Environ Chem Lett (2015) 13:239–249
DOI 10.1007/s10311-015-0508-4

REVIEW

Safe use of *Cry* genes in genetically modified crops

M. Rahman[1] · Muhammad Zaman[1] · Tayyaba Shaheen[2] · Samra Irem[1] · Yusuf Zafar[1]

Received: 16 May 2015 / Accepted: 19 May 2015 / Published online: 27 May 2015

Abstract Genomic technologies have been used to improve cultivated crop species. For example, *Bt* genes such as *Cry1Ac*, *Cry2Ab*, *Cry1F* and *Cry3Bb1* are derived from *Bacillus thuringiensis*, a soil bacterium. Such genes provide protection against lepidopteran insect pests. *Bt* genes have been introduced in corn, cotton, soybean, rice, potato and canola. Genetically modified (GM)-cotton, containing the *Cry1Ac* gene, was released for cultivation in the mid-1990s in the USA and later in 28 countries including China and India. Potential harmful effects of the Bt-crops on non-targets were assessed before release into the environment. Most commonly, cultivation of the Bt-crops was found safe. Safety was tested using various experiments including: the insertional impact of transgene and its regulatory elements on plant phenotype and agronomic performance; effect on non-target organisms; and nutritional impacts on multiple experimental models, albeit the studies were conducted for limited durations. However, skeptics always claim for conducting extensive clinical as well as field trials and also cast doubt on methods and procedures of calculating the ecological risks. This debate got further momentum especially after the publication of reports on substantial reduction in monarch butterfly caterpillars when exposed to Bt-maize pollen—though later nullified—and detection of traces of transgene in various tissues of experimental animals. It is generally accepted that procedures, methods and protocols for evaluating the potential risks of GM-crops and foods should be standardized for building confidence of all stakeholders. Efforts should be exerted in deploying genes of interest, marker genes and regulatory sequences invoking no or little issues of potential risks to the ecosystem.

Keywords GM-crops · Bt-crops · *Cry* genes · Risk assessment · Safety evaluation · Genotoxicity · Blood biochemistry · Allergic response · Non-target organisms · Mammals · Birds · Human

Abbreviations

GM	Genetically modified
GE	Genetically engineered
Bt	*Bacillus thuringiensis*
NTOs	Non-target organisms
PIPs	Plant-incorporated protectants
CaMV35S	Cauliflower mosaic virus 35S promoter
GFP gene	Green fluorescent protein gene
nptII gene	Neomycin phosphotransferase gene
IgE	Immunoglobulin E
IgG	Immunoglobulin G
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
MBC	Biomass carbon
MBN	Biomass nitrogen

Introduction

Genomic techniques, such as genetic engineering, emerged rapidly over the last two decades, have made possible the introduction of alien gene(s) into a plant

✉ M. Rahman
mehboob_pbd@yahoo.com

[1] Plant Genomics and Molecular Breeding Lab, National Institute for Biotechnology and Genetic Engineering (NIBGE), Jhang Road, P.O. Box 577, Faisalabad, Pakistan

[2] Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan

species, called as genetically engineered or genetically modified (GM) plant, and their product or by-products are used as food are referred as GM-food. Plant-derived GM-foods comprising staples such as soybean, maize, canola, rice and potatoes have been commercialized. Expression of desired traits which are beneficial for the consumers is attributed to this technology (Magaña-Gómez and de la Barca 2009).

First GM-crop was commercialized in 1996, and since that many other crops like GM-soybean, maize, cotton, potato, and canola have been made public. We have witnessed the rapid expansion in global area of GM-crops with a sustained growth 3–4 % (181.5 million hectares, James 2014). GM-crops have been classified on the basis of introduced trait. First-generation GM-crops are derived for enhanced production; however, the crops are not considerably different from their conventional equivalents except these crops have genes for combating plant disease, insect pests, viruses and herbicides, exhibiting that these are similar in taste, appearance and nutritional value for the consumers, while second generation of GM-plants is comprised of crops containing new traits of direct value to the consumers. It offers benefits to the processor, end user and consumer. Third generation of GM-plants by manipulating their genomes will have a greater ability to combat abiotic stress such as drought, high temperatures and salinity. Moreover, some modified crops are able to provide food with supplemental health benefits or renewable raw materials. This generation also includes "pharmaplants," which are used as biological production systems for producing high-grade active pharmaceutical elements (Magaña-Gómez and de la Barca 2009). This article is an abridged version of the chapter by Rahman et al. (2015).

Spectrum of *Bt* genes diversity

Discovery of *Bt* genes

The bacterium *Bacillus thuringiensis* (*Bt*) was first discovered by Japanese biologist, Shigetane Ishiwatari. Later in 1911, Ernst Berliner found a bacterium that killed a Mediterranean flour moth, named as *B. thuringiensis*, after the name of German town Thuringia where the moth was found. The presence of crystals was discovered in Bt in 1915 (Sanahuja et al. 2011), but its activity was described much later. In the USA, Bt was registered as a pesticide in 1961. In the 1980s, use of Bt sprays was substantially increased when insect pests became increasingly resistant to the synthetic insecticides (www.bt.ucsd.edu/bt_history.html).

Bacillus thuringiensis (*Bt*) produces insecticidal crystal proteins, solubilized in the larval midguts, are activated by the midgut proteases. Numerous kinds of Cry proteins found to be toxic for different orders of the insect family. A number of *Bt* genes have been introduced in crops around the world such as cotton (*Cry1Ac*, *Cry2Ab2*, *Cry1Fa2*), maize (*Cry1Ab*, *Cry1Ac, Cry1Fa2, Cry3Bb1, Cry9C*) and potato (*Cry3Aa)* (Hellmich and Hellmich 2012, Fig. 1). Engineered chimeric Bt toxins in PIPs (e.g., a *Cry1Ac/Cry1Fa* hybrid protein; Perlak et al. 2001), binary Bt toxins, as well as hybrid Bt toxins targeting multiple insect orders were introduced. Moreover, crops like apple, broccoli, cabbage, tobacco, tomato, soybean and rice have also been engineered to express *Bt* genes (Huesing and English 2004).

