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Insect-resistant transgenic crops: retrospect and challenges

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Abstract: The advent of genetic engineering has revolutionized agriculture remarkably with the development of superior insect-resistant crop varieties harboring resistance against insect pests. *Bacillus thuringiensis* (*Bt*) has been used as a main source for insect-resistant genes. In addition to Bt endotoxins, various plant lectins and other non-*Bt* genes from different sources have also been introduced in crop plants of economic importance. The insect-resistant crops have made a huge economic impact worldwide since their commercial release. The cultivation of insect-resistant cultivars has resulted both in increased crop productivity and in decreased environmental pollution. Although insect-resistant crops have been allowed to be commercialized following proper biosafety guidelines and procedures, still these crops face many challenges in order to be fully adopted and accepted. The degradation kinetics of Bt proteins, horizontal and vertical gene flow, effects on nontarget insects or organisms, antibiotic resistance, and some other unintended effects have been noted and discussed. Although no concrete evidence regarding any significant hazard of genetically engineered crops has been presented so far, the debate still remains intense. Impartial and professionally competent regulatory mechanisms for the evaluation of insect-resistant and other transgenic crops must be fully functionalized. The first part of this review focuses the development of different insect-resistant crops and various strategies adapted to delay resistance development in insect pests, while the second part addresses the challenges and future prospects of insect-resistant crops.

Key words: Transgenic Bt crops, adaptation, economic impact, safety assessment

1. Introduction

Conventional breeding methods have helped plant scientists to develop high-yielding crop varieties for centuries; however, certain unavoidable factors have led to a slowed pace in varietal developments, most importantly including the limitation of fertility barriers (Hussain, 2002). Modern recombinant technologies enabled researchers to move genes across species without any taxonomical limitations. Later on, advancements in plant transformation technologies helped to incorporate genes of interest in crop plants of economic importance (Khan et al., 2013).

Approximately 67,000 pest species able to damage crops have been reported; almost 9000 of these species are insects and mites (Ross and Lembi, 1985). Insect pests damage crops either by sucking sap or chewing plant parts like leaves, stems, roots, or fruits. Several pest species (larvae as well as adults) of Homoptera, Coleoptera, Lepidoptera, and Diptera fall into this category. The insect pest can also damage crops indirectly by acting as a vector for viral, bacterial, or fungal transmission (Rahman et al., 2012). According to an earlier report, the crop losses from insect pests and diseases were calculated at up to 37% in agricultural production globally, with 13% of losses incurred because of insects (Gatehouse et al., 1992). However, this can vary with climatic conditions and crop and pest type. Oerke (2006) reported actual crop losses in different crops, i.e. soybean (29%), wheat (28%), cotton (29%), maize (31%), rice (37%), and potato (40%).

Crop productivity has been affected by a variety of pests since the dawn of agriculture. Researchers and farmers adopt different means for crop protection against these pests (Oerke, 2006). With the introduction of synthetic insecticides, crop protection relied on the use of insecticides. However, such crop protection strategy has been proved unfriendly for the environment as well as for public health (Curry, 2002; Bakhsh et al., 2009). A study reported that 1%–3% of workers suffered from acute

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** Current address: Department of Field Crops, Faculty of Agricultural and Natural Sciences, Abant İzzet Baysal University, Bolu, Turkey pesticide poisoning while approximately 1 million required hospitalization annually, representing between 25 million and 77 million workers globally (EJF, 2007). Hence, to reduce the harmful side effects of insecticide application, genetically manipulated crops have been introduced using various plant transformation approaches.

The advent of recombinant DNA technology and successful plant transformation techniques led to the introduction of the first transgenic tomato, tobacco, and cotton in 1987 (Umbeck et al., 1987; Vaeck et al., 1987). *Cry* genes from *Bacillus thuringiensis* (*Bt*) have been widely used for the production of insect-resistant plants. These genes encode resistance against insect pests from Lepidoptera (Cohen et al., 2000), Coleoptera (Herrnstadt et al., 1986), and Diptera (Andrews et al., 1987). In addition to *cry* and *vip* genes from *Bacillus thuringiensis*, many other genes of bacterial, plant, or fungal origin encoding insect resistance have also been reported (Kereša et al., 2008).

Since commercialization, insect-resistant crops have widely been accepted and cultivated, and a gradual increase in cultivation has been witnessed (Figure 1). According to recent reports, the global area devoted to biotech crops has increased to 175.2 × 10^6 ha in 2013 from 1.7 × 10^6 ha in 1996. Transgenic soybean, cotton, maize, rice, oilseed rape, sugar beet, chickpea, tomato, and alfalfa crops have been developed successfully and some of them are already on the market (James, 2013). Transgenic technology and its successful utilization in agriculture have contributed significantly to global food security and poverty reduction. Reports show that this technology is advantageous for farming communities and consumers (Qaim, 2009).

The use of genetic engineering technologies in modern-day agriculture has been questioned and criticized. Many researchers as well as common people have raised concerns about the use of genetically modified organisms (GMOs), including insect-resistant crops (Godfrey, 2000). Organic agriculture supporters and activist journalists (anti-GMO campaigners) claim that transgenic crops are understudied and whatever studies that have been conducted came from seed companies who are producing GMOs. The fate of Bt protein in the soil, vertical and horizontal gene flow, effects on nontarget insects, antibiotic resistance, and some other unintended effects of transgenic crops have been highlighted in electronic and print media time and again (Bakshi, 2003; Séralini et al., 2007). There must be a pure scientific approach to evaluate the risks of insect-resistant crops for human health and the environment. Many countries have developed regulation and legislation procedures regarding GM crops to address public concerns about the food and environmental safety of transgenic crops (Perr, 2002; Singh et al., 2006).

