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Review Paper

Bacillus thuringiensis (Bt) transgenic crop: An environment friendly insect-pest management strategy

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Abstract: *Introduction of DDT (dichloro-diphenyl-trichloroethane) and following move towards indiscriminate use of synthetic chemical insecticides led to the contamination of water and food sources, poisoning of non-target beneficial insects and development of insect-pests resistant to the chemical insecticides. Increased public concerns about the adverse environmental effects of indiscriminate use of chemical insecticides prompted search of alternative methods for insect-pest control. One of the promising alternatives has been the use of biological control agents. There is well-documented history of safe application of Bt (B. thuringiensis, a gram positive soil bacterium) as effective biopesticides and a number of reports of expression of δ-endotoxin gene(s) in crop plants are available. Only a few insecticidal sprays are required on Bt transgenic crops, which not only save cost and time, but also reduce health risks. Insects exhibit remarkable ability to develop resistance to different insecticidal compounds, which raises concern about the unsystematic use of Bt transgenic technology also. Though resistance to Bt products among insect species under field conditions has been rare, laboratory studies show that insects are capable of developing high levels of resistance to one or more Cry proteins. Now it is generally agreed that 'high-dose/refuge strategy' is the most promising and practical approach to prolong the effectiveness of Bt toxins. Although many biosafety concerns, ethical and moral issues exist, area under Bt transgenic crops is rapidly increasing and they are cultivated on more than 32 million hectares world over. Even after reservation of European Union (EU) for acceptance of geneticaly modified(GM) crops, 6 out of 25 countries have already adopted Bt crops and many other industrial countries will adopt Bt transgenic crops in near future. While the modern biotechnology has been recognized to have a great potential for the promotion of human well-being, adoption of boisafety protocol is necessary to protect human health and environment from the possible adverse effects of the products of genetic engineering. The debate between proponents and opponents of GM technology has created major obstacles in harnessing benefits of the technology. It has now become clear that transgenics will be accepted by the public only when doubts related with general risks and environmental safety are adequately dispelled. Thus, there is need to organize public awareness and present the benefits of Bt transgenic crops to improve social attitude for their rational deployment. In this review, an attempt has been made to discuss social and environmental safety issues of Bt transgenic crops.*

Key words: *Bacillus thuringiensis, Transgenic crop, Insect-pest management, Resistance management, Biosafety*
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Introduction

Introduction of DDT (dichloro-diphenyl-trichloroethane) in 1940s as the first modern insecticide and its extensive use played a key role in protection of crops, forests and controlling insect-vectors of human diseases. As a contact poison against several arthropods, it was effectively used to combat mosquitoes spreading malaria, typhus and other insect-borne diseases. The following move towards indiscriminate use of synthetic chemical insecticides led to the contamination of water and food sources, poisoning of non-target beneficial insects and development of insect populations resistant to the chemical insecticides. Increased public concerns about the potential adverse environmental effects associated with the heavy use of chemical insecticides prompted search of alternative methods for insect-pest control. One of the promising alternatives has been the use of biological control agents, such as biopesticides and entomopathogenic microorganisms. An important benefit of biological control agents is that they can replace, at least in part, some hazardous chemical pesticides. At present, chemical control is more commonly used than biological control.

Farmers, who were basically organic farmers, have adapted to green revolution technology characterized by the use of high yielding varieties (HYVs), chemical fertilizers and pesticides. This technology has considerably increased the productivity, but has created problems of land degradation, pesticide residues in farm produces, gene erosion, environmental pollution *etc.* Continuous use of HYVs without proper crop rotation has resulted in enhanced pest incidences. Insect-pest management in HYVs by extensive use of chemical pesticides has certainly brought considerable protection to crops over the past decades. The need of today is to produce more food and livelihood opportunities from less per capita arable land and irrigation water to feed the burgeoning population. Providing ample food grain at affordable price for the ever-growing global population is only the first part of the challenge. The second and more important part is to produce this in a way that does not destroy the natural resources. Intensive agriculture, though provides sufficient food grains, yet treads heavily in the environment. An integrated crop management technology needs to be deployed to counteract the degradation of the agro-ecosystem due to the on-going intensive agriculture. This includes use of biological fertilizers, soil and water conservation practices, biodiversity conservation, integrated pest management, *etc.* If transgenic technology is integrated into the traditional system of crop husbandry, probably it holds great promise in augmenting agricultural production and productivity, while

Table - 1: Productivity loss caused by some important insect-pests

Pest		Productivity
Common name	**Scientific name**	**loss (%)**
Chilli midge	*Aspordylia capsici*	60 – 80
Cotton white fly	*Bemisia tabaci*	53 – 80
Pulse stem fly	*Ophiomyia* sp	50 – 72
Cotton boll worm	*Spodoptera* sp and others	50 – 60
Brinjal fruit and stem borer	*Lucinodes orbonalis*	48
Caster semilooper	*Achea janata*	40
Mustard aphid	*Lipaphis erysimi*	30 – 70
Sorghum shoot fly	*Atherigona soccata*	30 – 60
Sorghum midge	*Contarinia sorghicola*	30 – 50
Linseed budfly	*Dasineura lini*	30
Maize pink borer	*Sesamia inferens*	25 – 75
Rice stem borer	*Scirpophaga incertulas*	25 – 30
Groundnut leafminer	*Aproaerema modicella*	24 – 92
Lucerne weevil	*Hypera postica*	20 – 30*
Rice gall midge	*Orseolia oryzae*	15 – 60
Brown Plant Hopper	*Nilaparvata lugens*	10 – 70
Safflower aphid	*Dactynotus carthami*	9 – 67

Source: Pandey *et al.* (1995)

conserving biodiversity, natural resources and the environment for future generations (Kumar, 2001a). Insect-pest management in an ecofriendly manner is no longer a dream now. The tools and techniques of molecular biology and biotechnology can facilitate harnessing this in crop plants in a safe and sustainable manner.