Bt-crops

GM-crops are the most popular commodities in agriculture and at the same time are the most controversial from biosafety point of view (Tabashnik 2010). In the early 1980s, GM-plants were developed by multiple groups independently at the Washington University in St. Louis, Missouri, the Rijksuniversiteit in Ghent, Belgium, Monsanto Company in St. Louis, Missouri, and the University of Wisconsin (Framond et al. 1983). In the 1990s, a first commercially grown GM-tomato was produced by California-based company called the FlavrSavr, for improving the shelf life (takes longer to decompose after being picked). A variety of the tomato was used to make tomato puree that was sold in Europe in the mid-1990s, but later many safety concerns were raised over GM-crops. Since 1995, GM-crops including soybean, barley, potato, cotton and corn were commercialized (Rahman et al. 2012) (www.gmcrops.ewebsite.com/articles/history). Cotton and corn have been GM to mitigate the utility of insecticide sprays. Before the Bt-corn was introduced, insect pests have caused losses of $1 billion per year in the USA (Tabashnik 2010).

Targeted insect pests species

Bacillus thuringiensis produces a diverse group of Cry and Cyt proteins which act as toxin to a small range of insect pests, and this specificity is attributed to specific pH levels, enzymes and furthermore specific midgut receptors. This specificity can be explained by a "lock and key" theory. Insect death will only occur if the lock and key match. For example, midgut receptor can be considered as "lock" and the Cry protein can be considered as "key" (Hellmich and Hellmich 2012).

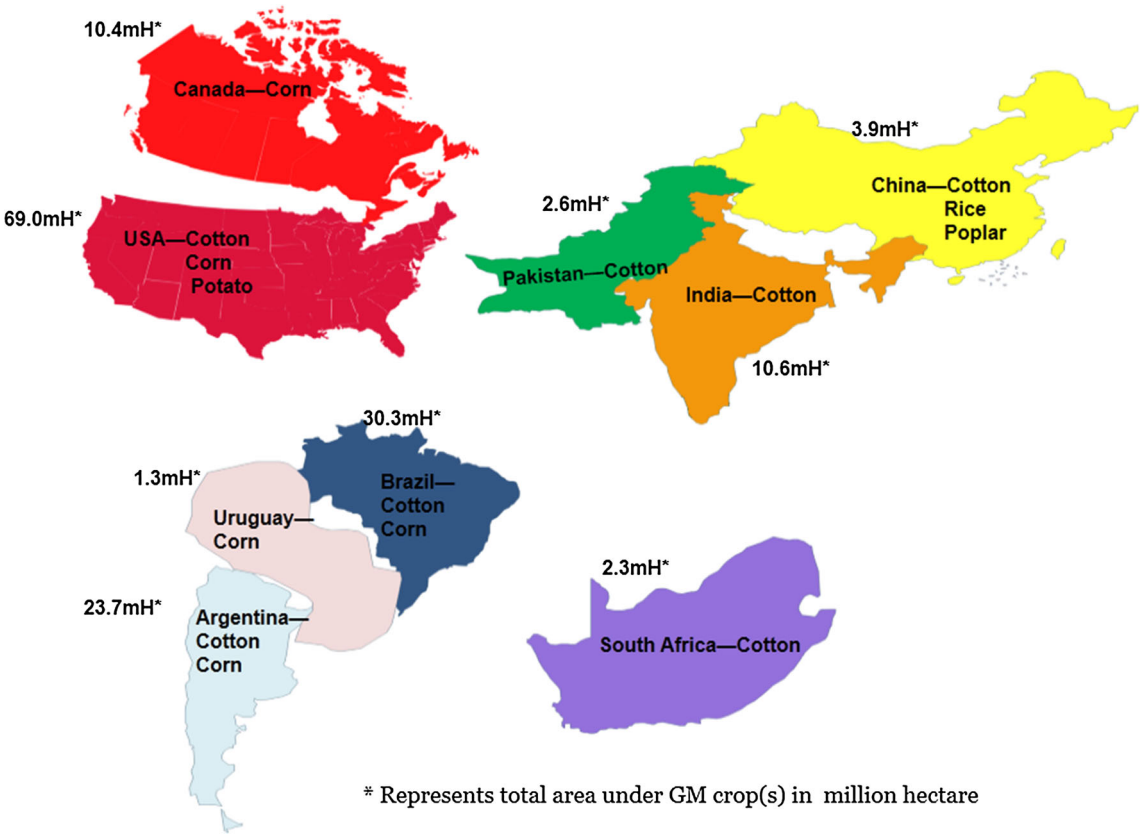

Fig. 1 Map showing the distribution of Bt-crops growing on more than 1 million hectare in different countries

Benefits of cultivating Bt-crops

GM-crops are being cultivated on 181.5 million hectares in about 28 countries worldwide (James 2014). An important trait used in GM-plants is resistance to insect pests. Development of GM-crops containing *Bt* genes is a step toward making agricultural system profitable for the producers including small farming community through increased earnings by reduction in chemical pesticides as well as farm labor required to protect crops from the insect pests infestation (Pray and Naseem 2007). Profitability earned by cultivating GM-crops may be diverted to improve the quality of life (Mal et al. 2011; Hellmich and Hellmich 2012). Also, the GM-technology helps in saving time of the women and children working as a farm labor in most developing countries, sparing them to engage in household and educational activities, may have high social significance for a society (Chen and Lin 2013).

