestation, but significant differences were detected later in the season due to serious damage on the non-transgenic control lines. The pink stem borer, *Sesamia inferens* (Walker) is highly susceptible to the *cry1Ac*-transgenic rice line in terms of larval density and infestation levels as compared to controls during the early-growing season, but showed a low potential for *S. inferens* population suppression later in the growing season, indicating that *S. inferens* may become a major pest of transgenic rice, and this needs greater attention while developing an effective alternative management strategy to control the rice stem borers in different regions (LanZhi et al. 2008).

Liu et al. (2002) observed that pink bollworm, *P. gossypiella* females did not discriminate between *Bt* and non-*Bt* cotton for oviposition. Significantly lower numbers of *Heliothis/Helicoverpa* spp., *Spodoptera* spp., and other Lepidoptera have been recorded in *Bt* cotton than in non-*Bt* cotton in Australia (Head et al. 2005; Whitehouse et al. 2005). The *Bt* cotton has resulted in a significant reduction in populations of bollworms such as *H. armigera* and *E. vittella* (Fab.) as compared to their non-*Bt* counterparts, while, no significant differences have been recorded in egg laying by the adult females on *Bt* and non-*Bt* cottons in India (Bambawale et al. 2004; Sharma and Pampapathy, 2006; Dhillon and Sharma 2009b). Since the introduction of *Bt*-cotton, the *Helicoverpa/Heliothis* populations have declined across *Bt*-cotton growing regions of the world. Feeding by the *H. armigera* larvae on *Bt* cotton results in a significant reduction in trehalose levels of diapausing pupae, and weakens the preparedness of cotton bollworm for overwintering, and reduces the survival of the overwintering generation, which in turn reduces the density of the first generation in the following year (Fang et al. 2011). Such effects of *Bt*-cotton on the overwintering generation of cotton bollworm appear to have significantly contributed to the suppression of cotton bollworm population in the *Bt* cotton growing countries of the world in the past decade.

The ability to synthesize genes in the laboratory and insert into plants has caused concern to the general public, and the promise of genetically engineered crops for increasing crop production has been dimmed by the debate on several issues such as intrinsic safety of the transgenic organisms, possible long-term impact on nontarget organisms, food safety, emergence of secondary pest problems, and development of resistance in the target species. Historically, crop plants have not been subjected to risk/safety analysis or risk management, and are improved by cross-pollination between plants with desirable traits or with species that are sexually compatible.

However, the concerns associated with follow up of the regulations for safety, quality and efficacy, long-term risks and benefits, and the environmental impact of genetically engineered crops are still inconclusive, and warrant careful and continual monitoring to harvest the benefits of genetic engineering technology for sustainable crop production. There are several issues associated with the commercial cultivation of transgenic crops such as effect of transgenic plants on population dynamics of target and nontarget insects, performance limitations, secondary pest problems, environmental influence on gene expression, issues arising from development of transgenic plants development of resistance, gene escape into the environment, effects on nontarget organisms, social and ethical issues, and unanticipated consequences, which need to be addressed while deploying transgenic crops for crop production and food security.

Effect of transgenic crops on nontarget pests and secondary pest problems

Under field conditions, most of the crops are damaged by a number of insect pests. There are no differences in abundance of aerial arthropod fauna including Aphididae, Cicadellidae, Araneae, and Coleoptera in *Bt* and non-*Bt* maize (Lozzia 1999; Bourguet et al. 2002; Hassell and Shepard 2002; Daly and Buntin 2005). The numbers of nontarget arthropods such as aphids [*Metopolophium dirhodum* (Walker), *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.)] and bugs, [*Orius insidiosus* (Say)] have been found to be similar in *Bt* and non-*Bt* maize, while thrips were more abundant on *Bt* than in non-*Bt* maize (Bourguet et al. 2002). A trend towards a community effect on flying arthropods has been observed with lower abundance of adult Lepidoptera and flies in the families Lonchopteridae, Mycetophilidae, and Syrphidae (Candolfi et al. 2004). Al-Deeb et al. (2001) did not observe any significant differences in the numbers of sap beetles in ears of *Bt* and non-*Bt* maize. However, the larvae and adults of sap beetles, *Carpophilus* spp. (mostly *C. lugubris* Murray) and larvae of the otitid fly, *Euxesta stigmatis* Loew have been found to be less abundant on *Bt* than on non-*Bt* maize (Daly and Buntin 2005). The abundance and diversity of most nontarget herbivorous arthropods representing plant, aerial, and surface-dwelling communities in *Bt* corn were comparable with the isogenic control, although the populations of nitidulid beetles and Cyclorrhapha dipteran immatures were significantly less abundant in *Bt* plants (Dively 2005).

Population densities of rice plant hoppers, *Sogatella furcifera* (Horváth), *Nilaparvata lugens* (Stål), and *Laodelphax striatellus* (Fallén) were not significantly affected by *Bt*-transgenic rice in comparison to the non-transgenic rice in China (Mao et al. 2007). Bai et al. (2006) observed that *N. lugens* ingested Cry proteins from *Bt* rice lines, but had no detectable negative effects on its fitness. *Bt* rice has no significant effect on the feeding and oviposition behavior of plant hoppers and leafhoppers (Chen et al. 2006a;

Tan et al. 2006). Studies across seasons have indicated that populations of plant hoppers and leafhoppers in *Bt* and non-*Bt* rice fields were similar (Chen et al. 2006a, b, 2007). However, Cheng and Zhu (2006) and Cheng (2009) argued that the deployment of lepidopteran-resistant GM rice in China may potentially reduce the competition pressure on plant hoppers in rice ecosystem, and further worsen their already severe pest status, as plant hoppers and leafhoppers have been identified as a key group of nontarget herbivores for *Bt*-transgenic rice in China. A recent two-year field trial has indicated that *cry1Ab / Ac Bt* Shanyou 63 rice harbored greater plant hopper population than did the non-*Bt* rice during the later growth stage of plant growth (Wang et al. 2010), which might be because of migration of these insects from nearby non-*Bt* rice fields, where non-*Bt* rice was severely damaged by rice stem borers and leaf folders.