There are an estimated 67000 pest species that damage agricultural crops, of which approximately 9000 species are insects and mites (Ross and Lembi, 1985). Insect-pests are the major cause of these losses (Table 1). Pest associated losses in major crops varies from 52% in wheat, 58% in soybean, 59% in maize, 74% in potato, 83% in rice to 84% in cotton (Oerke *et al.*, 1994). Insects not only cause direct losses to the agricultural production but also cause losses indirectly due to impaired quality of the produce and their role as vectors of various plant pathogens (Kumar *et al.*, 2006a). In addition to these losses, they increase cost of production by adding costs of pesticides and their application. Not only this, an extensive use of pesticides results in adverse effects on the beneficial organisms, pesticide residues in food and fodder, and environmental pollution. People are becoming more health conscious and aware of the pesticide residues in food, fodder and water. There is urgent need of replacing agrochemicals having harmful effects on human health and environment with safer alternatives and adopting integrated pest management (IPM) practices to provide adequate crop protection for sustainable agriculture (Hazra *et al.*, 1998). It is in this context that the use of insect resistant *Bt* transgenic crops will increase productivity as well as provide benefits to farmers, consumers and environment.

Although insect resistance in crop plants has been one of the major objectives of plant breeding, only a little about the mechanisms of natural insect resistance has been understood (Kogan, 1986). There has been increasing number of candidate genes that could be deployed for breeding insect-resistant crop varieties. Many insecticidal proteins and molecules are available in the nature, which are effective against agriculturally important pests but are safe for other organisms. Insecticidal proteins present in *Bacillus thuringiensis*, which have shown efficacy as spray formulations in agriculture over past few decades, have been expressed in crop plants. Genetic engineering technology offers the possibility of developing entirely new system of pest management that have advantages over classical biological control agents but have fewer of their drawbacks. Induced resistance using *Bt* gene is one of the first modern crop biotechnology applications where products are already in the world market. *Bt* is only the beginning of new and safer technologies to bring about sustainable agriculture and to protect the environment.

B. thuringiensis as biopesticide, commonly known as *Bt*, is a naturally occurring, gram-positive, spore-forming soil bacterium. *Bt* has been known to be reservoir of several insecticidal proteins, such as δ-endotoxins, cytolytic proteins, vegetative insecticidal proteins, *etc.* Among these, δ-endotoxins have been more efficiently utilized for protection of a variety of crops from various insect-pests. A number of commercial *Bt* formulations have been used for a long time as effective biopesticides to food crops, ornamentals, forest trees and stored grains (Meadows, 1993). Compared to chemical pesticides, *Bt* has certain advantages: it is highly toxic to insects and yet highly specific. *Bt* toxins are harmless to human beings, animals and a wide array of non-target pests. Therefore, it is ideally suited for incorporation into IPM programs (Nester *et al.*, 2002). In spite of these advantages, *Bt* based formulations have several disadvantages (McGaughey and Whalon, 1992). The biopesticide needs to be applied repeatedly and it is effective only against immature stages of target insects feeding on exposed plant surfaces. This limits its usefulness against insect-pests that feed inside the plants. These limitations can be removed by expression of *Bt* gene(s) in transgenic plants (Krattiger, 1997).

Bt δ-endotoxins constitute a family of related proteins for which more than 150 genes have been identified (Crickmore *et al.*, 1998). The crystalline (Cry) proteins are inactive until they get solubilized by proteases (Tojo and Aizawa, 1983; Milne and Kaplan, 1993) in the insect's midgut at high pH (>9.5), releasing proteins called δ-endotoxins. The toxicity is derived from the N-terminal half of the protein, which is composed of seven antiparallel α-helices. When insects ingest the crystalline inclusions, they are solubilized under alkaline conditions of insect midgut (Hofmann *et al.*, 1988a), releasing the δ-endotoxins which upon proteolytic activation exhibit a highly specific insecticidal activity. The activated toxin binds readily to specific receptors on the apical brush border of the midgut microvillae of susceptible insects (Hofmann *et al.*, 1988b). Then, α-helices penetrate the membrane to form an ion channel in the apical brush border membrane (Fig. 1) (Knowles and Dow, 1993). The formation of toxin-induced pores in the membrane allows rapid flux of ions (Sacchi *et al.*, 1986; Wolfersberger, 1989). This disruption of gut integrity leads to death of the insect due to starvation and/or septicaemia. The δ-endotoxins are highly insoluble in normal

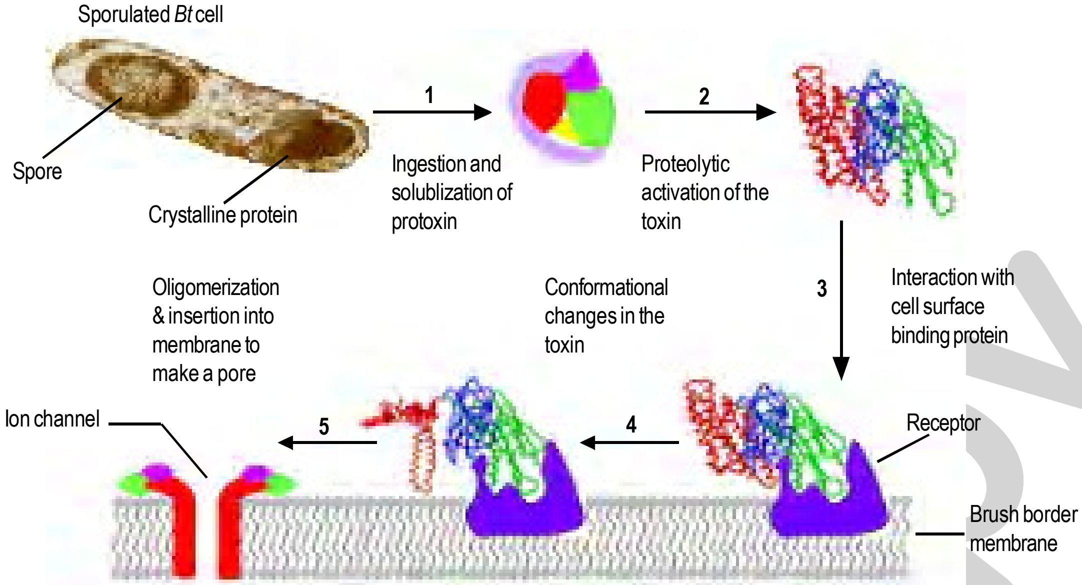

Fig. 1: Proposed mode of action of *Bt* Cry protein (Source: Knowles and Dow, 1993)

digestive conditions, therefore not harmful to mammals, birds, fish or to most of the beneficial insects. Moreover, mammals, including human, do not have δ-endotoxin receptors in their guts and all the δ-endotoxins tested so far (except Cry9) are unstable when heated and degrade within 20 seconds by the mammalian digestive enzymes (EPA, 1998). Although, the Cry9C protein is stable under stomach conditions, it is not glycosylated and does not cause any adverse effects characteristic of immune response in mammalian system. Safety of *Bt* toxin in terms of toxicity and allergenicity towards mammals and other non-target organisms is well documented (Glare and O'Callaghan, 2000). Lack of receptors that bind to *Bt* toxins and instant degradation of *Bt* toxin in human digestive system makes them innocuous to human beings. Community exposure to *Bt* toxins/ spray formulations over a period of six decades has not resulted in any adverse effects. Human volunteers consumed *Bt* toxin at very high concentration without any undesirable effects. Lack of homology to any allergenic protein/epitope sequence makes *Bt* toxin non-allergenic (Kumar, 2006). A report by the Food Safety Department of the United Nation's World Health Organization (WHO) concluded that GM foods currently in the international market do not pose any risk for human health (WHO, 2005).