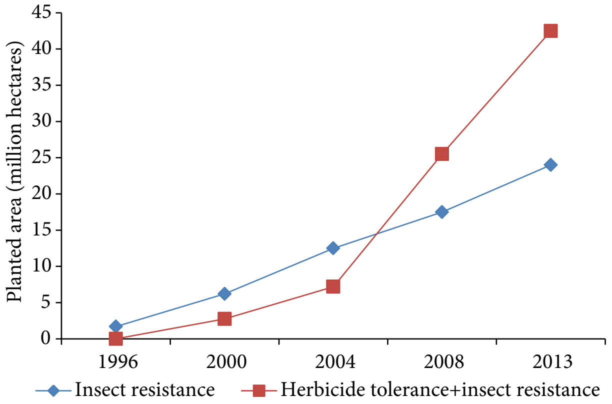

Figure 1. A trend in increased cultivation of commercialized insect-resistant crops worldwide. The graph also shows the data for insect-resistant crops in combination with herbicide tolerance trait (James, 2013).

2. Insect-resistant crops

The recent advances in the field of biotechnology have shown tremendous effects in improving agricultural crops by incorporating genes from different sources to build resistance against insect pests (Dhaliwal et al., 1998). As mentioned earlier, insect pests and diseases are serious threats to crops, causing approximately 37% loss of yield, while 13% losses have been reported only because of insect pests (Gatehouse et al., 1992). The genes from *Bacillus thuringiensis* have been extensively used in this context. A majority of *Bt* strains are harmful to insect pests from Lepidoptera; however, some of them are also lethal to insect pests from Coleoptera (McPherson et al., 1988) or Diptera (Yamamoto and Mclaughlin, 1981) (Table 1). It has been established that Bt proteins do not show any toxicity to beneficial insects, other animals, or humans (Klausner, 1984). The modification of *Bt* genes for better expression in plants was an important step towards obtaining insect resistance in plants (Perlak et al., 1991). The modified (codon-optimized) genes conferring protection against lepidopteran and coleopteran pests respectively were transferred to cotton and potato at first (Perlak et al., 1991). After initial reports of insect resistance, series of successful experiments were documented; a few such examples are compiled for the interest of readers in Table 2.

In addition to *cry* genes from *Bacillus thuringiensis*, many other genes of bacterial, plant, and other origins conferring insect resistance have been documented in crops (Kereša et al., 2008). Proteinase inhibitors (PIs) have been reported to show significant inhibitory activity against insect digestive enzymes. For the first time, use of a plant-derived PI gene by transforming tobacco plants with the trypsin inhibitor gene (*CpTI*) from *Vigna unguiculata* was reported (Hilder et al., 1987). Potato inhibitor II genes have been introduced in rice, cotton, and other crops, as well (Duan et al., 1996; Majeed, 2005).

Table 1. Examples of some important *cry* genes widely used that show toxic activity against insects pests from Lepidoptera, Coleoptera, and Diptera.

Cry gene	Targeted insect pests (common names)	Insect order
cryIA(a)	Silk worm, tobacco horn worm, European corn borer	Lepidoptera
cryIA(b)	Tobacco horn worm, cotton boll worms, cabbage worm, mosquito	Lepidoptera and Diptera
cryIA(c)	Tobacco budworm, cabbage lopper, cotton bollworm	Lepidoptera
cryIA(e)	Tobacco budworm	Lepidoptera
cryIB	Cabbage worm	Lepidoptera
cryIC	Cotton leaf worm, mosquito	Lepidoptera and Diptera
cryIC(b)	Beet army worm	Lepidoptera
cryID	Beet army worm, tobacco horn worm	Lepidoptera
cryIE	Cotton leaf worm	Lepidoptera
cryIF	European corn borer, beet army worm	Lepidoptera
cryIG	Greater wax moth	Lepidoptera
cryIIA	Gypsy moth, mosquito, cotton bollworm	Lepidoptera
cryIIB	Gypsy moth, cabbage lopper, tobacco horn worm	Lepidoptera
cryIIC	Tobacco horn worm, gypsy moth	Lepidoptera
cryIIIA	Colorado potato beetle	Coleoptera
cryIIIA(a)	Colorado potato beetle	Coleoptera
cryIIIB	Colorado potato beetle	Coleoptera
cryIIIC	Spotted cucumber beetle	Coleoptera
cryIVA	Mosquito (*Aedes* and *Culex*)	Diptera
cryIVB	Mosquito (*Aedes*)	Diptera
cryIVC	Mosquito (*Culex*)	Diptera
cryIVD	Mosquito (*Aedes* and *Culex*)	Diptera
cryV	European corn borer, spotted cucumber beetle	Lepidoptera and Coleoptera

Plant lectins have also been successfully utilized in crop protection against insect pests (Goldstein and Hayes, 1978). Various lectins have proved toxic towards members of Coleoptera, Lepidoptera (Czapla and Lang, 1990), and Diptera (Eisemann et al., 1994). Plant lectins are used to control sap-sucking insects belonging to the order Homoptera, which includes some of the most devastating pests worldwide. The lectins result in inhibited nutrient absorption or disruption of midgut cells by stimulating endocytosis and possibly other toxic metabolites present in the midgut (Czapla and Lang, 1990). The successful efficacy of plant lectins and other non-*Bt* genes against sucking insect pests has been successfully documented in transgenic crop plants (Table 3).