The diversity and abundance of arthropod community in transgenic cotton has been found to be similar to that of conventional cotton (Li et al. 2003; Men et al. 2003; Wu et al. 2003). However, the populations of *Aphis gossypii* Glover, *Thrips tabaci* Lind., and *Lygus lucorum* (Mayer-Dur) increased in *Bt* cotton fields under natural or chemical control as compared to that in the normal cotton fields (Cui and Xia 2000a, b; Sun et al. 2002, 2003). In Australia, the reduced use of insecticides for controlling bollworms in *Bt* cotton has resulted in increased incidence of secondary pests. The most significant of these is the green mirid, *Creontiades dilutus* (Stal) (Lei et al. 2003), and farmers now resort to insecticide sprays as many as three times per season to control this pest (Khan et al. 2006). This results in disruption of natural enemies, and also results in increased incidence of spider mites, *Tetranychus urticae* Koch; aphids, *A. gossypii;* and whitefly, *Bemisia tabaci* Gen. (Wilson et al. 1998; Farrell et al. 2006). In northern China, a number of mirid plant bugs, *Adelphocoris suturalis* Jakovlev, *A. lineolatus* Goeze, *A. fasciaticollis* Reuter, *L. lucorum*, and *Lygus pratensis* (Linn.) have become important in *Bt* cotton (Wu et al. 2002). Leafhoppers, *Amrasca biguttula biguttula* Ishida; cotton aphid; *A. gossypii;* and spider mites, *Tetranychus cinnabarinus* (Boisduval) have been observed to occur at higher levels in *Bt* cotton in the Henan Province (Deng et al. 2003; Men et al. 2005). Likewise, mirid plant bugs, *Lygus* spp., *Neurocolpus nubilus* (Say), and stinkbug, *Nezara viridula* L. have risen in pest status since the adoption of *Bt* cottons in the USA, particularly in the mid-southern and southeastern production areas (Williams 2006). Plant bugs also have become more problematic in South Africa (Gouse et al. 2004). There were no major differences in either species richness or diversity of the beneficial and nontarget communities between *Vip*-transgenic and conventional cotton, although cotton cultivar accounted for 2 - 7% of the variance in arthropod communities in Australia. However, the numbers of the mirid, *C. dilutus* and whitefly were greater in the *Vip*-transgenic cotton, which might be due to availability of more food (bolls) for feeding by the mirid in the *Vip* cotton, and differences in leaf hairiness between the cotton cultivars, which influences the damage by whitefly (Whitehouse et al. 2007).

A large numbers of insect species that are not susceptible to the *Bt* toxins expressed in transgenic cottons, and in the absence of competition from the major pests, the secondary pests may assume a major pest status and affect cotton production in India (Table 3). This will offset some of the advantages expected of the cultivation of transgenic crops. There is no evidence of increased susceptibility of *Bt*-cottons to nontarget insects such as leafhoppers, red cotton bugs, dusky cotton bugs, green bugs, and ash weevils. However, reduction in numbers of insecticide sprays, especially during the flowering and boll formation stages has led to resurgence of some minor pests such as tobacco caterpillar, *S. litura;* mealy bugs, *Phenacoccus solenopsis* Tinsley and *Maconellicoccus hirsutus* (Green); thrips, *T. tabaci;* aphids, *A. gossypii;* leafhoppers, *A. biguttula biguttula;* green

Table 3: Emerging pest problems on *Bt*-cotton in India.

Common name	Species	Remarks
Hemiptera		
Cotton leafhopper	*Amrasca biguttula biguttula*	Major pest of cotton with increased incidence across India.
Cotton aphid	*Aphis gossypii*	Emerging pest of *Bt*-cotton in South-central India.
White fly	*Bemisia tabaci*	Major pest across cotton growing regions of India, with outbreaks in 2010 in North and South-central India.
Red cotton bug	*Dysdercus* spp.	Major pest of cotton with increased incidence.
Green stinkbug	*Nezara viridula*	Emerging pest of *Bt*-cotton in South-central India.
Dusky cotton bug	*Oxycaraenus laetus* Kirby	Major pest of cotton with increased incidence.
Mealy bugs	*Phenacoccus solenopsis* & *Maconellicoccus hirsutus*	Emerged as major pests of cotton, including *Bt*-cotton since 2006.
Coleoptera		
Cotton stem weevil	*Alcidodes affaber* Auriv	Serious pest of cotton in southern India since 2003.
Grey weevil	*Myllocerus* spp.	Major foliage pest of cotton since 2002.
Cotton stem weevil	*Pempherulus affinis* Fst.	Major pest of cotton in southern India since 2006.
Blister beetle	*Zonabris pustulata* L.	Emerging pest on *Bt*-cotton.
Lepidoptera		
Tobacco caterpillar	*Spodoptera litura*	Emerging as a major foliage pest of cotton since 2007.
Pink bollworm	*Pectinophora gossypiella*	Resistance to *cry1Ac Bt*-cotton reported in 2009.
Diptera		
Serpentine leaf miner	*Liriomyza trifolii*	Major seedling pest of *Bt* cotton in South central India since 2006.
Thysanoptera		
Thrips	*Thrips* spp.	Emerging as major foliage pest of cotton since 2005.

stink bug, *N. viridula*; and the serpentine leaf miner, *Liriomyza trifolii* (Burgess) in India (Sharma et al. 2007; Karihaloo and Kumar 2009; Nagrare et al. 2009; Dhillon and Sharma 2010a). Parker and Huffman (1997) did not observe any significant differences between transgenic and non-transgenic cultivars in boll weevil or aphid damage. Mirid bug, *Creontiodes biseratense* (Distant) incidence has increased in severity on *Bt*-cotton in Haveri, Karnataka, India (Patil et al. 2006).