Both the macroeconomic level outcomes and microeconomic level effects of cultivating Bt-crops investigated in different countries showed that whole farming community in India including small and big farmers can reap benefits by cultivating Bt-cotton. Later, a significant impact of Bt-cotton cultivation to mitigate poverty was observed in India (Subramanian and Qaim 2010). Such commonalities of increased yield per hectare were observed in China (Huang et al. 2010) and Pakistan. Fluctuations in yield are largely due to weather conditions and pest pressure. Similarly, cultivation of other Bt-crops like eggplant and rice, compared to their non-Bt counterpart, will also add in the farm income by cutting down the cost of pesticides and farm labor. In Bangladesh, the Bt-brinjal was approved for cultivation on Oct 30, 2013 (James 2014).

Other indirect benefit of Bt-crops is the substantial reduction in lepidopteron insect pest populations, not requiring chemical pesticides to apply on non-Bt-crops. For example, lepidopteron populations in cotton have been substantially declined in China (Huang et al. 2010) as well as in corn in the USA (Hutchison et al. 2010).

Assessment of potential risks of Bt-crops to the ecosystem

Procedures and methods

Since the development of first transgenic plant, debates and discussions on the safe release and their usages have been initiated which resulted in formulating guidelines for assessing the safety of foods derived from GM-crops by a

group of international experts on food safety evaluation. Some non-GM activists still have divergent views, e.g., demand for long-term safety assessment by adopting high stringent conditions which are even more rigorous than for any other foods. Methods for testing the safety of GM-crops and their by-products have strengths as well as weaknesses. Guidelines designed to regulate the introduction of GM microbes and plants into the environment found to have some critical gaps in the scientific knowledge concerning the compositional effects of genetic transformation and also in the safety testing procedures (Prado et al. 2014). Similarly, the concept of substantial equivalence was introduced in 1993 for comparing the properties of GM-food with its conventional counterpart, and the GM-food will be regarded as safe as its conventional counterpart after establishing the substantial equivalence, and no further safety consideration is needed, concluded by the a Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) expert consultation on biotechnology and food safety. It was found imperative for growing GM and its parental varieties under similar conditions for making comparisons of key compounds as well as the genotypic and phenotypic differences. Secondly, unintended consequences of GM-crops have been reported. For example, higher lignin contents in Bt-maize than in non-Bt-maize, depleted plant flavonoids in herbicide tolerant soybean, etc. have been reported (Kuiper et al. 2001). Hence, the concept of substantial equivalence is not an acceptable method for GM evaluation because of its inability to detect unintended effects. Theoretically, the unintended changes may arise due to the insertion of genetic construct, gene regulation, gene–gene interactions and also possible interferences in metabolic pathways. For predicting such changes, DNA-based technologies such as DNA analysis, DNA/mRNA microarray hybridization, and proteomics and chemical fingerprinting (metabolomics) are required for quantifying the differences in GM-crops and their non-GM counterpart. Now it is accepted that for safety assessment of a GM-crop, substantial equivalence together with other parameters such as molecular characterization, phenotypic characteristics, key nutrients, toxicants and allergens should be considered, based on the guidelines prepared by the International Life Sciences Institute Europe and FAO/WHO in 1996. Despite the official standards for food safety evaluation published by the *Codex Alimentarius* Commission of FAO/WHO in 2003, risk assessment guidelines of GM-foods have not adopted as described. It is agreed that the safety evaluation of GM-crops will be conducted on a case-by-case basis (Pakistan National Biosafety Rules 2005). Also, it was emphasized that standardized methods, designs and statistical analysis for conducting animal feeding trials should be followed.

DNA-based genotoxity test

The comet assay, described by Singh et al. (1988), is used to detect the extent of DNA damage at individual cell level, one of the indicators for evaluating genotoxicity of GM-crops. In this test, the amount of sheared genomic DNA (degrades after exposing to various mutagens) that forms a tail-like structure is calculated by the fluorescence. A numerical value is assigned to each of the migrating genomic DNA for quantifying the extent of genotoxicity (Tice et al. 2000). In this particular test, tail length and the percentage of DNA-damaged cells are important parameters for estimating the impact of genotoxicity. A study was conducted using the organ samples of rabbits fed on Bt-cotton as well as its conventional type. No significant differences for damaged cell (2–3 %) within and between the normal and Bt-cotton exposed groups were found, highlighted that the transgenic cotton containing *Cry1Ac* gene is quite safe for other than target organisms (Rahman and Co-workers, unpublished results).

Potential harmful effects of Bt-foods to mammals

Globally, numerous studies for assessing the environmental risk of Bt technology have been conducted in different countries on multiple Bt-crop species such as maize, potato, soybean, brinjal, popular, rice and cotton (Prado et al. 2014). Earlier, rodents (rats) were exposed for 90 days to the semisynthetic diet containing 10 % (w/w) of lyophilized powder of Bt-tomato and non-Bt-tomato (Noteborn and Kuiper 1994). Based on multiple clinical, toxicological or histopathological studies, it was found that the group of rats fed on diet containing Bt-tomato is safe. Similarly, sheep were exposed to GM-corn (containing *Cry1A*) and conventional corn, and the GM-corn was found equally safe as the change in body weight gain and feeding were non-significantly different (Barriere et al. 2001). Such commonalities were found in many other studies conducted on different animals, such as chicken fed on GM-corn containing *Cry9c* gene (Yonemochi et al. 2002) and dairy cattle exposed to GM-corn containing *Cry1Ab* gene (Donkin et al. 2003). Also, in another set of experiments, the non-toxic impacts of Bt pesticidal protein to aquatic animals like fish, and mammals and invertebrates were reported (Xu-Chongren and Chang 2001). In multiple investigations, various experimental animals such as mice, zebra fish and eelworms were exposed to Bt-cotton plants/seeds/leaves. Based on acute and chronic toxicity trials, and also the genotoxicity experiments, each of the animals responded normally when fed on Bt-transgenic cotton plants/seeds/parts (Prado et al. 2014).