Beside the common strategies of achieving resistance such as applying toxic proteins, lectins, or inhibitors, plant-mediated RNAi technology has emerged as a new horizon to combat insects, and especially to address resistance development in targeted insect pests (Price and Gatehouse, 2008). RNAi, initially characterized in *Caenorhabditis elegans* (Fire et al., 1998), has emerged as an efficient gene-silencing approach in various organisms (Hannon, 2002). The gene knockdown of different insects has been achieved via orally fed dsRNA, including insects from Hymenoptera (Lynch and Desplan, 2006), Coleoptera (Tomoyasu et al., 2008), Diptera (Dzitoyeva et al., 2001), and Lepidoptera (Terenius et al., 2011). However, results from Mao et al. (2011), Zhu et al. (2012), and Mao and Zeng (2014) are more encouraging; using plant-mediated RNAi technology they knocked down the cytochrome P450 (*CYP6AE14*), ecdysone receptor (*EcR*), and hunchback (*hb*) genes to combat *Helicoverpa armigera*, *Spodoptera exigua*, and *Myzus persicae*, respectively. However, the technology is still in an early phase and being thoroughly investigated by different research groups worldwide.

2.1. Economic impact of Bt crops

The annual market of synthetic insecticides is approximately 8.11 billion US dollars; 30% of these insecticides are applied to vegetables and fruits while 23% and 15% are used to protect cotton and rice, respectively

Table 2. Examples of insect-resistant crops developed by different researchers using different resistance sources. Most are *cry* genes from *Bacillus thuringiensis*.

Plant/crop	Gene introduced	Target insects	Reference
Cotton	*cryIA(a)* *cry1A (b)* *cry1A (c)* *cryIIA* *cry1EC* Potato inhibitor *GNA*	Lepidoptera Homoptera	Perlak et al., 1990 Majeed, 2005 Tohidfar et al., 2008 Khan et al., 2011 Bakhsh et al., 2012 Pushpa et al., 2013
Potato/sweet potato	*cry3Aa* *cry1A (c)* Cowpea trypsin inhibitor *GNA*	Coleoptera Lepidoptera	Peferoen et al., 1990 Cheng et al., 1992 Adang et al., 1993 Perlak et al., 1993 Newell et al., 1995 Morán et al., 1998
Soybean	*cryIA(b)* *cryIA(c)*	Lepidoptera	Parrott et al., 1994 Dufourmantel et al., 2005 Dang et al., 2007
Rice	*cryIA(b)* *cryIA(c)* PinII *cry1C* sbk+sck	Lepidoptera	Fujimoto et al., 1993 Wunn et al., 1996 Cheng et al., 1998 Bashir et al., 2005 Tang et al., 2006 Zhang et al., 2013
Maize	*cry3Bb1* *cry1Ab* *cry1Ab* (MON810) *cry19c*	Lepidoptera	Koziel et al., 1993 Vaughn et al., 2005 Gassmann et al., 2011
Canola	*cry1A (c)*	Lepidoptera	Tabashnik et al., 1993 Stewart et al., 1996 Ramachandran et al., 1998 Halfhill et al., 2001
Chickpea	*cry1A (c)* *cry2Aa* *cry1A (c) + cry1A (b)*	Lepidoptera	Sanyal et al., 2005 Indurker et al., 2007 Acharjee et al., 2010 Mehrotra et al., 2011
Tomato	*cry1A (c)* *cry1A (b)*	Lepidoptera	Mandaokar et al., 2000 Kumar et al., 2004 Koul et al., 2014
Alfalfa	*cry3a*	Coleoptera	Tohidfar et al., 2013

(Krattiger, 1997). Almost 92% of the world's rice is produced in Asia, and the bulk of insecticides, calculated to one billion dollars approximately, is used to protect this crop from insect pests. Cotton is another favorite crop of insect pests, consuming insecticides that annually cost approximately 1.9 billion dollars. The efficacy of insect-resistant crops through Bt has been effective and an ideal alternative to synthetic insecticides (Bakhsh et al., 2009). The development of insect-resistant cotton resulted in a reduction of 49.8% of insecticide use worldwide, Mexico and China being at the top with 77% and 65% reductions of insecticide use, followed by Argentina (47%), India (41%), and South Africa (33%), respectively (Qaim, 2009). The reduction in insecticide use resulted in increased crop productivity. On average, 22.5% increase in yield has been recorded worldwide by the introduction of insect-resistant crops. Biotech cotton in China brought economic benefits valued at over $15 billion between 1996 and 2012, with $2.2 billion gained during the past year. India increased farm income using Bt cotton by $5.1 billion in the period

Table 3. Important examples of insect-resistant crops developed using plant lectins. Targeted pests and transformation methods are also presented. BPH: Brown planthopper, GLH: green leafhopper, SBPH: small brown planthopper, WBPH: whitebacked plant hopper.

Crop	Gene	Insect type	Method/applied explant	Result	Reference
Rice	*GNA*	*Nilaparvata lugens*; BPH	Electroporation of rice protoplast and particle bombardment of the immature rice embryo	Decrement in survival and fecundity of BPH	Rao et al., 1998
Rice	*GNA*	Sap-sucking insects including BPH	Particle bombardment of mature seed-derived callus	Resistance against BPH and bacterial blight	Tang et al., 1999
Rice	*GNA*	Sap-sucking insects including BPH and GLH	Particle bombardment of immature rice embryos	Resistance against BPH and GLH	Foissac et al., 2000
Rice	*GNA*	Sap-sucking insects including SBPH	Particle bombardment of mature seed-derived callus	Expressing *GNA* of over 0.3% of total soluble protein	Wu et al., 2002
Rice	*GNA*	Sap-sucking insects including BPH and GLH	*Agrobacterium*-mediated genetic transformation of embryogenic calli	Significant resistance towards BPH and GLH insects with minimal plant damage	Nagadhara et al., 2003
Rice	*GNA*	Sap-sucking insects including BPH, GLH, and WBPH	*Agrobacterium*-mediated genetic transformation of embryogenic calli	Substantial resistance against three major sap-sucking insects of rice	Ramesh et al., 2004
Rice	*ASAL* (*Allium sativum* agglutinin)	Sap-sucking insects including BPH and GLH	*Agrobacterium*-mediated genetic transformation of scutellar calli	Reduction in fecundity and survival	Saha et al., 2006
Rice	*ASAL*	Sap-sucking insects including BPH, GLH, and WBPH	*Agrobacterium*-mediated genetic transformation of embryogenic calli	Surpassing the resistance BPH, GLH, and WBPH	Yarasi et al., 2008
Rice	*ASAL*	Sap-sucking insects including BPH and GLH	*Agrobacterium*-mediated genetic transformation of the calli	Radical reduction in survivability and fecundity of BPH and GLH	Sengupta et al., 2010
Rice	*DB1/ G95A-mALS*	Sap-sucking insects including BPH	*Agrobacterium*-mediated genetic transformation of the calli	Decrement in fecundity and survival of BPH	Yoshimura et al., 2012
Rice	*ASAL*	BPH	*Agrobacterium*-mediated genetic transformation of scutellum-derived embryogenic calli	Significant resistance towards BPH with minimal plant damage	Chandrasekhar et al., 2014
Indian mustard	*WGA-B*	Mustard aphid	*Agrobacterium*-mediated genetic transformation of hypocotyl	Decrement in survival and fecundity of mustard aphid	Kanrar et al., 2002