Bt also produces certain cytolytic (Cyt) proteins and vegetative insecticidal proteins (VIPs) which can be deployed to synergies the insecticidal activity of Cry proteins. The Cyt proteins, encoded by the *cyt* genes of *B. thuringiensis* sub sp *iseraelensis*, differ from Cry proteins in its smaller size and high cytolytic activity against a wide range of cell types, including those of vertebrates (Drobniewski and Ellar, 1988). More than 8 types of *cyt* genes have been cloned and sequenced so far (Agrawal and Bhatnagar, 2003). VIPs are produced in extremely small amounts by *Bt* during its vegetative growth and excreted out into the medium, therefore VIP is an exotoxin. Estruch *et al.* (2001) provided compelling evidence that VIP3A protein contains 60 to 70 amino acid long motif, called 'death domain', which is involved in protein-protein interaction and induces apoptosis in insect cells. The VIP3A protein targets different receptors in mid-gut lining and binding results in the formation of ion-channels distinct from those formed by δ-endotoxins. Only certain insect species possess VIP binding sites on the surface of their gut epithelia, therefore, other beneficial insects, animals and human are not targeted by this insecticidal protein. A number of *vip* genes (*vip1*, *vip2* and *vip3*) have been identified and cloned (Estruch *et al.*, 1996; Selvapandiyan *et al.*, 2001).

Bt transgenic Crops

Expression of *Bt* gene in tobacco and tomato provided the first example of genetically engineered plants for insect resistance (Barton *et al.*, 1987; Vaeck *et al.*, 1987). Subsequently, several *Bt* genes have been expressed in transgenic plants, including tobacco, potato, tomato, cotton, brinjal, rice, *etc.* Delannay *et al.* (1989) for the first time reported field performance of transgenic tomato plants expressing δ-endotoxin gene. Though Cry1Ab protein was effective against tobacco hornworm, higher level of gene expression was needed for the control of tomato fruit worm (*Helicoverpa* sp). Results of field trials of *Bt* transgenic tobacco (Hoffman *et al.*, 1992) and cotton (Wilson *et al.*, 1992) expressing truncated δ-endotoxin genes were encouraging. Since then, there have been several reports of field-tested δ-endotoxin expressing transgenic crops (Table 2). Syngenta Seeds Inc. reported development of transgenic cotton plants expressing *vip3Aa* gene and their evaluation across the US cotton-belt during 2000-2002. The transgenic cotton plants were reported to provide excellent protection against lepidopteran insects throughout the season and resulted in significantly higher yields (Artim, 2003). Some of the potential benefits of transgenic insect resistant crops include reduced applications of broadspectrum insecticides (Carpenter *et al.*, 2002), increased or protected yields

Table - 2: Reports of field-tested *Bt* transgenic crops

Crop	*Bt* gene used	Against the pest	References
Corn	*cry1Ab*	*Heliothis zea*	Koziel *et al.* (1993), Armstrong *et al.* (1995)
	cry1Ac	*Pectinophora gossippiella*	Buschman *et al.* (1998)
Potato	*cry3A*	*Leptinotarsa decemlineata*	Perlak *et al.* (1993)
	cry1Ab	Potato tuber worm	Duck and Evola (1997)
	cry1Ac	Potato tuber worm	Ebora *et al.* (1994)
	cry5	Potato tuber worm	Douches *et al.* (2004)
Cotton	*cry1Ac*	Cotton ball worm	Perlak *et al.* (1990), Jenkins *et al.* (1997)
	cry1Ab	Pink ball worm	Wilson *et al.* (1992), Benedict *et al.* (1996).
	vip3Aa	Lepidopteran insects	Artim (2003)
Tomato	*cry1Ab*	Tobacco hornworm	Delannay *et al.* (1989)
Brinjal	*cry1Ab*	Fruit borer	Kumar *et al.* (1998)
	cry3B	Fruit borer	Iannacone *et al.* (1997)
Chickpea	*cry1Ac*	Pod borer	Kar *et al.* (1997)
Sugarcane	*cry1Ab*	Shoot borer	Arencibia *et al.* (1997)
Rice	*cry1Ab*	Yellow stem borer, Striped stem borer	Datta *et al.* (1998), Shu *et al.* (2000), Ye *et al.* (2001)
	cry1Ac	Yellow stem borer	Nayak *et al.* (1997)
	cry2A-1Ac-gna	Rice weevil, Rice hispa	Maqbool *et al.* (2001), Loc *et al.* (2002)
	cry1B - 1Aa	Yellow stem borer	Raina *et al.* (2002)
	Cry1Ac – 2A	Yellow stem borer, Rice leaf folder	Bashir *et al.* (2004)

Source: Kumar (2007)

due to season-long control of the target insect pest (Rice and Pilcher, 1998); protection of stored grain from insect pests (Giles *et al.*, 2000); and lower mycotoxin levels due to reduction in fungal pathogens associated with *Ostrinia nubilalis* (Hubner) feeding on corn (Munkvold *et al.*, 1999). Perceived disadvantages of *Bt* transgenic crops may be: 1) potential impact on nontarget species; 2) increase in toxin levels in the soil may affect soil microflora; 3) exchange of genetic material between the transgenic crop and related plant species leading to the development of so called "Super weed" and 4) evolution of new and more virulent biotypes of the pests (Kumar, 2002).