Recently, a study was conducted for evaluating the safety assessment of Bt-cotton (containing *Cry1Ac*,

Mon531) in Pakistan. In this study, various clinical trials such as sign of allergenicity, weekly weight gain, hematological parameters and histopathological studies were conducted on two groups of rabbits (one group fed on Bt-cotton leaves/seeds, and the other was exposed to non-GM seeds and leaves of cotton), and it was declared that Bt-cotton has no toxic impact on the health of rabbit (Rahman and Co-workers published data).

Potential impact on non-targeted organisms

Harmful impact of Bt-crops, particularly on non-target organisms, was a major apprehension. A unique quality of *Cry* genes is their specificity for killing certain orders of insects. After the development of Bt-crops, relative impact of transgenics and their control was estimated on the population of NTOs (Mendelsohn et al. 2003), and generally, no toxic impact of the transgenic crops was observed. For example, the harmful effect of Bt-rice on the populations of NTOs was not found (Chen et al. 2006; Rahman et al. 2007). Similarly, no harmful impact on arthropod community was found while comparing the data (family composition, diversity index etc.) collected from the transgenic and non-transgenic rice (Chen et al. 2006). Such commonalties were also found while comparing the influence of Bt-corn versus its conventional counterpart on communities of NTOs such as predators and parasitoids. A total of five predator populations were monitored in various Bt and non-Bt-corn plots of Iowa State; a significant depression (29–60 %) of the *M. cingulum* population was found in Bt-corn field (Pilcher et al. 2005).

Only one major contrary report showed substantial reduction in the population of monarch butterfly caterpillars, *Danaus plexippus*, due to feeding on milkweed leaves treated with Bt-maize pollen (Mendelsohn et al. 2003). However, later insignificant influence on the population of monarch butterfly was reported (Hellmich and Hellmich 2012). Also, a positive impact of Bt-corn cultivation was found on biodiversity in comparison with the corn treated with chemical insecticides (Romeis et al. 2008). In subsequent years, a comprehensive study conducted jointly by the scientists of USA and Canada showed no acute toxic effects at different pollen densities in laboratory as well as in the field due to low level of Bt protein expression in pollens of Bt-hybrids http://www.isaaa.org/resources/publications/pocketk/6/default.asp.

Potential threats to human health

Bt proteins are target specific, and their specificity lies in their receptor-mediated responses. Thus, the Bt protein can harm the organisms having receptor sites in their gut, making the protein receptor mediated. By chance, most of the beneficial insects and human lack these receptors.

Prior to commercialization, stringent regulatory tests for evaluating the toxicity and allergic responses of Bt-crops are mandatory requirement. Bt proteins have been assessed at high dosage for evaluating their toxicology by the US Environmental Protection Agency (US-EPA). Also, the Extension Toxicology Network (Extoxnet), multi-universities project in the US dealing with the pesticide information, did not report any complaint of toxicity/poison when a group of 18 humans were exposed to 1 g of commercial Bt preparation for five but on alternate days or for three consecutive days. Moreover, in vitro studies revealed a rapid degradation of Bt proteins in human gastric fluid (Mendelsohn et al. 2003).

Potential risk of the introduced gene cassette

In most GM-crops, cauliflower mosaic virus 35S promoter (CaMV35S) has been used that can be transferred horizontally which may cause disease, carcinogenesis and mutagenesis. In few cases, the promoter sequences may lead to reactivate the dormant viruses as well as can generate new viruses (Hodgson 2000). In contrary to this hypothesis, CaMV, present in the normal food, cannot cause infections and thus mammals cannot absorb it (Ho et al. 2000). No disease or recombination with human viruses has ever been reported irrespective of the fact that humans have been ingesting high levels of CaMV and its 35S promoter (Paparini and Romano-Spica 2004). Recent studies conducted using mice as an experimental animal were unable to detect DNA transfer as well as transcriptional activity of the CaMV35S quantified through real-time PCR (Paparini and Romano-Spica 2006).

In most Bt-crops, antibiotic resistance genes have been used as selectable markers which may potentially transfer to microflora, comprising of 500–1000 distinct bacterial species, of human gastrointestinal tract, thus reducing the efficacy of antimicrobial treatment. However, under very stringent laboratory conditions, very low frequency of plant DNA transfer to bacterial species has been demonstrated between the homologous sequences (De Vries et al. 2001). In another study, it was shown that without introducing homologous sequences in the recipient strain, uptake of the transgene is not possible. These phenomena were demonstrated on multiple crop species like sugar beet, tomato, potato and oilseed rape containing the *nptII* gene (De Vries et al. 2001). Also, a jellyfish green fluorescent protein (*GFP*) gene, another marker gene, was utilized but did not find any risk of toxicity and allergenicity (Richards et al. 2003).

There are concerns about the aforementioned potential impacts of genes that can cause gene silencing, changes in

expression level, or can turn on the existing silent genes (Conner and Jacobs 1999). Alternatively, expression of the Bt proteins may potentially alter the metabolism and biochemical pathways of the plants. For example, interaction of two genetically produced foods, tryptophan and g-linolenic acid, has created new toxic compounds (Sayanova et al. 1997). Also, the epigenetic changes may occur in GM-organism that may raise concerns like unpredictability of genetic modifications, non-reproducible results and instability of the products, and thus together suggest that in animals, toxicity assessment of whole food should be evaluated instead of the single novel protein. Though it is very well conceived, it is difficult to generate a dose–response relationship (Kuiper et al. 2004).