Table 3. (Continued).

Indian mustard	*ASAL*	Mustard aphid	*Agrobacterium*-mediated genetic transformation of the calli derived from hypocotyl	Sustainable resistance against mustard aphid	Dutta et al., 2005
Indian mustard	*ACA* (*Amaranthus caudatus* agglutinin) *ACA-ASAL*	Mustard aphid	*Agrobacterium*-mediated genetic transformation of the apical meristem	Giving resistance against mustard aphid by reducing survival and fecundity	Hossain et al., 2006
Indian mustard	*ASAL*	Mustard aphid	*Agrobacterium*-mediated genetic transformation	Resistance against mustard aphid	Bala et al., 2013
Potato	*ConA*	Peach-potato aphid	*Agrobacterium*-mediated genetic transformation of leaf pieces	Protection against aphid was documented	Gatehouse et al., 1999
Wheat	*GNA*	Grain aphid	Particle bombardment of the calli	Decrement in fecundity	Stoger et al., 1999
Maize	*GNA*	Corn leaf aphid	*Agrobacterium*-mediated genetic transformation of the embryogenic type II calli derived from immature embryos	Fecundity of the insects reduced depending on strong *GNA* expression	Wang et al., 2005
Chickpea	*ASAL*	Cowpea aphid	*Agrobacterium*-mediated transformation of single cotyledon with half embryo explant	Resistance against aphid by reducing the survival and fecundity of aphids	Chakraborti et al., 2009
Cotton	*ACA*	Cotton aphid	*Agrobacterium*-mediated transformation	Transgenic cotton plants showed resistance to aphids	Wu et al., 2006
Cotton	*ASAL*	Jassid and whitefly	*Agrobacterium*-mediated transformation	Transgenic cotton resistant against major sap-sucking pests, Jassid, and whitefly insects and glufosinate	Vajhala et al., 2013

of 2002–2008 and $1.8 billion only in 2008 (Brookes and Barfoot, 2010), while $1.7 billion was reported from Pakistan (Kouser and Qaim, 2012).

3. Delaying strategies for resistance development

Earlier researchers believed that insect pests would not able to develop resistance against cry toxin proteins. However, based on laboratory selection and field data, different species of insects were found resistant to cry proteins (Tabashnik, 1994; Ferré et al., 1995). A strain of European corn borer that required 70-fold more toxin for its mortality could not survive when fed on transgenic maize harboring the same toxin (Huang et al., 2002). The laboratory-maintained insects are supposed to have lower genetic diversity as compared to field insects.

The multiple introductions of different insecticidal genes in crops at one time is believed to result in efficient pest management. Resistance management includes the use of multiple toxins, i.e. pyramiding or stacking (Salm et al., 1994; Zhao et al., 2003). Bt proteins binding to different receptors in the same insect pests are used to avoid resistance development. Simultaneous introduction of three insecticidal genes, *cry1Ac*, *cry2A*, and *GNA*, in indica basmati rice conferred protection against yellow stem borer, rice leaf folder, and brown leaf hopper (Maqbool et al., 2001). Tobacco was transformed with *cry1Ac* and *GNA* (Zhao et al., 2001) and tomato with *cry1Ab* and *cry1Ac* (Salm et al., 1994) to achieve full protection against pests by using dissimilar genes. Cotton larvae fed with fresh plant tissue indicated that dual toxin *B. thuringiensis* cultivars expressing *cry1Ac* and *cry2A* endotoxin were more toxic to bollworm (*Helicoverpa zea*), army worm (*Spodoptera frugiperda*), and beet worm (*Spodoptera exigua*) than a single toxin (Stewart et al., 2001).

Another practical approach to prolong the effectiveness of Bt crops has been refugia strategy (Cohen et al., 2000) by dedicating a portion of a field to a nontransgenic crop (conventional counterpart); however, with the advent of dual toxin insect-resistant crops, companies like Monsanto have requested the elimination of non-Bt refugia (Christou et al., 2006). The different approaches used to delay resistance in insects are summarized in Figure 2.

The recent approach to avoid resistance development in insect pests is confining the expression of insecticidal genes in particular plant tissues, other parts of the plants serving as a spatial refuge (Schnepf et al., 1998; Shelton et al., 2000; Bakhsh et al., 2011b). Although crops with constitutive Bt expression have shown sustainable resistance in crop plants, gene expression driven by tissue-specific stress and wound inducible promoters is also desirable in order to address biosafety concerns (Özcan et al., 1993; Garg et al., 2002; Bakhsh et al., 2011a, 2012).