Bt toxins are highly specific against insects without affecting predators and other beneficial insects (Christou, 2005). Efficacy of *Bt* transgenic crops has been found to be comparable to or even better than any of the currently used control methods. Moreover only a fewer insecticidal sprays are required on *Bt* transgenic crops, which not only save money and time, but also reduce health risks to farmers and consumers. Studies have demonstrated that the number of insecticidal spray can be reduced significantly by using *Bt* transgenic crops, particularly *Bt* cotton (Carpenter *et al.*, 2002; Edge *et al.*, 2001). Using insect resistant *Bt* transgenic crops, mainly the *Bt* cotton, United States reduced insecticide use by 1,870,000 pounds of active ingredients in 2001 (Gianessi *et al.*, 2002). Similarly, insecticide application was reduced by 67% in China (Huang *et al.*, 2002) and 66% in south Africa (Ismael *et al.*, 2002a) by the use of *Bt* transgenic cotton. Use of *Bt* transgenic crops can have indirect positive impacts on beneficial insects by reducing the use of chemical pesticides and thus can preserve their populations (Head *et al.*, 2001). In addition, they can have positive environmental impacts such as saving of raw materials needed to manufacture chemical insecticides, conserving fuel-oil required to manufacture, distribute and apply such insecticides and eliminating the need to use and dispose-off insecticide containers (Leonard and Smith, 2001).

Better pest control strategy can improve quality of life of farmers by reducing inputs on insecticides, sprayers and labour-charges thereby increasing their incomes and offering time savings (Ismael *et al.*, 2002b; Pray *et al.*, 2002). Water saving due to *Bt* cotton, by reduced insecticidal sprays, is another benefit to the farmers. Plantation of *Bt* cotton in 1.0 ha may result in reduction of more than 7 days of labour needed for spraying pesticides, eliminates 60 km of walking and saves about 600 liters of water (James, 2002). *Bt* corn has been reported to have lower levels of mycotoxins and carcinogenic chemicals produced by fungi that colonize the pest infested crop/produce. The reduction of mycotoxins by *Bt* corn was found significant enough to have an economic impact in terms of international trade (Wu, 2006). In the countries where certain mycotoxins are significant contaminants of food, adoption of *Bt* corn may even improve human and animal health. These cumulative benefits may have significant social relevance for a society. However,

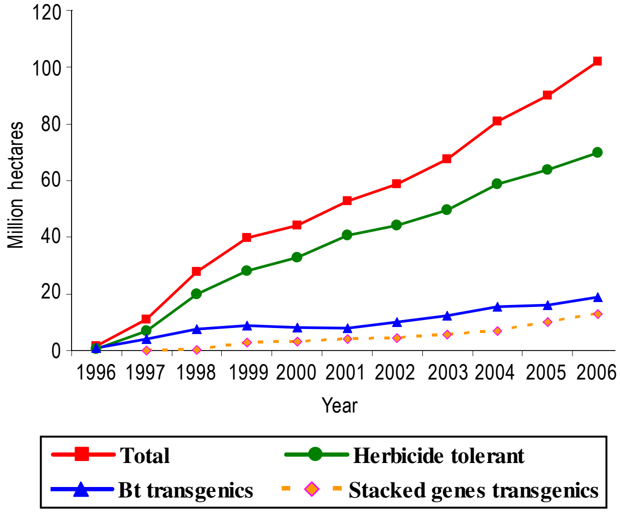

Fig. 2: Global adoption of transgenic crops. Stacked genes include those for herbicide tolerance and insect resistance (James, 2004, 2006)

long-term impact can only be sustained if deployment strategies are designed to maintain the effectiveness of *Bt* genes.

Bt transgenic crops have been used successfully to provide resistance against a number of insect-pests for a decade or so. The year 1996 marked a milestone in agricultural biotechnology, when for the first time *Bt* transgenic varieties of potato, cotton and corn were released for commercial cultivation. Since then the global area of transgenic crops continued to grow at a sustained double-digit growth rate. In 2006, out of 102 million ha of transgenic crops grown by 10.3 million farmers in 22 countries, 19 million ha (19%) were planted with *Bt* crops and 13.1 million ha (13%) with stacked genes for herbicide tolerance and insect resistance (James, 2006). Insect resistance has been the second most widely used trait in transgenic crops (after herbicide resistance) in global agriculture (Fig. 2). The rate of adoption of GM crops in European Union (EU) agriculture is slow compared to other agricultural countries. The conventional explanation for the US-EU difference in GM regulations is that Europeans care more about the natural environment than do Americans, and have less trust on their food safety regulators. However, this seems incomplete for two reasons. One is that consumers and even environmentalists typically do not wield a great deal of political clout relative to producer interests (Bernauer, 2003). The other is that there is no hard evidence to date to justify the concerns reflected in the precautionary stance taken by EU member countries. Their main worries on the consumer or food safety side have been that GM food may be more toxic or carcinogenic, more allergenic or nutritionally less adequate and that transgenes might escape digestion and alter genome of the person or animal consuming them. But such concerns are inconsistent with statements made by the European Commission scientific community (EC, 2001) and according to a report commissioned by the UK government (King, 2003).

Only one GM crop is currently authorized for commercial cultivation in EU. Spain being the leading EU country planted 60,000 hectares of *Bt* maize in 2006. On the other hand, EU has become the world's largest importer of GM crops, mostly for animal feed. In the near future EU farmers may adopt GM crops for feed or industrial uses rather than for food (Gomez-Barbero and Rodriguez-Cerezo, 2007). *Bt* cotton is in many ways an ideal candidate for introduction into cotton-growing countries as a pilot and model transgenic crop. Its basic use as fibre crop facilitates its regulation and ready acceptance by the public. The biosafety concerns are minimum because of the limited movement of sticky, heavy pollen and natural genetic barriers that preclude outcrossing between tetraploid *Bt* hybrid and diploid native cotton, having no compatibility with any wild relatives (Kumar, 2006). However, a case-by-case approach is necessary while evaluating other *Bt*-crops for their introduction in the centers of crop origin.