Horizontal gene transfer to the consumer species

Another important potential hazard of the GM-crops or GM-foods is associated with their capability to transfer the transgene to animals including humans through their guts. GM-soybean containing glyphosate-resistant gene was fed to pigs; however, DNA fragments were not detected in tissues of the pigs (Jennings et al. 2003a, b), whereas short DNA fragments were detected in the gastrointestinal tract of pigs when exposed to Bt-corn but were absent in the blood stream (Chowdhury et al. 2003) suggesting that a very small proportion of the transgene cassette is not degraded in the digestive tract, and this small quantity is difficult to amplify with PCR from the genomic DNA isolated from the blood because of their low level, but can easily be amplified in animal tissues (Pusztai 2001). It shows that the PCR assays may affect the interpretations (Murray et al. 2007) and thus need to be optimized. In spite of the fact that DNA fragments were detected but it is much unlikely that the DNA taken up by the cells of gastrointestinal tract will be integrated into the host genome that usually degrades in the cell (Flachowsky et al. 2005). Possibilities of horizontal gene transfer from Bt-crops to soil microflora were also explored because of the evidence of such transfer reported after conducting several planned experiments to facilitate the transfer. However, such conditions are not possible to occur in open environment. Furthermore, gene incorporated in Bt-crops is already present in most of the soil bacteria. Therefore, it was concluded that horizontal gene transfer is a rare event in Bt-crops (Mendelsohn et al. 2004).

Potential allergic response

GM-foods derived from GM-crops including GM-soybean expressing methionine (Nordlee et al. 1996) and GM-corn expressing Bt protein (Bernstein et al. 2003) may cause allergic hypersensitivity (Taylor and Hefle 2002). It has also been conceived that the transgene expressing non-allergenic protein such as GM field pea, expressing alpha-amylase inhibitor-1, may have potential to produce product with allergenicity (Prescott et al. 2005). Thus, each of the GM case should be treated separately (case-to-case basis).

In order to assess the allergic response of GM-crops, GM *Brassica juncea* was added in the diet of mice and low IgE response was observed because of the expression of choline oxidase gene (transgene) in *B. juncea,* whereas, in another study, expression of the gene did not cause any allergic hypersensitivity (Singh et al. 2006), highlighting the need to undertake safety evaluation test on multiple experimental models for establishing a valid correlation between IgE response and toxicity. Farmers may have allergic sensitivity when exposed to GM-crops containing various *Bt* genes as skin sensitization and IgG antibodies were detected in farm workers exposed to Bt pesticide (Bernstein et al. 2003).

Allergenicity assessment

According to a decision tree approach, formulated in 1996 and later it was revised (FAO/WHO 2001; Metcalfe 2003), if the conventional counter part of the GM-plant species is known for causing allergy and or toxicity, then the whole GM-plant of that particular species should be evaluated for quantifying the chances, if any, of increasing in toxicity. A 90-day-long study for toxicity testing is required in rodents through comparing concentration of allergens in non-GM-crop versus GM-crop. Possibilities for the differential accumulations of toxic compounds or allergens in GM-crops containing single transgene with GM-crops containing stacked events should be considered as in each case interaction with the host genomes may vary. For example, interaction of the transgene conferring regulatory proteins if transferred into an entirely different background may fluctuate (De Schrijver et al. 2007). At this point of time, sequence analysis of amino acids is difficult to predict, thus limiting their utility for comparing their sequences with the known allergens (Prescott and Hogan 2006). Also quantifying their degradation in vitro system has been the major challenge in establishing valid correlations with allergens (Bannon et al. 2003) which set a stage for conducting such experiments in vivo systems (Pusztai et al. 2003). It has also been shown that no single animal model can help in testing allergenicity responses of various GM-foods as different animal species respond differentially to the allergens, indicating that animal models should be validated (Tryphonas et al. 2003). A comprehensive study addressing the allergenicity in human in response to GM-food has been discussed by Germolec et al. (2003).

Potential impact to the environment

Pollen flow

Gene flow or transmission of genetic material from GM-crops to their wild types is a potential threat (Messeguer 2003). Maize is an open pollinated crop, and pollen can travel miles of distances through air currents. Thus, cultivation of GM-maize should be separated from the related species that have tendency for hybridizing with maize. In another study, chances of pollen-mediated gene flow from transgenic lines of rice to their untransformed counterparts through natural cross-pollination were found to be very low (0.14 %) (Rahman et al. 2007).

Bt-cotton, another important crop commercialized in 1996, is predominantly a self-pollinated crop (usually 2–5 % cross-pollination was reported mainly through insects) in most of the cotton-growing countries. However, in a few countries like Panama, the cross-pollination rate may even increase to 80 %. Its pollens are sticky, thus eliminating the chances of traveling through wind (Poehlman 1994). Hence, crossing is only possible when honeybees collect pollens (Oosterhuis and Jernstedt 1999). Also, propensity of shifting pollen from one flower to another could substantially be minimized by increasing the distance between the two cotton genotypes. We studied that pollen transfer rate reached to <1 % if the distance between the two genotypes is more than 100 ft (Rahman and Co-workers, unpublished result). In another study, chances of gene flow between transgenic lines and their untransformed counterparts through cross-pollination are found to be low (0.14 %; Rahman et al. 2007). Secondly, a chance of transfer of pollen to other species even with in the same genus is extremely low as phyletic barriers exist among different species. Lastly, Bt-crops have no sound potential of transferring transgene to nearby cultivated wild relatives because of difference in chromosome number, phenology and territory. In few states of America, Bt-cotton cultivation was restricted to Hawaii, Florida, Puerto Rico and the US Virgin Islands due to chances of transfer of the *Bt* gene from the cultivated Bt-cotton to their wild relatives (Mendelsohn et al. 2003).

Grain yield

Grain yield is one of the most important parameters for studying the agronomic performance of crop species. In multiple studies, Bt-crop varieties out yielded their non-Bt counterparts. For example, Bt-corn hybrids exhibited 11 % more grain yield than the non-Bt-corn hybrids (Subedi and Ma 2007), while few reports have shown no differences in any of the parameters including grain yield and chemical composition from non-Bt-corn hybrids (Yanni et al. 2011).