4. Challenges and risk concerns

Although insect-resistant crops have been on the domestic and international market since their commercialization, many ecological and other health concerns have been raised in spite of their beneficial potential (Godfrey, 2000). The major concerns raised are degradation kinetics of Bt proteins, horizontal and vertical gene flow, effect on nontarget insects, antibiotic resistance, and some other unintended effects. The aforementioned challenges

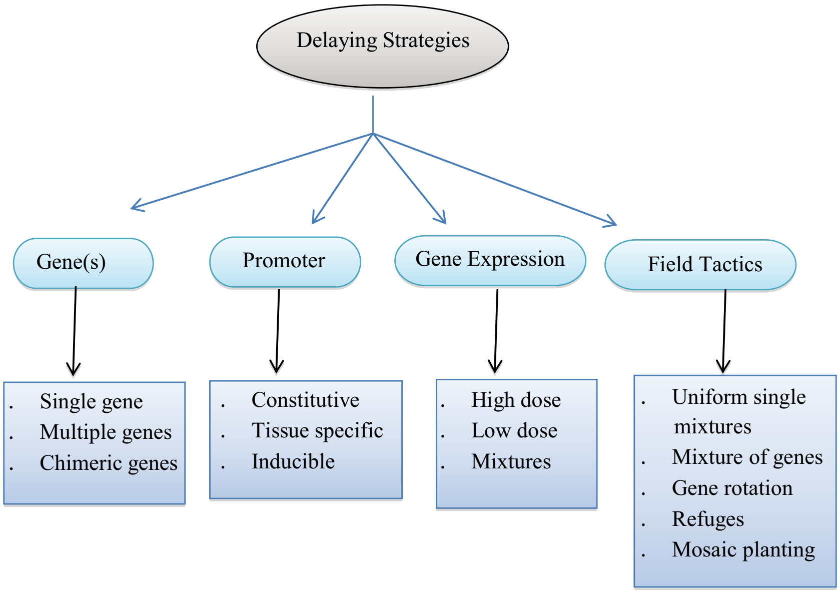

Figure 2. A sketch of different strategies/approaches proposed and adopted to delay the evolvement of resistance in targeted insect pests against *cry* and other genes.

and concerns are discussed here in view of the available literature.

4.1. The degradation kinetics of Bt proteins

Transgenic technology has emerged as a powerful tool to develop insect-resistant crops; however, the fate and effects of the introduced *Bt* gene(s) in soil ecosystems continue to be of concern (Stotzky and Saxena, 2009). The residues of Bt crop plants after harvest could result in the accumulation and persistence of *cry* genes (proteins) in the soil due to their binding on soil components (Stotzky, 2004). The Bt toxin is introduced in the soil by different field operations like postharvesting or is released from plant roots (Saxena and Stotzky, 2000). According to one estimate, an amount of 196 g/ha or 1.6 µg/g of insecticidal Bt proteins is released in soil (Sims and Ream, 1997).

Different reports on the persistence or degradation kinetics of Bt proteins in soil are available. Palm et al. (1994) reported a dissipation rate of 80% of *cry1Ab* within 7 days of experiment, while Donegan et al. (1995) estimated 28 days to 56 days for dissipation of *cry1Ac* in soil. The studies conducted by Tapp and Stotzky (1998) showed relatively longer persistence (more than 6 months) of Bt protein in the soil while, based on bioassay, the half-life of *cry1F* in soil was estimated as less than 1 day (Herman et al., 2002). Wang et al. (2006) reported that the half-life of *cry1Ab* ranged from 11.5 to 34.3 days in soil containing Bt rice straw.

Li et al. (2007) reported rapid degradation of *cry1Ac* (50%) in the initial month after harvesting of rice while the degradation rate slowed afterwards. The rates of dissipation varied greatly between the experiments due to differences in soil type and starting amounts of protein. A comprehensive study by Feng et al. (2011) helped to understand the degradation kinetics of cry1Ab proteins in soil. The effects of water contents (20%, 33%, 50%), soil temperature (15, 25, 35 °C), and pH (4.5, 7.0, and 9.0) were evaluated on the degradation of Bt proteins released from corn straw in soil. The trend of degradation of *cry1Ab* in soil from two Bt corn cultivars was the same. It rapidly degraded in the earlier stage while a slowed degradation was observed at middle and later stages. The trend in corn cultivars is shown in Figure 3.