Resistance Management

Insects exhibit remarkable ability to develop resistance to different insecticidal compounds. The nonjudicious use of chemical insecticides has led to the development of resistance to one or multiple insecticides in over 1,000 insect/insecticide resistance combinations, and at least 17 species of insects that are resistant to major classes of insecticides (Bellinger, 1996). The problem of resistance development raises the concerns over the indiscriminate use of biopesticides also (Ferre *et al.*, 1995). However, products based on *Bt* toxin have been widely used in agriculture, forestry and civil society, so far there have been very few documented cases of resistance development in insects against *Bt* (Marrone and Macintosh, 1993). This does not mean that insects will not develop resistance under field conditions in future also. It will be very unfortunate if the effectiveness of transgenic crops is nullified within a few years due to inappropriate deployment strategies. Therefore, it is prudent to devise deployment strategies to assure long-term usefulness of insect resistant transgenic crops. Presently when organic pesticides are increasingly proving ineffective against many of the insect-pests, the *Bt* transgenic technology should be utilized to its full potential by its strategic deployment. The durability of insect resistance in transgenic crops can only be ensured if IPM practices are followed (Ranjekar *et al.*, 2003). Deployment of IPM practices can further delay resistance development in insects so that the resistant crop varieties can be used effectively for a comparatively longer duration. Van Emden (1999) proposed analogy between the enthusiasm currently focused by multinational agrochemical and seed companies as well as by the farmers on insect resistant transgenic crops and the enthusiasm that greeted DDT in the 1940s. Any indiscriminate use, like DDT was used, may lead to the situation what happened with DDT, which resulted into resistance development in mosquitoes just after 4 years of its non-judicious use.

Scientists and environmentalists have been debating about how resistance development in insect pests can be delayed. Widespread agreement emerged that 'high-dose/refuge strategy' is the most promising and practical approach for prolonging the effectiveness of *Bt* transgenic crops. Farmers in USA growing *Bt* transgenic crops need to allocate 4-20% of their land for refuge planted with non-*Bt* cultivars and the refuge fields must be within 0.8 km from *Bt* fields (EPA-USDA, 1999). Unfortunately, enforcing a similar system for small and marginal farmers may not be possible in most of the developing countries. This does not mean that refuge strategy is not required to be adopted in developing countries. In a typical village of developing country like India, it is unlikely that all the farmers will be growing *Bt* transgenic varieties on all of their land. Thus unstructured refuge might be present on small land holdings due to diverse choice of cultivar, cost of seed and market demand *etc.* (Ranjekar *et al.*, 2003). But, as the *Bt* crops/varieties will gain popularity, the area under *Bt* crop will increase in a village. This will make strategic plantation of refuges absolutely necessary.

The tactics available for sustainable deployment of insect resistance genes in transgenic crops can be grouped into four strategies. These are not essentially mutually exclusive. Two or more strategies can be combined together by deploying one or several genes (Gene strategies) expressed constitutively or in tissue-specific manner (Expression strategies), produced at high dose of the endotoxin (Dose strategies) and may be grown along with refuges, as mixtures of genes or rotation of genes (Field tactics). Use of multiple genes or gene pyramiding aims at reducing the likelihood of resistance development due to mutation in insects since multiple mutations would have to occur simultaneously in individual insect. High-dose expression of a toxin in all plants aims at killing most of the insects, including the resistant (Rr) insects. Targeted expression of *Bt* toxin in vulnerable parts of crop plants or at specific stage of plant development aims at reducing the period of exposure of insects to the toxin. Whereas refuge strategy aims at minimizing the chances of evolution of homozygous resistant (RR) insect by preventing resistant insect (Rr) from mating with other resistant insects.

A large number of *Bt* genes have been cloned and sequenced. It has been suggested that any two *Bt* toxins that are proposed to be used in combination in transgenic plant must not be homologous to each other, otherwise a single mutation could confer cross-resistance to both the toxins. In most of the cases that have been studied so far, resistance to *Bt* toxins is caused by mutations in receptor proteins of the insect midgut (Frutos *et al.*, 1999). Based on studies by Fiuza *et al.* (1996) and Lee *et al.* (1997), it has been found that Cry1Aa and Cry1Ac should not be used in combination. Also, to delay evolution of insect populations resistant to a *Bt* toxin, it is important to deploy dissimilar *Bt* genes or genes with different modes of action. With this objective and in keeping with the strategy of resistance management, fusion toxin genes were successfully used for rice transformation (Tu *et al.*, 1998; Raina *et al.*, 2002, Kumar *et al.*, 2006c). Maqbool *et al.* (2001) co-transformed rice with two dissimilar *Bt* genes (*cry1Ac* and *cry2A*) and a snowdrop lectin gene (*gna*). Co-transformants for all the three genes showed 100% protection against rice leaf folder and yellow stem borer (YSB), and 25% reduction in the survival of brown plant-hopper (BPH). Loc *et al.* (2002) used the same three genes for rice transformation with minimal gene-expression-cassette bombardment. Simpler transgene integration, higher level accumulation of the insecticidal proteins and enhanced resistance against BPH and stripped stem borer (SSB) was observed. In an alternate strategy for sustainable pest-resistance in genetically modified crops, Mehlo *et al.* (2005) used a fusion protein of N-terminal δ-endotoxin and C-terminal galactose-binding domain of nontoxic ricin B-chain. Transgenic rice and maize plants expressing the fusion protein were significantly more toxic than those containing the *Bt* gene alone.

Tissue specific expression of *Bt* genes is another strategy to manage resistance development and also to address certain biosafety concerns. Enhanced stem borer resistance was observed by Ghareyazie *et al.* (1997) in indica rice transformed with *cry1Ab* under the regulatory control of *pepc* promoter. The gene expression was detected in leaf blades but not in dehulled mature grains and provided effective resistance to first-instar larvae of SSB and YSB. Datta *et al.* (1998) transformed rice varieties using *cry1Ab* gene driven by green-tissue specific and/or pith-specific promoters. Insect bioassays showed up to 100% mortality of YSB larvae. High level Cry proteins expression in green tissues (leaves and stem) of the plants resulted in enhanced YSB resistance. Frutose and group at the Center for International Co-operation in Agricultural Research for Development, France, used *cry1Aa* or *cry1B* gene under the control of wound-inducible promoter (*mpi*) for rice transformation. Insect bioassays of the transgenic plants provided full protection against SSB larvae in T_0 to T_4 generations (Breitler *et al.*, 2000). Accumulation of Cry1B toxin in leaf and pith tissues upon wounding, and its absence in pollen grains and endosperms was demonstrated in *mpi-cry1B* transgenic plants.