Like other Bt-crops, cultivation of Bt-cotton has also got popularity among the farming community because of the increased protection against lepidopteron insect pests, ultimately resulted in high yield especially in developing countries like China (Huang et al. 2010), India (karihaloo and kumar 2009) and Pakistan (Zaman 2015). It has been shown that the Bt-cotton cultivar is much like their parental varieties by comparing traits like germination rate, establishment, rate of vegetative growth, flowering duration, fruiting potential, fiber yield and fiber quality. In another study, Bt-cotton variety IR-NIBGE-901 was grown along with its conventional variety FH-901 for a period of 4 years in Pakistan, and it was found that Bt-cotton and the parental variety were similar in all morphological and quality characteristics (Zaman and co-workers, unpublished results).

Differences in morphological parameters such as plant height, flowering duration and lodging resistance have been reported in Bt-rice developed in China (Jiang et al. 2000) and Pakistan (Bashir et al. 2005). Such fluctuations in morphological traits are generally attributed to the insertion of transgene in the host genome which may cause gene silencing (Matzke et al. 2000). However, somaclonal variations are the much likely cause of creating variations in transgenic lines (Kaeppler Shawn et al. 2000). Also, chemicals like hygromycin may also induce variations in rice (Wu et al. 2000). In contrary to this, characters like panicle length, aroma and flag leaf area were found to be similar in Bt-rice/non-Bt-rice, where fluctuations in average number of tillers, plant height and maturity were reported (Rahman et al. 2007). Small differences in physiochemical properties between the transgenic and non-transgenic lines were observed due to fluctuation in the prevailing environmental conditions, late maturing lines find slightly different environment than the early maturing lines (Rahman et al. 2007).

Weediness

Weediness indicates that if a cultivated crop species establishes as a weed, survival beyond economic life, in the succeeding crop or neighboring crop. Potential indicators of weediness can be numerous. For example, seed-related characters (prolonged and high seed production with discontinuous germination under different environmental conditions), and physiological and morphological traits, together lead to evolve or enhance the capability of producing allelochemicals, special seed dispersal mechanisms, unusual high growth rate etc. Chances of producing such traits are very much unlikely as most domesticated crops species have lost, if not all, many traits which add in weediness traits (Mendelsohn et al. 2003). Also, the traits

make the plant species to be domesticated render them unsuitable for sustaining in a wide range of environments. Detailed experimental studies revealed that Bt trait did not add in the fitness of the Bt-plant, except that *Bt* gene confers resistance to the lepidopteron insect pest species. For example, in Pakistan, a study was conducted for a period of 4 years, investigated the potential weediness trait of Bt-cotton, showed non-significant differences in agronomic characteristics between Bt-cotton and its parental variety. Bt-cotton meets all morphological, yield and quality characteristics of non-Bt-cotton varieties produced in Pakistan. Based on such mechanistic arguments and field experiences, insertion of the *Cry1Ac* gene into the cotton genome would not add any effect toward the weediness trait of the cotton.

Persistence of Cry proteins in soil

Persistence of Bt protein in the soil is moderate and thus considered immobile due to its less mobility and leaching with groundwater. However, it is not persistent in acidic soils as it degrades rapidly upon exposure of UV radiations in the sunlight (http://www.isaaa.org/resources/publications/pocketk/6/default.asp).

In another experiment, the presence of Cry1Ac protein, assayed by ELISA and bioassay, was not detected in soil samples collected from Bt-cotton fields (Head et al. 2002). A substantial rapid degradation of Bt proteins in soil cultivated with Bt-cotton (*Cry1Ac*), Bt-potatoes (*Cry3Aa*) and Bt-corn (*Cry1Ab*) is a major cause for not reaching the concentration of biologically significant levels (Palm et al. 1994; Sims and Holden 1996; Head et al. 2002). In few countries like Australia, where cotton is cultivated on soils with pH ranging from 7.5 to 8.5 (Tapp and Stotzky 1998) that helps in rapid degradation of Bt endotoxins by soil microorganisms. Pakistan, another important cotton-growing country, where pH of the soil is in the range of 8.5–9.5, is likely to degrade Bt proteins relatively faster. It may be concluded that there are meager chances of accumulation of Cry1Ac proteins in soils as a result of repeated rounds of Bt-cotton cultivation.

It is much likely that soil microorganisms can be exposed to Bt proteins because of the occurrence of root exudations or during the decomposition of Bt-plant in the soil as this phenomenon has been reported in Bt-corn containing *Cry1Ab* gene (Saxena et al. 1999; Stotzky 2000). Some studies also confirmed the release of Bt protein in soil cultivated with Bt-cotton (Gupta et al. 2002).

Multiple studies conducted to evaluate the impact of Bt-crops on soil organisms showed that Bt proteins have no harmful impact on the soil microbes even at far higher concentration of the Bt proteins. In another study, variations were not found in the soil microbiota of the fields with Bt-plant material versus the fields with conventional plant material http://www.isaaa.org/resources/publications/pocketk/6/default.asp. Also, substantial changes in the counts of soil microbes were not found from the fields cultivated with Bt-cotton and non-Bt-cotton in Pakistan (Zaman et al. 2015).

However, recently a report appeared, showing a significant reduction in actinobacteria (17 %), bacterial (14 %) count as well as acid phosphatase (27 %), phytase (18 %), nitrogenase (23 %) and dehydrogenase (12 %) activities in the Bt-cotton fields versus non-Bt-cotton fields of India. Fungal and nitrifier counts, and esterase and alkaline phosphatase activities were not affected by the introduction of Bt-cotton in fields. Nonetheless, substantial decline between 8 and 9 % in biomass carbon (MBC) and biomass nitrogen (MBN) was observed (Jagadish et al. 2012).