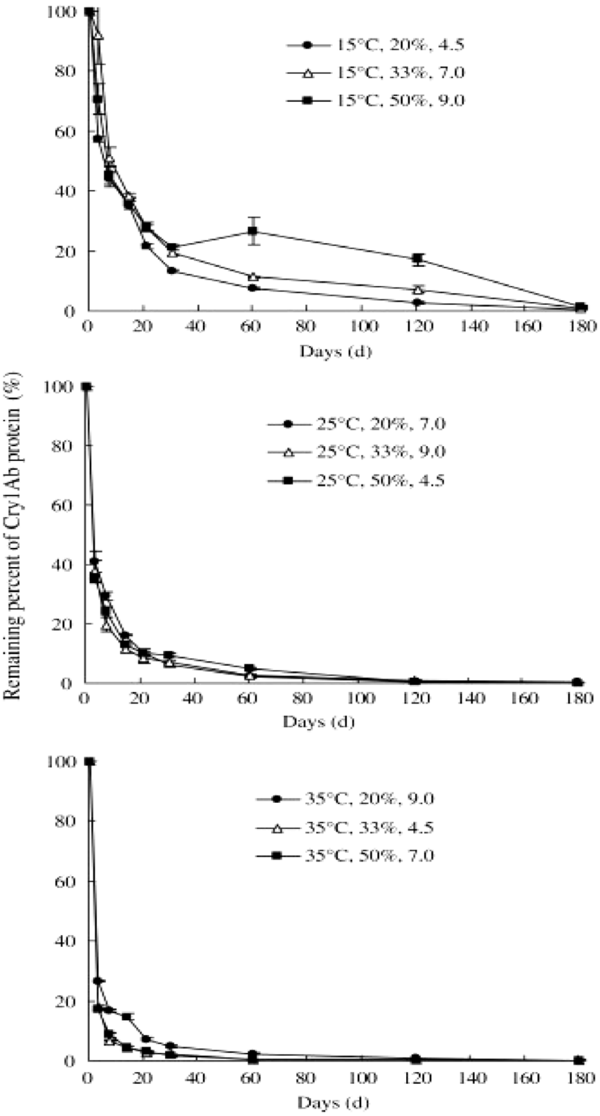

Figure 3. The degradation kinetics of *cry1Ab* gene from 34B24 (Bt Corn) straw in soil. Bt protein degraded in an earlier stage while a slowed degradation was observed in middle and later stages. Figure by Feng et al. (2011), used with permission.

There are some reports of detection of cry proteins (small amounts) in soil even a long time after incorporation of Bt straw in the soil (Feng et al., 2011). It is important to investigate biological activities of residual cry proteins to understand the effect of these proteins on soil microorganisms. The exposure of Bt proteins in soil can be avoided by using wound-inducible or green tissue promoters in transgenic crops (Özcan et al., 1993; Bakhsh et al., 2012).

4.2. Vertical and horizontal gene flow from transgenic Bt crops

One of the major concerns regarding insect-resistant crops is associated with vertical and horizontal gene flow (Stewart et al., 2003). While commercializing GM crops at large scale, the monitoring of transgene flow and its downstream concerns are of significant importance (Lu and Snow, 2005). The transgene spread in environments depends largely on possible fitness (Lee and Natesan, 2006). Seed impurity of varieties may occur as transgenes flow from GM to non-GM crop (Messeguer, 2003). The measurement of transgene flow between crops can help to understand the transgene flow from crop to weeds or wild plants, thus facilitating establishment of control measures (Lu and Snow, 2005).

Zhang et al. (2005) showed that a buffer zone of 60 m can avoid or reduce pollen dispersal from Bt cotton. They estimated a maximum outcrossing frequency of 10.48% when transgenic Bt cotton was surrounded by non-Bt cotton. The Bt pollen dispersal frequency decreased to 0.08% as distance increased to 20 m. Varying outcrossing estimates (0%–2%) in Bt rice crop have also been reported (Jia, 2002; Messeguer et al., 2004) at different distances and methods (Bashir et al., 2004). The adjacent plantation of Bt and non-Bt rice cultivars resulted in higher pollen-mediated transgene flow.

Londo et al. (2010) established the possibility of hybrid formation between transgenic Bt crops and wild relatives. Studies showed that such gene flow can lead to permanent incorporation of transgenes into wild relatives as a result of introgression (Warwick et al., 2008). In the case of insect-resistant crops, *Bt* gene flow to wild relatives may result in their fitness advantage. However, features of the transgene(s) introduced in genetically modified crops must be taken into consideration prior to evaluating the risk of gene introgressions to wild relatives (Nicolia et al., 2013). No negative results of such introgressions have been reported to date. The various strategies proposed to reduce chances of introgression from GM crops to wild relatives include delayed flowers, male sterility, and use of gene-reducing fitness (Kwit et al., 2011).

Gay et al. (2001) reported horizontal gene transfer as the transfer of genetic material from one organism to another sexually incompatible organism. The likelihood of horizontal gene transfer from plants to bacteria has been based on the established mechanisms in bacteria including transduction, conjugation, and natural transformation (Davison, 1999). The transfer of mobile sequences (plasmids, transposons, and mobilized chromosomal genes) between bacterial cells can mediate horizontal gene transfer among bacterial population residing in soil and rhizosphere, on plant surfaces, and in water (Normander et al., 1998). Weber and Richert (2001) could not detect the *Bt* gene or an endogenous corn gene in pork loin samples. PCR and Southern blot analysis of the Bt transgene and endogenous gene were uniformly negative.

The possible transfer of DNA from transgenic crops to soil microorganisms has been investigated (Droge et al., 1998). Badosa et al. (2004) examined soil bacteria collected from commercial biotech maize fields and an attempt was made to detect the ampicillin resistance gene (*bla*); no transgene was detectable by PCR. Based on laboratory experiments, de Vries et al. (2003) reported that soil bacteria can uptake very low levels of exogenous DNA (10^{-4} to 10^{-8}), while no evidence of horizontal gene transfer was found in the case of field experiments (Ma et al., 2011).

4.3. Effects of Bt crops on nontarget insect

A technology is considered successful if its benefits exceed any potential risk (Waltz, 2009). One of the important concerns of transgenic Bt technology is its impact on nontarget organisms (predators and other nontarget insects). The debate started when Losey et al. (1999) reported that Bt maize pollen is harmful for the monarch butterfly on the basis of their laboratory experiments. However, the study was criticized and questioned after repeated large-scale field trials by researchers (Oberhauser et al., 2001; Gatehouse, 2002). Since then, many studies have been conducted to investigate the impact of Bt crop on natural enemies (predators). To date, no concrete evidence has been reported about the negative impact of Bt crops on nontarget insects.