Four practical recommendations for sustainable use of *Bt* transgenic crops have been recommended by Cohen *et al.* (2000). These are: (i) not to release *Bt* transgenic crop varieties which do not have a high-dose of the toxin. (ii) release only those *Bt* crop varieties that harbour two dissimilar *Bt* genes both expressed at high-dose. (iii) not to release too many *Bt* transgenic varieties of a crop. Some popular non-*Bt* crop varieties must remain be available to improve availability of refuges in the area. (iv) implementation of a resistance monitoring program. Resistance monitoring programs can provide valuable information for deployment and improvement of future insect-resistant varieties. Use of sentinel plots, in which insect damage is monitored in unsprayed fields of *Bt* transgenic crops, is perhaps the most practical method of monitoring insect-pest populations for evolution of resistance to *Bt* toxin (Andow and Hutchinson, 1998).

Other biosafety concerns: The rapid development of transgenic crops brings about the questions of their environmental safety. It is likely that a wide range of modifications, other than insect resistance, will be targeted in transgenic plants in the future, thus intensifying the need of biosafety considerations (Kumar, 2001b). In fact, biosafety concerns are among the major reasons for non-adoption of transgenic crops in most of the countries. One of the issues of concerns pertains to the inclusion of superfluous and undesirable DNA sequences as a consequence of the plant transformation protocols currently in use. Two commonly used transformation techniques, *viz.* Direct DNA transfer and *Agrobacterium*-mediated transformation, generally leads to the integration of vector backbone sequences into the host genome (Kononov *et al.*, 1997). The integration of vector backbone may exert undesirable effects in *cis* (Artelt *et al.*, 1991) as well as promotes transgene rearrangements. Although, it has been a common practice for all the vector backbone sequences to be removed before microinjecting the foreign DNA into animal eggs or embryos (Palmiter and Brinster, 1986), whole plasmid bombardment has been a common practice for plant transformation. Therefore, Fu *et al.* (2000) investigated utility of 'clean DNA' transformation technique for rice following microprojectile-mediated direct DNA transfer. Results revealed that such transformants had predominantly 'simple' integration events, produced transgenic plants with low-copy number and a low frequency of transgene re-arrangements. In a subsequent study, Loc *et al.* (2002) confirmed the utility of 'clean DNA' transformation technique in rice transformation. Higher levels of transgene product accumulation were found in transgenics derived from minimal gene-cassette transformations, in comparison to those derived from whole plasmids (Loc *et al.*, 2002). Co-bombardment with two 'clean DNA' gene-cassette fragments, one carrying the gene of interest and plant selectable marker gene on the other, allows integration of transgenes at different loci of the host genome. Such integration facilitates separation of unwanted marker gene from the gene of interest during segregation of the genes in the progenies to obtain marker free clean DNA transgenic plants (Kumar *et al.*, 2006b). In an attempt towards generation and evaluation of *Bt* transgenic indica rice, translational fusion genes of *cry1B-1Aa* and hygromycin selectable marker gene (*hpt II*) were used for biolistic-mediated 'clean DNA' transformation. The 'clean DNA' transgenic rice plants showed high level protection against YSB infestation. Independent assortment of the *Bt* gene and selectable marker gene was observed in the segregating progenies.

The concern has also been expressed that pest damage in non-*Bt* fields will increase after introduction of *Bt* transgenic crops and farmers will be less interested to grow non-*Bt* crops. In contrast, it has been envisaged that after introduction of *Bt* crop, say *Bt* rice, the damage caused by the insect (stem borers) in non-*Bt* crop will probably be unchanged or it may reduce. If we look at biology of the insect, we find that there is no evidence that stem borer moths can detect whether a rice plant contains *Bt* toxin or not, therefore moths will not prefer to lay their eggs on *Bt* or non-*Bt* plants (Hellmich *et al.*, 1999). Studies show that caterpillars move away from *Bt* rice plants faster than from non-*Bt* plants, but only few stem borer larvae move from one field to another (Cohen *et al.*, 2000). On the other hand, stem borer moths move very fast from one field to another (Khan *et al.*, 1991). Many of the moths emerging from non-*Bt* rice field will disperse and lay their eggs in *Bt* fields. Because only a few moths will be emerging from *Bt* fields, very few moths will move from *Bt* fields to non-*Bt* fields. As a consequence, the amount of stem borer damage in non-*Bt* fields will reduce. Such reduction in damage in refuge fields, also known as 'halo effect', has been reported (Riggin-Bucci and Gould, 1997) in case of the damage caused by European corn borer on *Bt* maize (Andow and Hutchinson, 1998).

Some environmentalists argue that once modified crops are released into the environment, they could have unforeseen and undesirable effects. Although *Bt* transgenic crop produces very specific insecticidal protein intended to kill only pests that feed on the crop, in 1999 researchers at Cornell University found that pollen from *Bt* corn could kill caterpillars of the harmless Monarch butterfly. When they fed milkweed dusted with pollen from *Bt* corn in the laboratory to Monarch caterpillars, half of the larvae died. But follow-up field studies showed that under real-life conditions Monarch butterfly caterpillars rarely come in contact with pollen from corn. Serious biosafety concerns are being expressed about the use of *Bt* toxin gene in food crops. Acceptance of *Bt* transgenics wherein the toxin gene is constantly expressed in the edible part of the plant, such as grains, is questioned. This led to the search for temporal and spatial expression of the toxin. It has been argued that transgenic plants can directly or indirectly affect natural enemies if the toxin expressed in the plants is toxic for them or prey are fewer, smaller, less nutritious or behaviourally unacceptable (Schuler, 2000). Any human interference to protect crops from pests may have some negative impact on the insects that depend on the pests (Schuler, 2000). Many pesticides indiscriminately kill both pests and natural enemies, but *Bt* toxins are safer in the sense that they show specificity in their action. Another issue frequently cited as a potential risk is the possible movement of the inserted transgene from crops to wild or weedy relatives, and the consequences of such movement. Among several strategies to control transgene-flow, plastid transformation has been advocated for the purpose but there are reports that transgene present in the plastid genome can rapidly relocate itself into the nuclear genome (Stegemann *et al.*, 2003; Huang *et al.*, 2003). On the other hand, transgene expression in transplastomic plants is generally high which is desirable to increase the level of expression of the *Bt* protein for their effective and sustainable deployment.