Environmental influence on gene expression

There have been some failures in insect control through the transgenic crops in certain seasons/regions. Expression of transgene is influenced by site of gene insertion, gene construct, epistasis, somaclonal mutations, and the physical environment. Expression of *cry1A* is influenced by genetic and environmental factors. Corn earworm, *H. zea* destroyed *Bt* cottons due to high tolerance to CryIAc in Texas, USA (Kaiser 1996). Similarly, *H. armigera* and *H. punctigera* 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 (Hilder and Boulter 1999). Possible causes for the failure of insect control may be inadequate production of the *Bt* toxin, environmental influence on transgene expression, locally resistant insect populations, and development of resistance due to inadequate management. Cotton crop flooded with 3 to 4 cm deep water for 12 days lost resistance to insects as compared with the plants irrigated normally (Wu et al. 1997). Similar reaction has been observed in *Bt* cotton, which grew under overcast and rainy weather continuously for 21 days. When the water logging 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.

The levels of *cry1Ac* expression decreased consistently throughout the growing season, and season-long expression differences among varieties can vary as much as 2-fold, which in turn affects plant resistance to insects (Finnegan et al. 1998; Adamczyk and Sumerford 2001; Adamczyk et al. 2001). Adamczyk and Sumerford (2001) reported that the factors such as parental background had a stronger impact on the expression of *cry1Ac* than the environment. Plant structures such as terminal leaves, express more Cry1Ac δ-endotoxin compared to certain flower structures (Greenplate 1999; Adamczyk et al. 2001; Gore et al. 2001). However, factors that influence expression of *Bt* toxin proteins in transgenic plants are still not fully understood, but site-of-gene insertion, cultivar or parental background, and decreased overall expression of the Cry1Ac δ-endotoxin have been implicated (Sachs et al. 1998). Finnegan et al. (1998) concluded that part of the decline in *cry1Ac* expression was related to reductions in the levels of mRNA production. Reduction of *Bt* protein content in late-season cotton could also be due to the over-expression of *Bt* gene at earlier stages, which leads to gene regulation at post-transcription levels and consequently results in gene silencing at a later stage (Dong and Li 2007). Methylation of the promoter may also be involved in the reduced expression of endotoxin proteins. As a part of total protein, the insecticidal protein in plant tissues changes its level through inhibited synthesis, degradation or translocation to developing plant parts, particularly under environmental stresses, which is closely correlated to N metabolism (Dong and Li 2007). Therefore, appropriate evaluation and selection procedures should be used in a breeding program to develop crop varieties with pest-resistant traits conferred by the foreign genes.

Development of resistance

Development of resistance in insect pests

Insect pest populations have shown a remarkable capacity to develop resistance to chemical pesticides. Over 500 species of insects have developed resistance to insecticides (Moberg 1990). Therefore, there are concerns that the deployment of transgenics will lead to development of resistance in insect populations. While some of these concerns may be real, the others seem to be highly exaggerated. 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 (Harris 1991). However, several site or tissue specific promoters have been developed in the recent past. Toxin production may also decrease over the crop-growing season, which 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. Since most *Bt* toxins have a similar mode of action, resistance developed against one toxin can also lead to development of cross-resistance to other toxins. However, there are reports that insects selected for resistance to one *Bt* toxin may not be resistant to other *Bt* toxins (Sharma and Ortiz 2000).

Under laboratory conditions, development of resistance to *Bt* toxins has been demonstrated in several insect species. A 10,000-fold resistance has been obtained in a colony of *H. virescens* to Cry1Ac (Gould et al. 1995), which also exhibited resistance to Cry1Ab and Cry1Fa, but no resistance to Cry1Ba and Cry1Ca. Following continued selection for resistance to Cry1Ac or Cry1Ab, moderate levels of resistance were recorded against Cry2a (Stone et al. 1989; Kota et al. 1999). Larvae of *H. armigera* have also shown potential to develop resistance to Cry1Ac under selection pressure, and 31.4-fold resistance was observed after six generations (Chandrashekar and Gujar 2004), and 76-fold resistance after nine generations (Kranthi et al. 2000). However, Akhurst et al. (2003) observed that resistance ratio (RR) in

H. armigera to Cry1Ac peaked at 300-fold at generation 21, after which it declined and oscillated between 57- and 111-fold. However, the first-instar *H. armigera* larvae from generation 25 were able to complete larval development on transgenic cotton expressing *cry1Ac*, and produce fertile adults. Selection of *P. gossypiella* larvae for resistance to Cry1Ac resulted in 300-fold resistance, and cross resistance to Cry1Aa and Cry1Ab, low levels of resistance to Cry1Bb, but no resistance to Cry1Ca, Cry1Da, Cry1Fa, Cry2Aa, and Cry9Ca; and the larvae from the resistant colony showed 40% survival on the *cry1Ac* expressing *Bt*-transgenic cotton (Tabashnik et al. 2000). In Australia, the estimated frequency for alleles conferring resistance to Cry1Ac is <0.0003. In contrast, the *R* frequency for alleles conferring resistance to Cry2Ab is over 10× higher at 0.0033 (Mahon et al. 2007a, b, 2008).