It is well established that *Bt* genes are active against particular classes of insects (Fitt et al., 1994). Comparing nontarget insects on Bt crops and non-Bt crops can help to understand whether transgenic Bt crops can influence nontarget insects (Sims, 1995; Orr and Landis, 1997). Bashir et al. (2004) found no significant differences in a number of nontarget insects in transgenic Bt rice lines and their conventional counterparts. Likewise, Bakhsh et al. (2009) collected nontarget insects from Bt and non-Bt cotton fields and found no significant differences (Figure 4). Transgenic Bt cotton expressing *cry1Ac* and *cry2Ab* genes had no harmful effects on the ladybird beetle (Li et al., 2011). The laboratory results of Lovei et al. (2009) showed a negative impact of Bt on arthropods, which was later challenged and reported as a misleading conclusion by Shelton et al. (2009). In some instances, more nontarget insects were found in Bt crops as compared to non-Bt crops where insecticides were applied, suggesting transgenic Bt technology to be quite safe in this context. A comprehensive and conclusive review by Gatehouse et al. (2011) described the effect of Bt crops on biodiversity/predators in detail.

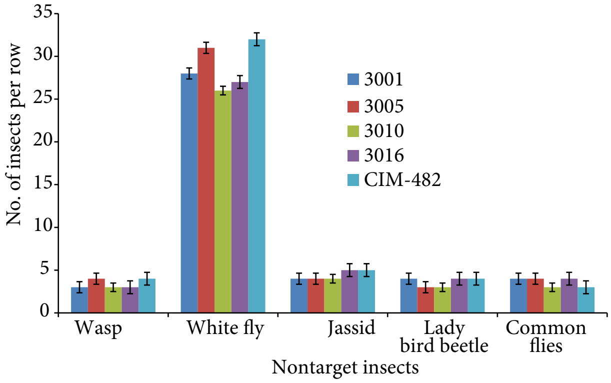

Figure 4. Nontarget insects were collected from Bt and non-Bt cotton. The difference in number of insects visiting Bt and non-Bt cotton was nonsignificant (Bakhsh et al., 2009). Transgenic lines 3001, 3005, 3010, and 3016 express *cry1Ac* and *cry2A* genes while CIM-482 is the control non-Bt cotton variety grown within transgenic lines.

4.4. Risk assessment of Bt crops using animal models

Transgenic Bt crops have gone through risk assessment studies using various animal models, feeding times, and other parameters (Domingo and Bardonaba, 2011), like other GMOs. The concept of substantial equivalence was developed in 2003 by the Society of Toxicology such that any particular food found equivalent in composition and nutritional characteristics to an existing food should be regarded as being as safe as the conventional food (Hollingworth et al., 2003). This concept enabled researchers/toxicologists to investigate the potential differences between already available food and new products (Domingo and Bardonaba, 2011). Interestingly, most of the studies performed to assess the biosafety of GMOs lacked this concept of substantial equivalence.

Several risk assessment studies of insect-resistant Bt crops have been documented in recent years following guidelines given by the World Health Organization to conduct 90-day feeding studies in animal models (WHO, 2002). Recently Nicolia et al. (2013) reviewed the scientific literature available on biosafety assessments in the last 10 years and concluded that not a single scientific hazard has been reported directly because of GM food; however, the debate continues as many research groups think otherwise. Séralini et al. (2007) found significant variations in body weights of male and female rats fed with a corn diet harboring *cry3Bb1*. Signs of hepatorenal toxicity and an increase (24%–40%) in female triglycerides were also reported. The study was reinforced by another report from de Vendômois et al. (2009), who also found signs of hepatorenal toxicity in an animal feeding assay. Furthermore, Séralini et al. (2012) also reported the presence of tumors and the early death of experimental rats compared to controls when fed with glyphosate-tolerant corn. However, these aforementioned results have been questioned and criticized because of poor experimental design, statistical analysis, and misleading conclusions (Doull et al., 2007; Arjó et al., 2013). Moreover, many reports are suggestive of the safety of Bt crops being the same as that of their conventional counterparts (Table 4).

4.5. Antibiotic resistance

Most vectors contain antibiotic-resistant genes known as selectable marker genes to be used for the selection of transformed plant cells that uptake the foreign DNA (Rao et al., 2009). Although this technology has proven to be of great benefit (Qaim, 2009), there are still some concerns regarding the safe use of genetically modified crops containing antibiotic genes as selectable markers along with genes of interest. A general approach is the recombination of these antibiotic genes with disease-causing bacteria in the surroundings or with bacteria in the GI tract of mammals using genetically modified products. Effectiveness of antibiotics can be reduced, hence making humans impervious to antibiotics (Azadi and Ho, 2010).

The neomycin phosphotransferase gene has been widely used as an antibiotic resistance marker to develop transgenic plants. Various in vitro and in vivo experiments conducted have proven it safe (Bakshi et al., 2003). Earlier, Ciba-Geigy (Novartis) Bt corn was rejected by the European Union based on the assumption that the *bla* gene (the marker gene used) can make animals resistant to β-lactam antibiotics (D'Agnolo, 2005). However, a series of later evaluations proved the *bla* gene quite safe even if animals ingested it for a long time.

The production of marker-free transgenic crops is an appreciable effort to increase wider acceptability in this context. Marker-free transgenic plants have been developed using different approaches of cotransformation of two transgenic site specific recombination and transposon-based marker excision methods (Puchta, 2003; Upadhyaya et al., 2010).

The incorporation of genes from various sources into plant genomes is a random process; therefore, it can give rise to unintended and unpredictable effects. Such introductions in plant genomes may interrupt a plant's own genes and may change endogenous plant proteins (Svitashev and Somers, 2001). Irregularities/unintended effects in transgenic Bt crops have been recorded (Hernández et al., 2003). Such unintended and unpredictable effects could impact the environment and animal and human health seriously. In a short communication, Rischer and Oksman-Caldentey (2006) emphasized that unpredictable and unintended effects of GMOs can be connected to changes in metabolite levels in plants. Analysis of the overall metabolite composition of genetically modified plants has been a challenge; metabolomics can play an important role here in the identification and quantification of small molecules in GM and non-GM plants (Hoekenga, 2008). The metabolomic profiles of GM foods along with transcriptomic and proteomic studies showed some differences between GM and control lines; however, some differences were also recorded within conventional lines (Ricroch et al., 2011).