Allelopathic impact

To explore the allelopathic effects of Bt-crops is important especially in developing countries because most farmers adopt crop rotations. For example, in subcontinent, cotton wheat or cotton rice rotations are very popular for harvesting maximum profitability per unit area of land. Multiple planned experiments were conducted for testing the allelopathic effect of Bt-crops including rice, cotton, and it was shown that the cultivation of Bt-rice has no harmful effect on the germination of wheat (Rahman et al. 2007). Similarly, field experiments were conducted for 3 years to assess the impact of plant residue containing Bt protein on weed population of the Bt-cotton field at various intervals. Weeds were allowed to grow in one big plot of Bt-cotton field and non-Bt-cotton field in various locations of Pakistan. Non-significant differences were observed between the weed populations of Bt and non-Bt-cotton fields (Zaman 2015).

Conclusions

Cultivation of GM-crops has been gaining popularity worldwide every year among the farmer community. Beneficial impact of cultivating Bt-crops has been found relatively high in developing countries than in the industrialized countries. The indirect benefit of cultivating Bt-crops is a substantial suppression in insect pest populations which may help in controlling pests on their non-Bt counterparts with fewer inputs. However, cultivation of Bt-crops may help minor pests to emerge as major pests because of reduced insecticides application on Bt-crops. Thus, this phenomenon may arise much faster in developing countries where farmers are not much educated

about IPM programs. For harvesting maximum benefits of the Bt-crops, public sector organizations should make deliberate efforts to educate farmers for controlling insect pests by supplementing with some other control measures.

So far, numerous crop varieties modified with genes expressing Cry toxins have been developed, and no detrimental impacts of the Bt-crops on NTOs populations were found in experiments conducted at laboratory scale as well as in the field. Furthermore, populations of beneficial insects are increasing on Bt-crops, further strengthening the defense umbrella of crops. In future, new genes derived from different wild species, preferably belonging to the same genus, should be kept on incorporating in major domesticated crops. It will help in releasing crop varieties with little potential risks of developing resistance in the target insect pest species. Secondly, the efficacy of the *Cry* genes in various genetic backgrounds should be tested as the expression of the gene(s) fluctuates in different backgrounds; it will facilitate in designing strategies regarding "when to introduce new genes or stacked genes with different mode of action." It will help in cultivating crops containing diverse genes which will set a stage for designing IPM strategies for combating resistance concerns in insect pests. Thirdly, even after two decade of Bt toxins deployment, their mode of action is not fully explored. Many Bt toxins are active against insects of more than one order. Thus, it is vital to characterize thoroughly any new *Bt* gene before introducing into a crop variety.

Antibiotic-resistant gene has been used extensively as a marker gene in most Bt-crops, which needs to be replaced with other reporter genes like green fluorescent protein gene and herbicide tolerant gene that will set a stage for building confidence of most of the skeptics regarding the safe use of GM-foods. It is suggested that potential risk of every marker gene should be tested for a longer period of time by exposing a significant number of animals to draw trustworthy conclusions.

For studying harmful impact of Bt-crops on soil microbial communities, it is imperative to carry out experiments in different ecological zones as we know that microbial communities fluctuate in various ecological zones. Also, for each of the new gene of the same family, such studies should be carried out individually.

For assessing the safety of Bt-food, evaluation of the allergic responses should be treated case-to-case basis. Also, the individuals having some allergic issues should orally be given GM-foods expressing known allergens. While studying the allergic response of GM-foods in human, both allergy history and immunodeficiency problems of individuals should be considered to avoid erroneous conclusions.

Genotoxicity studies should be undertaken on each of the animal species without considering the specific toxic properties. Some antagonistic effects of two genes of the same family have been reported. Thus, it is imperative to study the interactions of the genes not only in the GM-plant but also in the GM-food. Similarly, synergistic effects of Bt toxins with chemicals such as pesticides were reported.

In few studies, small traces of ingested DNA were found. It is likely that the ingested DNA may get into the blood stream or be excreted. For addressing such issues, intensive scientific inputs as well as the influx of funds are required for predicting and exploring the possible consequences on NTOs including humans and animals. Also, the post-release monitoring of the GM-crops should be undertaken stringently for studying allergic issues, especially in infants and individuals. Thus, the debate initiated on risk associated with GM-crops over the last two decades educated the end user which paved the way toward the rapid adoption of this technology instead of many controversies raised by the skeptics. The present knowledge about the genes and their functions of different important plant species would further accelerate the progress for introducing new genes (of plant origins) in domesticated crops which would ultimately improve the socioeconomic conditions of the resource-poor farming community, especially living in developing countries.

Acknowledgments We are extremely grateful to the funding agency Pakistan Science Foundation for providing funds through a project "Exploration of Cotton Germplasm Potential Against Drought Stress Using Genomic Approaches"—Project No. PSF/NSLP/P-NIBGE (19).

References

Bannon G, Fu TJ, Kimber I, Hinton DM (2003) Protein digestibility and relevance to allergenicity. Environ Health Perspect 111:1122–1124

Barriere Y, Verite R, Brunschwig P, Surault F, Emile JC (2001) Feeding value of corn silage estimated with sheep and dairy cows is not altered by genetic incorporation of Bt1376 resistance to *Ostrinia nubilalis*. J Dairy Sci 84:1863–1871

Bashir K, Husnain T, Fatima T, Riaz N, Makhdoom R, Riazuddin S (2005) Novel indica basmati line (B-370) expressing two unrelated genes of *Bacillus Thuringiensis* is highly resistant to two lepidopteron insects in the field. Crop Prot 24:870–879

Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, Rubin C (2003) Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect 111:1114–1121

Chen H, Lin Y (2013) Promise and issues of genetically modified crops. Curr Opin Plant Biol 16:255

Chen M, Ye GY, Liu ZC, Yao HW, Chen XX, Shen ZC, Hu C, Datta SK (2006) Field assessment of the effects of transgenic rice expressing a fused gene of *cry1Ab* and *cry1Ac* from *Bacillus thuringiensis* Berliner on nontarget planthopper and leafhopper populations. Environ Entomol 35(1):127–134