Many environmentalists are concerned about the loss of biodiversity due to adoption of transgenic crops. Increased use of HYVs raised similar concerns in the past century, which led to the extensive efforts towards germplasm collection and maintenance. These collections are maintained and used by the plant breeders. Biotechnology has also significantly improved *in vitro* long-term storage and preservation of genetic materials. While transgenic crop will ensure reliable supply of basic foodstuffs, markets for specialty crop varieties and locally grown cultivars will help in maintaining the diversity. With the kind of concerns witnessed among the public, keeping GM and non GM products separately with appropriate labeling, perhaps also through colour codes for illiterate people, may be absolutely necessary. Another related issue is the need for a certification agency specialized in certifying the GM nature of the products. The debate between proponents (scientists and companies involved in development of GMOs) and opponents of GM technology

(NGOs like greenpeace, gene campaign *etc.*) has created major obstacles in harnessing benefits of the technology. In order to maximize the trust, it is essential that relevant and reliable information about the GM products is communicated to the consumers and stake holders. The media, scientists, administrators, politicians and NGOs have the responsibility to educate people about the benefits of GM products.

Biosafety Measures

While biotechnology has been recognized to have great potential for the promotion of human well-being, particularly in meeting critical needs for food and healthcare, the concept of boisafety is necessary to protect human health and environment from the possible adverse effects of the products of genetic engineering. Biosafety is one of the issues addressed by the convention on biological diversity (CBD), which was finalized in Nairobi in May 1992 and entered into force on 29 December 1993 (Anonymous, 2004a). Today, the convention is the main international instrument for addressing biodiversity issues. It provides a comprehensive and holistic approach to the conservation of biological diversity, sustainable use of natural resources, fair and equitable sharing of benefits deriving from the use of genetic resources. In the second meeting of the convention, held in November 1995, an open-ended Adhoc working group on biosafety was established to develop a draft protocol on biosafety, focusing specifically on transboundary movement of any genetically modified organism (GMO) resulting from modern biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity. After several years of negotiations, the protocol, known as the Cartagena protocol on biosafety, was finalized and adopted in Montreal on 29 January 2000 in a meeting of the conference of the parties (Anonymous, 2000). The protocol thus created an enabling environment for the environmentally sound application of biotechnology, making it possible to derive maximum benefit from modern biotechnology, while minimizing the possible risks to the environment and to human health.

The protocol promotes biosafety by establishing rules and procedures for the safe transfer, handling, and use of GMOs, with specific focus on transboundary movements of GMOs. It features a set of procedures including one for GMOs that are to be intentionally introduced into the environment and one for GMOs that are intended to be used directly as food, fodder or processing. Parties to the protocol must ensure that GMOs are handled, packaged and transported under conditions of safety. Furthermore, the shipment of GMOs subject to transboundary movement must be accompanied by appropriate documentation specifying identity of GMOs and contact point for further information (Anonymous, 2000). The protocol has been signed by 103 countries. The Biosafety protocol was signed by India on 23rd January 2001. Subsequent to the cabinet's approval on 5th September, 2002, India has acceded the Biosafety protocol on 17th January 2003.

Indian strategy for development of transgenic crops: In India, Department of Biotechnology (DBT), under Ministry of Science and Technology (MOST), is the nodal agency for biotechnology research and promotion. Transgenic crops that need commercialization have to undergo and pass extensive safety trials with regard to potential for food toxicity, food allergenicity, cross pollination and effect on non-target beneficial organisms including biological control agents. Proactive measures need to be taken up to delay the development of pest resistance to *Bt* proteins expressed *in-planta*. The major responsibility for regulation of GMOs lies with the MOST and Ministry of Environment and Forests (MOEF). Other ministries and institutions, for example, the Ministry of Agriculture, Indian Council of Agricultural Research (ICAR), Agricultural Universities, State Department of Agriculture etc. may be involved for issues related to agricultural biotechnology (Manjunath, 2005). Regulatory mechanisms, based on interaction between committees and different Government departments, for the development and release of transgenics are in place (Fig. 3). Two important committees, namely Institutional Bio-Safety Committee (IBSC) and Review Committee on Genetic Manipulation (RCGM), work under the guidance of DBT. Another major committee, namely Genetic Engineering Approval Committee (GEAC), is constituted under MOEF. GEAC is the competent authority to grant permission to conduct large-scale field trials of transgenics (Ghosh, 1997).

India made its long-awaited entry into commercial agricultural biotechnology when the GEAC, at its 32nd meeting held in New Delhi on 26th March 2002 approved three *Bt* cotton hybrids for commercial cultivation (Kumar, 2002). This was a historic decision as *Bt* cotton became the first transgenic crop to receive such an approval in India. The *Bt* cotton hybrids approved were MECH 12, MECH 162 and MECH 184 of Mahyco-Monsanto Biotech Limited, which were planted in six states of south and central India to cover about 29,415 ha (Manjunath, 2005). Following its success, the area under *Bt* cotton increased to 86,240 ha in 2003 and to 3,800,000 ha in 2006 (James, 2006). India made record increase in its adoption with almost tripling of area under *Bt* cotton from 1,300,000 ha in 2005. This tripling in area was the highest year-on-year growth for any country in the world. Realizing the potential of *Bt* cotton, 19 other Indian seed companies have started introducing the *Bt*-gene into their own cotton hybrids developed for different agroclimatic regions and will seek regulatory approvals.

The GEAC examines, under the Environment Protection Act-1986, from the viewpoint of environmental safety and issues clearance or release of genetically modified organisms and products into the environment (Sharma *et al.*, 2003). To address the biosafety concerns of public on GM food, it is of utmost importance that the regulatory system is followed strictly. The environmental effects of transgenic crops need to be assessed and compared with non-transgenic crops grown with conventional practices. The field performance and drift of transgene to wild relatives of the crop or to non-transgenic varieties need to be monitored over a considerable period of time. While introducing transgenic varieties, it is necessary to maintain the conventionally bred varieties for use as germplasm for future plant breeding. Major initiative in transgenic research and