The inflamed public discussion about unintended effects of GMOs can be considered as a result of a mere concern, unawareness of the technology, or propaganda stemming from the objectives of particular groups, individuals, or organizations that intend to delay the commercial development of this great technology. It is well established that insect-resistant crops have played significant roles in increasing crop productivity and have been declared safe after going through proper regulatory procedures. Almost 2 decades have passed since the commercialization of transgenic crops, and not a single report with significant effects has been presented (Nicolia et al., 2013).

Table 4. Some examples of risk assessment studies using Bt as an ingredient in the diet of model animals. No evidence of negative impact of Bt diet in animals has been reported or established to date.

GM crop	Gene	Model	Effects	Reference
Corn	*cry19c*	Chicken	There were no differences among conventional and GM diets	Yonemochi et al., 2002
	Bt endotoxin (*Bt-176*)	Mouse	There were no differences among conventional and GM diets	Brake et al., 2004
	cry3Bb1	Rat	Slight increase in white blood cell count and glucose level, and decreased cardiomyopathy	Hammond et al., 2006
	cry1Ab	Salmon	Small changes in stress protein level and activities, changes in white blood cell counts	Sagstad et al., 2007
	cry3Bb1	Rat	Increase in body weight, signs of hepatorenal toxicity, increase in triglycerides	Séralini et al., 2007
	cry1Ab (MON810)	Salmon	There were no differences among conventional and GM diets	Bakke-McKellep et al., 2008
Potato	*cryI*	Mouse	Several villi with abnormally large enterocytes, hypertrophied and multinucleated	Fares and El-Sayed, 1998
	GNA	Rat	Gastric mucosa proliferation, thinner cecal mucosa	Ewen and Pusztai, 1999
Rice	Cowpea trypsin inhibitor	Rat	No maternal toxicity, embryo toxicity, or teratogenicity was noted	Zhuo et al., 2004
	cry1Ab (KMDI)	Rat	Higher sodium, urea, and glucose levels; reduced protein and adrenal levels, white blood cell counts	Schrøder et al., 2007
	GNA	Rat	Lower potassium, protein, albumin, creatinine; increased small intestine weight	Poulsen et al., 2007a
	PHA-E lectin	Rat	Increased weight of small intestine, stomach, and pancreas	Poulsen et al., 2007b
	cry1Ac and *sck*	Rat	No unintended adverse effects of GM diet was found in rats after 78 weeks of study	Zhang et al., 2013
Tomato	*cry1Ab*	Rat	Normal body weight and diet consumption; microscopy revealed no adverse effects	Noteborn et al., 1995

5. Conclusion and future prospects

There is no doubt that conventional plant breeding played a significant role in crop improvement in past centuries, but the advent of genetic engineering technologies revolutionized breeding methods by breaking hybridization barriers among species and genera. The transgenic technology to develop genetically modified plants is about to celebrate its 30th anniversary. The productivity of agricultural crops worldwide has been severely affected by insect pests. The commercialization of insect-resistant crops expressing *Bt* genes has been outstanding in terms of crop productivity and economic benefits to the farming community. However, it is important to note here that almost all commercialized insect-resistant crops contain genes from *Bacillus thuringiensis*. Although pilot-scale field trials of crops expressing genes other than *Bt* were reported by public-sector universities and research organizations, no report of commercialization of such insect-resistant crops has been documented to date, not even from multinational companies. In view of increased resistance development in insects, there is an urgent need to investigate other sources of pest resistance in addition to adopting resistance-delaying strategies. The incorporation of genes from other origins (lectins, proteinase inhibitors, etc.) or the use of RNAi technology seem to be promising alternate options for sustainable resistance against crop pests, but this technology is still in its infancy.

Despite the economic benefits of transgenic crops, insect-resistant crops are under criticism by a group of researchers, nongovernment organizations, and consumers. Scientific reports are quite clear about the gradual degradation of Bt proteins in the soil. To date, there has been no threatening report regarding the vertical and horizontal gene flow from transgenic Bt crops, while the misperception of negative impacts of Bt crops on nontarget insects has been addressed rationally. Most studies concluded that Bt crops were safer for predators compared to nontransgenic crops where heavy insecticides were applied. However, the heated debate over the application of transgene technology has continued since the introduction of the first genetically modified organism. A deadlock has been observed, rather than formulation of agreed-upon policies regarding GMOs. The favoring and opposing parties advocate contrasting views about GMOs from every available platform. Risk assessment studies of GM food have been described critically in articles by different research groups in a very concise, focused, and informative way, although negative reports about GM food have also been reported. The animal feeding results opposing the use of GMOs have been questioned and criticized by different researchers scientifically. The impartial and professionally competent regulatory mechanisms for the evaluation of risks and benefits of insect-resistant crops must be fully functionalized. More farm trials should be conducted. In developing countries, policy makers and scientists should assess risks associated with GMOs carefully. Efforts should be directed to gain public confidence. The risk assessment debate should be converted to risk benefit as every technology has shortcomings along with its benefits. A trial and safety assessment system must be established to answer the concerns of nongovernmental organizations who oppose the technology.

The increasing world population, to reach 9.7 billion in 2050, is a true challenge for the scientific community. We cannot feed tomorrow's population with yesterday's technology. Therefore, we cannot ignore the huge potential of transgenic technology to enhance the food supply for an increasing population. Following proper biosafety guidelines, integration of modern technologies to develop insect-resistant crops in conventional breeding methods and their economic benefits downstream are quite promising for the future of agriculture.

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