Many studies have predicted development of resistance to transgenic crops at a fast rate as in case of synthetic insecticides, and selection for resistance to *Bt* Cry proteins under laboratory conditions is quite high. Kranthi and Kranthi (2004) estimated that it would take 11 years for resistance gene frequency to reach 0.5 in *H. armigera* populations, if no pest control measures are adopted. If control operations cause 90% mortality, then it would take 45 years for resistance allele frequency to reach 0.5. Using a single locus simulation model, Zhao et al. (2000) showed that resistance allele frequency of *H. armigera* to Cry1A toxin will increase from 0.001 to 0.5 after 38 generations (9 years) in China, where corn fields act as a natural refuge for *Bt* cotton. Similar views have been expressed for development of resistance in *H. zea*, which is less susceptible to *Bt* cotton (Han and Caprio 2002). There is considerable variation in the 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 agro-climatic conditions (Gujar et al. 2000, 2004; Kranthi et al. 2001). However, this variability in *H. armigera* tolerance to Cry1Ac has not been reflected evolution of resistance in *H. armigera* to *Bt* cottons under field conditions in China and India (Wu et al. 2006; Gujar et al. 2007). Although, increases in the frequency of resistance alleles caused by exposure to *Bt* crops in field have not yet been documented (Tabashnik et al. 2003), there is a need to take a 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 (Sharma et al. 2004).

In India, there are sufficient alternate bollworm crop hosts such as chickpea, pigeonpea, pearl millet, sorghum, tomato, and sunflower that are grown during the same or in the subsequent season, which will provide sufficient refuge population to delay the development of resistance to *Bt* transgenic crops (Manjunath 2005; Dhillon and Sharma 2007). However, developmental asynchrony of bollworms on *Bt* transgenic and *Bt* sprayedcrops due to variation in toxin expression, weather, and overlapping generations may favor assortive mating among resistant moths from *Bt* plants (Perez et al. 1997; Liu et al. 1999), and lead to development of resistance to *Bt* toxins in the target and nontarget insect pests. In addition to use of refuge and other tactics for pre-empting resistance development, there is an active program for monitoring resistance development in the target pests to *Bt* toxins (Kranthi et al. 2005). Large-scale monitoring of insects in regions with high adoption of *Bt* cottons has not yet led to detection of resistance in field populations of target insect pests in USA, Australia, China, and India.

Development of resistance to antibiotic genes

The antibiotic genes used as a marker to select for gene transfer may lead to development of resistance in pathogens infecting human beings. However, general scientific view is that the risk of compromising the therapeutic value of antibiotics through transgenic plants is almost negligible. Hypothetically, antibiotic resistance genes may move from a crop into bacteria in the environment. Since bacteria exchange the genes readily, the antibiotic resistance genes may move into disease-causing bacteria. Gene transfer from plants to microorganisms has been demonstrated under laboratory studies (Gebhard and Samalla 1998), and possibly has happened during evolution (Doolittle 1999). Several studies have shown that there is a little chance that such a transfer would occur (Calgene 1990), but there is a continuing debate whether such a gene should be present in the commercial varieties. Methods have been developed for removing selectable marker genes after selection of the transgenics (Yoder and Goldsbrough 1994; Ebinuma et al. 1997). There are alternatives to the antibiotic markers, and systems are also available to carry out the transformation without involving any markers. The marker gene can also be excised after two lines are crossed (Dale and Ow 1991).

As transgenic-crop DNA will not be released as plasmids, conjugal transfer can be totally ruled out. Although DNA in decaying plant cells is rapidly degraded, DNA of appropriate length can survive in some soils and aquatic environments (Lorenz and Wackernagel 1992) or in the digestive tract of mice (Schubbert et al. 1997) long enough to be available for uptake. The intestinal tract of cows and other ruminants is likely to be more hostile towards free DNA (Duggan et al. 2000; Gay 2001). Competence of bacteria in natural surroundings is difficult to assess, but is unlikely to approach the efficiencies reached in optimized laboratory conditions. The maintenance and integration of incoming DNA is mediated by, and may require sequence homology with DNA of the recipient bacterium (de Vries and Wackernagel 1998). The given transfer of a plant gene to a bacterium does not imply functionality in the bacterium. Regulatory sequences (promoters and enhancers) may not work, and introns, if any, may not be recognized in the recipient species.

Gene transfer

The greatest risk of a transgenic plant released into the environment is its potential spread beyond the plant area to become a weed. Plant breeding efforts 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. However, 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.
- Adequate management practices to mitigate any risks inherent in the crop plants.

The introgression of transgenes into the wild relatives is of potential concern (Gregorius and Steiner 1993; Serratos et al. 1997). One of the hazards of large-scale deployment of transgenic crops is the possibility of gene transfer from the transgenic plants to the wild relatives, if the wild relatives are also under selection pressure (biological control) from the pest. If the target pest does not play any role in population regulation of the wild hosts, the gene transfer will not constitute to any hazard. The buildup of resistance in the wild relatives can also act as a component of pest management to the target pest, if the wild relative acts as an alternate or collateral host for the target pest. Inter-specific hybridization is a common process, but the hybrids are rare and most are sterile, as a result, there is very low probability of gene introgression into the wild relatives (Fitter et al. 1990). Transgenic plants may also become weeds, except in the context of their normal agricultural environment. Gene escape may occur when the plant invades a semi-natural habitat or transferred into the wild relative, and persist in the uncultivated land. Its' spread can be checked by methods similar to any other single trait. There are differences among plant species to disperse in the environment other than the one in which they are released, and their ability to establish feral populations. Such an event has to be compared with that of the original plant. Resistance to abiotic stress factors may present additional challenge, as this would enable the plants to grow in environments where they were unable to do well earlier (Fraley 1992). This confers additional advantage to the transgenic plant, and there are chances for gene transfer through cross-pollination. The risk assessment in such cases requires more information, and the nature of competitive advantage conferred by the transgene under specific conditions. Assessment of realistic risk for gene transfer through pollen is available for many crops (Raybould and Gray 1993), and good agriculturally sound procedures need to be developed for different regions (Boulter 1995).