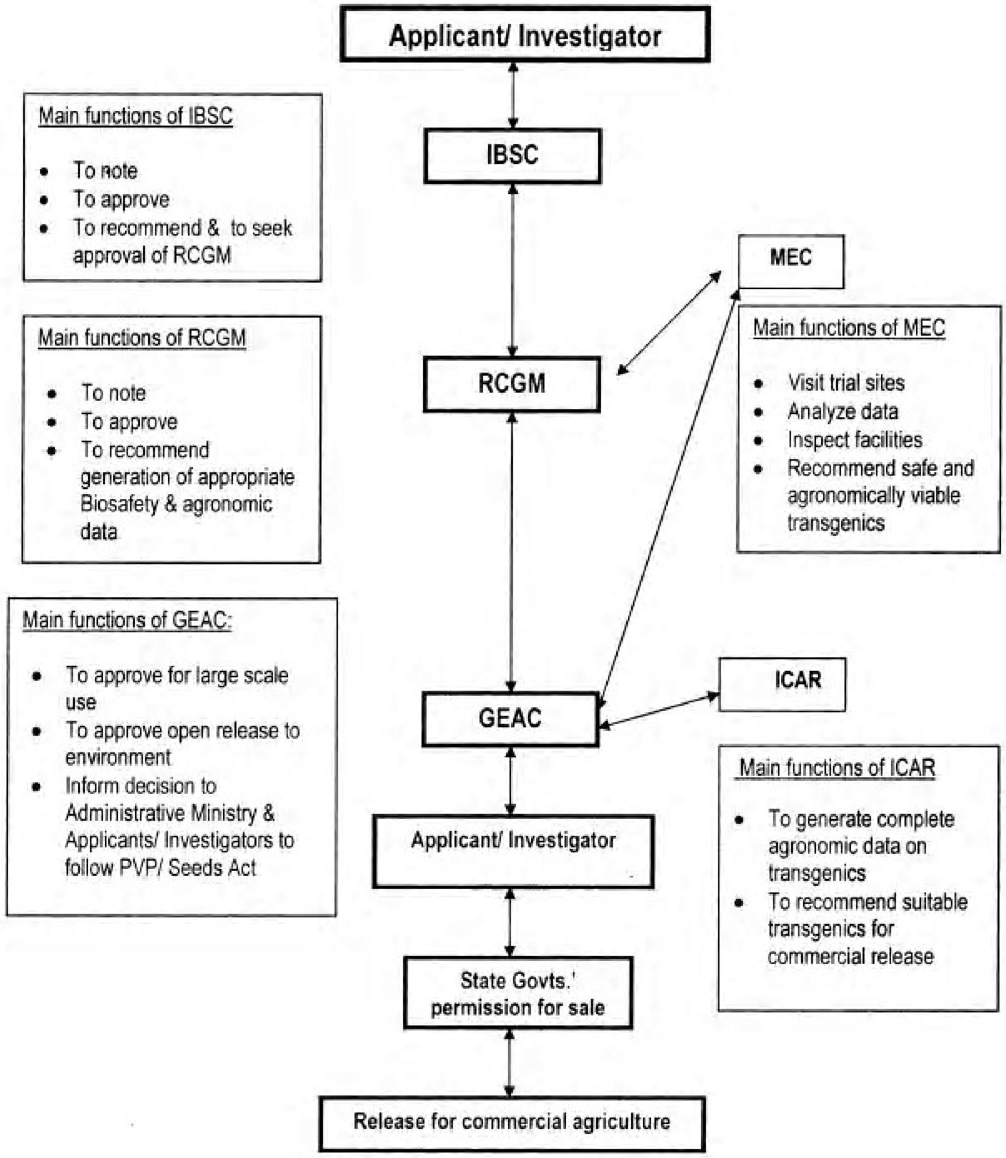

Fig. 3: Procedures involved in commercialization of indigenously developed transgenic crops in India (Reproduced from Sharma *et al.*, 2003)

development in public and private sector has been discussed by Sharma *et al.* (2003).

Task Force on Agricultural Biotechnology chaired by Dr. M.S. Swaminthan, set up by the Ministry of Agriculture in 2003, prepared a detailed framework on the application of biotechnology in agriculture. The report rightly laid emphasis on the judicious use of biotechnology for the economic well-being of farm families, food security of the nation, health security of the consumer, protection of the environment, and security of national and international trade in farm commodities (Anonymous, 2004b). Priority in agricultural biotechnology must be based on social, economic, ecological, ethical and gender equity issues. An indicative list of priority crops and traits was suggested by the task force. Priority target crops has been suggested as wheat, maize, sorghum, pigeon pea, chickpea, moongbean, groundnut, mustard, soybean, cotton, sugarcane, potato, tomato, cole crops, banana, papayas and citrus, and the traits have been yield increase, pest and disease resistance, abiotic stress tolerance, enhanced quality and shelf-life, engineering male sterility and deployment of apomixes (Anonymous, 2005). With special reference to basmati rice, soybean and Darjeeling tea, it was suggested that transgenic research should not be undertaken in crops/commodities where our international trade may be affected. However, its use may be allowed for generation of proof of principle, strictly for research purpose. Also, in priority crops equal emphasis was sought to be given on GM hybrids and new varieties. The varieties, in contrast to hybrids, are preferred by small farmers as they can use their own farm saved seeds for at least three to four years. In case of hybrids, research on the introduction of genetic factors for apomixis was suggested so that resource-poor farmers can derive benefits from hybrid vigour without having to buy expensive seeds every cropping season (Anonymous, 2005).

The indiscriminate use of chemical insecticides has resulted into the development of insecticide-resistant pest populations and contamination of water and food sources. Technologies and chemical inputs that have proven harmful for human health and environment need to be replaced with safer alternatives to manage insect pests in agricultural ecosystem. Increased public concerns about the potential adverse environmental effects of chemical pesticides led to the search of alternative methods for insect-pest control. Transgenic technology has facilitated insect control in crop plants in a safe and sustainable manner. It offers the possibility of introducing *Bt* gene(s) into crop plants to develop entirely new system of pest management that have many advantages of classical biological control agents. The resistance needs to be protected by taking lessons from our past experiences with chemical pesticides. *Bt* may play a central role in protecting crop plants in combination with other insecticidal proteins such as proteinase inhibitors, thereby it will drastically reduce the consumption of chemical pesticides and protect the environment.

Although *Bt* gene expression in transgenic plants has tremendous effects on insect-pest management as well as environmental safety, equally important is the need to follow biosafety regulations to make the technology sustainable. The durability of insect resistance in transgenic crops can only be ensured if IPM practices are followed. The judicious expression of multiple insecticidal proteins that differ in their mechanism of toxicity will provide formidable barriers for insects to develop resistance. In spite of the advantages of *Bt* based insect resistance in crop plants, doubts and uncertainty about general risks and hazards, long term safety to human health, nutrition and environment, and sustainability to agriculture exist, which need to be properly dispelled by generating scientific data on the issues. Thus, there is need to adopt biosafety protocol and resistance management strategies, organize public awareness, and present the benefits of *Bt* transgenic crops to improve social attitude for their rational deployment. If deployed appropriately, *Bt* transgenic technology could be one of the best environment friendly insect-pest management strategies.

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