Horizontal gene transfer (HGT) occurs from one organism (the donor) to another (the recipient), which are sexually incompatible (Gay 2001). The HGT between bacterial species is particularly common when it involves plasmids and transposons (Courvalin 1994; Lorenz and Wackernagel 1994; Landis et al. 2000). With the availability of full genomic sequences of organisms, more and more potential candidates for HGT between species, genera, and even kingdoms are being identified. The HGT is considered as a significant source of genome variation in bacteria (Ochma et al. 2000), and may be a common route for evolution of bacterial populations and possibly eukaryotes (De la Cruz and Davies 2000). Detailed phylogenetic analyses based on the presence of specific DNA sequences have not supported the involvement of HGT (Stanhope et al. 2001). Of particular concern are putative recipient microorganisms in soil or in the digestive track of humans and livestock (Dröge et al. 1998, 1999). Several studies have failed to demonstrate HGT from transgenic plants to bacteria (Schlüter et al. 1995; Nielsen et al. 1998; Bertolla and Simonet 1999; Gebhard and Smalla 1999). Kanamycin resistance gene from transgenic maize could be retrieved in an *Acinetobacter* strain (de Vries and Wackernagel 1998). Transgenes in genetically modified crops constitute only a fraction of the total plant DNA, whereas all plant-derived DNA will be subjected to the same likelihood of decay and HGT.

Interaction of transgenic crops with the nontarget organisms

One of the major concerns of transgenic crops is their effects on the nontarget organisms, where bulk of the evidence to date suggests that *Bt* crops are highly selective and the negative effects, if any, are relatively minor in magnitude as compared to the conventional pest control technologies. 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, and needs a greater attention to harvest the benefits of *Bt* technology in future (Dhillon and Sharma 2009b). 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. *Bt* proteins may have some adverse effects on the beneficial insects, although such affects are much less severe than those of the broad-spectrum insecticides (Sharma and Ortiz 2000). 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. The major effect of transgenic crops on the activity of nontarget organisms have been summarized below.

Nontarget insects

The information that use of genetically modified corn may have toxic effects on the 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).

Pollinators

Studies have been carried out in the US and Australia on pollinating species and pollen dispersal in cotton under intensified large-scale cropping systems (Van Deynze et al. 2005; Cattaneo et al. 2006; Llewellyn et al. 2007), however, little is known about the biodiversity of pollinators in transgenic cotton in different parts of the world. Flower-visiting and pollinating species from family Meloidae have been recorded from cotton flowers (Tomimatsu and Ohara 2003; Blanche and Cunningham 2005). Some Nitidulidae are involved in very short distance dispersal of cotton pollen (Li 1981), while Hymenoptera (57%) and Hemiptera (21%) constitute the bulk of all flower-visiting insects on the main crops, including cotton in Sudan (El-Sarrag et al. 1993). Presence of pollinating species on cultivated cotton flowers raises the issue of pollen dispersal by insects between *Bt* and non-*Bt* cotton, and impact of *Bt* cotton on pollinator species. Studies on prevalence of honeybees, *Apis mellifera* L., various Nitidulidae and Meloidae species, and *Mylabris oculata* Thunberg on *Bt* and non-*Bt* cotton plants, suggested no impact of *Bt*-cotton on their abundance and diversity (Hofs et al. 2008).

There are no significant effects of transgenic crops on the honeybees. Transgenic rape with *Bt* genes does not appear to have harmful effects on the lifespan and behavior of honeybees, but further tests may be necessary (Pham and Jouanin 1997). Chitinase in genetically modified oil seed rape does not affect the learning performance of honeybees, but beta-1, 3 glucanase affected the level of conditioned responses (the extinction process occurring more rapidly as the concentration increased), while cowpea trypsin inhibitor (CpTI) induced marked effects in both conditioning and testing phases, especially at very high concentrations (Picard Nizou et al. 1997). 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. Serine proteinase inhibitor from soybean, cysteine PI (OCI) from rice, chicken egg white cystatin, and Bowman-Birk soybean inhibitor (SBTI) do not produce harmful effects on honeybees at the concentrations expressed in transgenic plants (Bottino et al. 1988; Girard et al. 1988; Pham and Jouanin 1997). Consumption of high doses of protease inhibitors induces proteinase overproduction

(Jouanin et al. 1998). Trypsin endopeptidase inhibitor, 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 (Malone et al. 1995). As a result of reduced insecticide use in *Bt*-cotton in India, beekeepers are keeping their beehives in *Bt*-cotton fields at Sirsa and Dabawali in Haryana, and Sriganaganagar in Rajasthan, India. These beekeepers are producing good amount of honey, fetching good market price of their honey, and have not reported any negative impact of *Bt*-cotton on the population buildup of their bee colonies (Dhillon MK, Unpublished).

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. No major differences have been observed in the abundance of predators between the transgenic and non-transgenic crops (Hoffman et al. 1992; Sims 1995; Wang and Xia 1997; Sharma et al. 2007). 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* (Boisd.) fed on *Bt*-maize (Hoffmann et al. 1992; 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). Laboratory studies have shown no adverse effects of the *Bt* based insecticide on the Colorado potato beetle predator, *Coleomegilla maculata* (DeGeer) (Giroux et al. 1994). Cry3A-intoxicated *Leptinotarsa decemlineata* (Say) can be eaten by *C. maculata* without any observable adverse effects on survival or predation potential (Riddick and Barbosa 1998). Its predatory activity also decreases the rate at which *L. decemlineata* adapted to the *Bt* toxins if mixed plantings are used (Arpaia et al. 1997). However, under choice conditions, the predator showed a distinct preference for the untreated eggs than those treated with *Bt* (Gillard et al. 1999). The predator activity was not affected by pure transgenic and mixed seed potato fields (Riddick et al. 1998). No acute toxicity to the ladybird beetles 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. Lozzia et al. (1998) did not observe any adverse effects on pre-imaginal development or mortality of *C. carnea* when reared on *Rhopalosiphum padi* L. that had fed on *Bt*-maize. However, abundance of *Labia grandis* Hentz was lower in pure and mixed plants of transgenic potatoes than in pure non-transgenic potato plants (Riddick et al. 1998). 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 (Donegan 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 *S. litura* larvae fed on *Bt*-transgenic cotton (Zhang et al. 2006a). 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 *Aphis craccivora* Koch, *H. armigera*, *S. litura*, *Spodoptera exigua* (Hub.), or some other lepidopteran larvae, and soft bodied insects feeding on transgenic chickpea plants (Dhillon and Sharma 2009a).

Direct effects of plant lectins and protease inhibitors have also been reviewed for arthropods belonging to Coleoptera, Lepidoptera, Acaridae, Orthoptera and Neuroptera (Dhillon et al. 2008a). Birch et al. (1999) reported that the fecundity, egg viability and longevity of ladybirds decreased significantly when fed 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. 2008a), suggesting that additional tests under more realistic exposure conditions need to be conducted for safe deployment of lectin-transgenic plants. Although, no major differences have been observed in the abundance of predators viz., coccinellids, chrysopids, and spiders in *Bt*-transgenic and non-transgenic cotton fields under Indian conditions (Sharma and Pampapathy 2006; Sharma et al. 2007; Dhillon and Sharma 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, and therefore, long-term field studies need to be continued.

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 insect 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 development and survival of the parasitoids. 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 2010b). 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 2010b). 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).

Increased levels of parasitism by *Campoletis sonorensis* (Cameron) have been observed on the transgenic plants compared to the non-transgenic plants, which may be due to fewer larvae on the transgenic plants. *Campoletis sonorensis* and transgenic plants act synergistically, decreasing the larval survival beyond the level expected for an additive interaction (Johnson and Gould 1992). Synergistic increases in mortality and parasitism have been detected when development rates on toxic plants and control plants were equal, indicating existence of another type of interaction between natural enemies and transgenic crops, suggesting that *Bt*-transgenic crops are compatible with natural enemies for the control of *H. virescens*.

The parasitoid, *Cardiochiles nigriceps* Viereck does not reduce the survival of the host larvae significantly, and its' activity is not influenced by the transgenic plants (Johnson 1997; Johnson et al. 1997). Egg parasitism of third-generation noctuids in *Bt*-transgenic cotton has been observed to be lower than in the conventional cottons (Wang and Xia 1997). In natural and integrated control plots, the parasitoids *C. chlorideae* and *Microplitis* sp. density decreased by 79.2 and 87.5, and 88.9 and 90.7%, respectively, and the activity of *Lysiphlebia japonica* (Ashmead) increased by 85.1 and 90.2%, respectively (Cui and Xia 1998). Parasitism by the parasitoid *Diadegma insulare* (Cresson) was not significantly different between the mixed and pure stands of transgenic crop (Riggin Bucci and Gould 1997). There is no effect of transgenic corn on the parasitization of European corn borer, *O. nubilalis* by *Eriborus tenebrans* Gravenhorst and *Macrocentrus grandii* Goidanich (Orr and Landis 1997). Intra-field mixtures could serve to decrease the density of target pests 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 may be due to differences in pest abundance between the transgenic and the non-transgenic crops. 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.

Zhang et al. (2006b) 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 numbers 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). 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). Reduced longevity and fecundity was reported for the aphid parasitoids, *Aphidius colemani* Viereck and the caterpillar parasitoid, *Eulophus pennicornis* (Nees) when fed on 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 the host insect, rather than direct effects (Arora et al. 2007). Although, *Bt*-transgenic chickpea has been found to be compatible with entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin 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* need to be tested under realistic conditions.

Soil microflora

Potential effects of genetically transformed crops on nontarget species are not restricted only to the environment above ground, but also on those inhabiting the soil rhizosphere (Jepson et al. 1994). Cry proteins produced by *Bt*-transgenic plants might persist in soil, and may pose a hazard to nontarget 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 loses its insecticidal activity against *H. armigera* (Padmaja et al. 2008). 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. Biomass of *Bt* canola, cotton, potato, rice, and tobacco also decomposed at a lower rate than the biomass of respective near-isogenic non-transgenic plants.

Toxins from the transgenic plants are introduced into the soil primarily through the crop residues after crop harvest or through the root exudates. Insecticidal proteins produced by *Bt* bind rapidly and tightly on clays, both pure clay minerals and soil clays, on humic acids extracted from soil, and on complexes of clay and humic acids, which reduces the susceptibility of the *Bt* proteins to microbial degradation (Stotzky 2004). The toxins produced in *Bt* plants retain their biological activity when bound to the soil, and the accumulation of these toxins in the soil is likely to occur in the ecosystem. Organisms in the rhizosphere such as Collembola, nematodes, protozoa, fungi, bacteria, nematodes, and earthworms should be included in risk assessment studies, but have received little attention (Groot and Dicke 2002).

The *Bt* toxins released from the root exudates of *Bt*-maize remain in the soil rhizosphere throughout the crop growth, and for several months after crop maturity (Saxena and Stotzky 2000). Transgenic *Bt* corn decomposed at a slower rate in soil than the non-transgenic corn, possibly because the *Bt* corn had more lignins than the non-transgenic corn. There are no significant differences in mortality or weight of the earthworm, *Lumbricus terrestris* L. after 40 days in soil planted with *Bt* maize or after 45 days in soil amended with *Bt* maize (Saxena and Stotzky 2001). 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. A 200-day study revealed no lethal effects of transgenic *Bt* corn on immature and adult earthworms (Zwahlen et al. 2003). There were no adverse effects on cultured bacteria, fungi, protozoa, and nematodes from the *Bt*-maize fields (Saxena and Stotzky 2001). The *Bt* transgenic corn cultivation appears to have no significant influence on the nematofauna, either at the level of genus composition or with regard to biodiversity (Manachini and Lozzia 2002). The Cry1Ab protein was not taken up from soil by non-*Bt* corn, carrot, radish, or turnip grown in soil in which *Bt* corn had been grown or into which biomass of *Bt* corn had been incorporated (Stotzky 2004). Studies on the possible impact of Cry proteins released from living or decaying roots of *cry3Bb1* transgenic corn on soil microbial communities revealed that the coleopteran-active *cry3Bb1*-transgenic corn does not affect nontarget ecological processes, such as decomposition or the function of the associated saprophytic microbial community of soil and decaying roots (Lawhorn et al. 2009).

The level of Cry1Ac protein in samples collected 3 months after the previous season revealed no detectable Cry1Ac protein in any of the soil samples collected from within or outside the Bollgard cotton fields (Head et al. 2002). Cry1Ac protein accumulated as a result of continuous use of transgenic *Bt* cotton, and subsequent incorporation of plant residues into the soil by postharvest tillage is extremely low and does not exhibit detectable biological activity. Persistence of *Bt* toxins in the soil might improve the insect control or lead to development of resistance in insects inhabiting the soil. 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 (Donegan et al. 1996). There were no significant differences between genetically modified tobacco plants with protease inhibitor bovine spleen trypsin inhibitor (BSTI) and the non-transgenic counterparts for survival and fecundity of the collembolan, *Folsomia candida* Willem, and mortality and growth of earthworms, *Aporrectodea caliginosa* (Savigny), *Lumbricus rubellus* Hoffmeister, and *Eisenia foetida* (Savigny), indicating that the inhibitor had no effect on the overall function of the decomposer community of microflora and fauna in the soil (O'Callaghan et al. 2007). The assessment of the effects of transgenic eggplants on soil quality and microbial diversity has indicated that there were significant differences in microbial respiration and diversity among transgenic and non-transgenic eggplant plots at the beginning, but such difference disappeared after 6 and 12 months, suggesting a strong correlation between plants and microorganisms, as well as a short-term impact on microflora (Mocali et al. 2009).

Although the decomposition dynamics, and bacterial and fungal communities associated with decomposition of rice crop residues were strongly affected by surface and incorporated placements, but no significant differences were observed between *Bt* and non-*Bt* rice variety in either decomposition dynamics or in the soil microbial communities associated with residue decay (Lu et al. 2010a, b). The *cry1Ab* transgenic rice also had no significant effect on the residual decay and decomposition-associated microbial community compositions in the rapeseed-rice cropping system. There were no significant differences in the bacterial community composition profiles in root decomposition between *Bt*-transgenic and non-*Bt* rice, regardless of the litterbags being placed on the surface or buried in the soil (Lu et al. 2010a). However, there were some significant differences in fungal community composition between *Bt*-rice root and non-*Bt* rice root treatments at the early stage of root decomposition in the paddy field, suggesting that more research should be conducted to evaluate the ecological effects of the *Bt*-rice residues returned to paddy field after grain harvesting (Lu et al. 2010b).

Social and Ethical Issues

Genetically engineered foods may be unacceptable for ethical or religious reasons. Some people may be concerned about genes from animals or species that are proscribed by certain religions. Labeling allows those consumers to choose according to their conscience without imposing that view on others. Many consumers are suspicious of who is controlling a technology that promises to revolutionize agriculture. Biotechnology enables agricultural production to become more vertically integrated, consolidated, and centralized, largely in the hands of multinational corporations. No technology, no matter how much beneficial, is risk free. And with any new technology, there may be unanticipated consequences. Genetic engineering of a plant to have a particular trait can have unexpected effects on the ecosystem that cradles it. It is consumers' grasp of this fundamental phenomenon that underlines much of the concern over biotechnology. Expression of virulence from a pathogen in a transgene in a plant may trigger an uncontrolled hypersensitive response, which is potentially lethal to the plants (de Wit 1995). But such a genetic disease can be eliminated early in research and development.

BIOSAFETY OF FOOD FROM TRANSGENIC PLANTS

There is a need for the new technologies to be tested rigorously for potential allergenic, toxic, and antimetabolic effects in a transparent manner (Gillard et al. 1999). The *Bt* proteins are rapidly degraded by the stomach juices of vertebrates. Most *Bt* toxins are specific to insects as they are activated in the alkaline medium of the insect gut. No major changes have been observed in the composition of the transgenic tomatoes and potatoes. The levels of the antinutrients *viz.*, gossypol, cyclopropenoid fatty acids, and aflatoxins in the seed from the insect-protected lines were similar to or lower than the levels present in the parental variety and reported for other commercial varieties. The seed from the *Bt* transformed cotton lines is compositionally equivalent to, and as nutritious as seed from the parental and other commercial cotton varieties (Berberich et al. 1996). Very little amount of *Bt* toxins may remain in plant parts to be consumed by human beings or dairy cattle, e.g. the raw seed of line 81 [with *cry1Ab* gene] showed 14.00 mg per g active protein, and line 531 [with *cryIAc* gene] contained 2.22 µg per g of active protein by ELISA method. Processing removed >97% of the active proteins in the transgenic cottonseed (Sims and Berberich 1996). Cry1Ab protein as a component of postharvest transgenic maize plants dissipates readily on the surface of, or cultivated into soil (Sims and Holden 1996), and has not been detected in silage prepared from transgenic plants (Fearing et al. 1997).

There are no specific receptors for *Bt* protein in the gastrointestinal tract of mammals, including man (Kuiper and Noteborn 1994). Histopathological effects have been observed in the gut mucosa, but no systemic adverse effects have been observed in mice and rabbits following oral administration. There are no major changes in composition of the transgenic tomatoes, which pose 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 (Noteborn et al. 1996). There were no differences in the survival and body weight of broilers reared on meshed or pelleted diets prepared with *Bt* transgenic maize and the diets prepared using control maize (Brake and Vlachos 1998). Several protein families that contribute to the defense mechanisms of food plants are allergens or putative allergens, and some of these proteins have been used to confer resistance to insect pests. These include α-amylase and trypsin inhibitors, lectins, and pathogenesis-related proteins (Franck and Keller 1997). Some of the secondary metabolites are toxic to mammals, including humans. This may result in a tradeoff between nature's pesticides produced by transgenic plants or varieties from traditional breeding programs, synthetic pesticides, and mycotoxins, or other poisonous products of pests. 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 (Pusztai et al. 1992). Although most of the cowpea trypsin inhibitor (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 in 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 stimulation of growth and metabolism of pancreas. Thus, the nutritional penalty for increased insect-resistance after the transfer of the cowpea trypsin inhibitor gene into food plants is quite low in the short-term.

Level of GNA expression that provides insecticidal protection for plants does not reduce the growth of young rats, has a negligible effect on weight and length of the small intestine, but a slight hypertrophy of this tissue (Pusztai et al. 1996). However, the activity of brush border enzymes was affected. Sucrase-isomaltase activity was nearly halved, and those of alkaline phosphatase and aminopeptidase increased significantly. Incorporation of N-acetylglucosamine-specific agglutinins from wheat germ (WGA), thorn apple, *Datura stramonium* L., or nettle, *Urtica dioica* L. 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 (Pusztai et al. 1993). As a result of their binding and endocytosis by the epithelial cells of the small intestine, all the three lectins interfered with its metabolism and function to varying degrees. WGA also induced the hypertrophic growth of the pancreas and caused thymus atrophy. The presence of this lectin in the diet may harm higher animals at concentrations required to be effective against most pests.

Expression of a new gene in a crop could also introduce new allergens, normally not present in the non-transformed plants (Lehrer 2000). Allergic reactions to foods are hard to predict, but they can be life-threatening. Virtually every gene transfer in crops results in some protein production, and proteins trigger the allergic reactions. Genetic engineering can introduce new proteins into food crops, whose allergenicity may be unknown. This might lead people to needlessly avoiding foods that are actually safe. If the indigenous proteins or the new proteins are from the known sources of allergens, then assessing the allergens within the genetically modified plants is easier. If the source of the allergenic protein is known, and is related to the introduced gene from sources that have not been used as a human food, then one has to rely on the criteria with which to assess their potential allergenicity. Eight commonly allergenic and 160 less allergenic foods have been identified, and scientists can avoid the transfer of genes with known allergenic effect (Lehrer 2000).

RISK ASSESSMENT AND RISK MANAGEMENT OF GENETICALLY MODIFIED ORGANISMS

Genetically modified plants have been released in several countries, but the regulations governing the use of transgenic plants vary considerably in different countries (OECD 1992, 2007; APHIS 1997; EPA 1997, 2001; DBT 1998; EU 2003). The decisions that address the concerns associated with the application of biotechnology to agriculture must be science based. The regulatory agencies should assure credibility and use a rational basis for decision-making. Long-term ecological risk can be determined from the probability that an initially rare transgene might spread into the ecosystem, resulting in vertical gene transfer as a result of gene introgression into feral populations, invasion of new territories as a result of introduction of an exotic species, and horizontal gene transfer mediated by microbial agents, or a combination of these. Therefore, the deployment of transgenic plants should assess the risks for their safe deployment on a commercial scale. A biosafety working group has been formed by Food and Agricultural Organization (FAO), United Nations Environment Program (UNEP), United Nations Industrial Organization (UNIDO), and World Health Organization (WHO), and guidelines for handling and release of genetically modified organisms have been published by EPA, OECD, DBT, etc. (Tzotzos 1995). Different committees with well-defined roles and protocols have also been formed in India to enforce biosafety regulations for the safe deployment of genetically modified organisms and post commercialization monitoring and use (DBT 1998; Dhillon et al. 2008b).

CONCLUSIONS

Insect-resistant transgenic plants have been deployed for pest management in several countries, which will continue to expand, and gene pyramiding might become very common in future. However, concerns have been raised about the possibility that large-scale deployment of GMOs for pest management might impact the environment in the sense that they might influence the activity and abundance of nontarget herbivore arthropods, natural enemies, fauna and flora in the rhizosphere and aquatic systems, toxin flow in the insect fauna through different trophic levels, development of resistance in target insect pests, pollen flow to closely related wild relatives, antibiotic resistance, etc. However, we should also consider the risk of not using GMOs for pest management and crop production, when the need to increase food production is most urgent. To ensure a sustainable deployment of transgenic insect-resistant plants, it is important that they are compatible with other control methods, including biological control agents, are commercially viable and environmentally benign, easy to use in diverse agro-ecosystems, and have a wide-spectrum of activity against the target insect pests, but harmless to nontarget organisms. However, it is also important to follow the biosafety regulations properly, and there is a need for better presentation of the benefits of biotechnology to the general public for rational deployment of the transgenic plants for pest management. At the same time, we should also follow the risk assessment and risk management strategies for the safe deployment of genetically modified organisms in the environment for sustainable crop production and food security.

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