Chowdhury EH, Kuribara H, Hino A, Sultana P, Mikami O, Shimada N, Guruge KS, Saito M, Nakajima Y (2003) Detection of corn intrinsic and recombinant DNA fragments Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J Anim Sci 81:2546–2551

Conner AJ, Jacobs JME (1999) Genetic engineering of crops as potential source of genetic hazard in the human diet. Mutat Res 443:223–234

De Schrijver A, Devos Y, van den Bulcke M, Cadot P, De Loose M, Reheul D, Sneyers M (2007) Risk assessment of GM stacked events obtained from crosses between GM events. Trends Food Sci Technol 18:101–109

de Vries J, Meier P, Wackernagel W (2001) The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. FEMS Microbiol Lett 195:211–215

Donkin SS, Velez JC, Totten AK, Stanisiewski EP, Hartnell GF (2003) Effects of feeding silage and grain from glyphosate-tolerant or insect-protected corn hybrids on feed intake, ruminal digestion, and milk production in dairy cattle. J Dairy Sci 86:1780–1788

FAO/WHO (2001) Evaluation of allergenicity of genetically modified foods report of a joint FAO/WHO expert consultation on foods derived from biotechnology, 22–15 January 2001, Rome Italy. Food and Agriculture Organisation of the United Nations, Rome http://www.fao.org/es/esn/allergygm.pdf

Flachowsky G, Chesson A, Aulrich K (2005) Animal nutrition with feeds from genetically modified plants. Arch Anim Nutr 59:1–40

Framond AJ, Bevan MW, Barton KA, Flavell F, Chilton MD (1983) Mini-Ti plasmid and a chimeric gene construct: new approaches to plant gene vector construction. Adv Gene Technol Mol Genet Plants Anim Miami Winter Symp 20:159–170

Germolec DR, Kimber I, Goldman L, Selgrade M (2003) Key issues for the assessment of the allergenic potential of genetically modified foods: breakout group reports. Environ Health Perspect 11:1131–1139

Gupta VVSR, Roberts GN, Neate SM, McClure SG, Crisp P, Watson SK (2002) Impact of Bt-cotton on biological processes in Australian soils. In: Akhurst RJ, Beard CE, Hughes PA (Eds.) Proceedings of the fourth Pacific Rim conference on the biotechnology of *Bacillus thuringiensis* and its environmental impacts. CSIRO, Australia, pp 191–194

Head G, Surber JB, Watson JA, Martin JW, Duan JJ (2002) No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollgard) use. Environ Entomol 31:30–36

Hellmich RL, Hellmich KA (2012) Use and impact of Bt maize. Nat Educ Knowl 3(5):4

History of Bt. http://www.bt.ucsd.edu/bt_history.html

Ho MW, Ryan A, Cummins J (2000) Hazards of transgenic plants containing the cauliflower mosaic virus promoter. Microb Ecol Health Dis 12(3):189–198

Hodgson J (2000) Scientists avert new GMO crisis. Nat Biotechnol 18:13

http://www.gmcrops.ewebsite.com/articles/history.html

Huang J, Mi JW, Lin H, Wang Z, Chen R, Hu R, Rozelle S, Pray CE (2010) A decade of Bt cotton in Chinese fields: assessing the direct effects and indirect externalities of Bt cotton adoption in China. Sci China Life Sci 53:981–991

Huesing J, English L (2004) The impact of Bt crops on the developing world. AgBioForum 7(1&2):84–95

Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster LV, Hunt TE, Wright RJ, Pecinovsky K, Rabaey TL, Flood BR, Raun ES (2010) Area wide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330:222–225

Jagadish CT, Indira R, Vandana S (2012) Effect of Bt-transgenic cotton on soil biological health. Appl Biol Res 14(1):5–23

James C (2014) Global status of commercialized biotech/GM crops. ISAAA, Ithaca, NY

Jennings JC, Albee LD, Kolwyck DC, Surber JB, Taylor ML, Hartnell GF, Lirette RP, Glenn Monsanto KC (2003a) Attempts to detect transgenic and endogenous plant DNA and transgenic protein in muscle from broilers fed yieldgard corn borer corn. Poult Sci 82:371–380

Jennings JC, Kolwyck DC, Kays SB, Whetsell AJ, Suber JB, Cromwell GL, Lirette RP, Glenn KC (2003b) Determining whether transgenic and endogenous plant DNA and transgenic protein are detectable in muscle from swine fed roundup ready soybean meal. J Anim Sci 81:1447–1455

Jiang J, Linscombe SD, Wang J, Oard JH (2000) Field evaluation of transgenic rice (*Oryza sativa* L.) produced by *agrobacterium* and particle bombardment methods. In: Plant and animal genome VIII conference (9–12 January 2000, Town and County Hotel, San Diego, CA; Abstr)

Kaeppler Shawn M, Kaeppler Heidi F, Rhee Y (2000) Epigenetic aspects of somaclonal variation in plants. Plant Mol Biol 43:179–188

Karihaloo JL, Kumar PA (2009) Bt cotton in India—a status report, 2nd edn. Asia-Pacific consortium on agricultural biotechnology (APCoAB), New Delhi

Kuiper HA, Kleter GA, Noteborn HPMJ, Kok EJ (2001) Assessment of the food safety issues related to genetically modified foods. Plant J 27:503–528

Kuiper HA, Konig A, Kleter GA, Hammes WP, Knudsen I (2004) Concluding remarks. Food Chem Toxicol 42:1195–1202

Magaña-Gómez JA, de la Barca AMC (2009) Risk assessment of genetically modified crops for nutrition and health. Nutr Rev 67:1–16

Mal P, Manjunatha AV, Bauer S, Ahmed MN (2011) Technical efficiency and environmental impact of Bt cotton and non-Bt cotton in north India. AgBioForum 14(3):164–170

Matzke MA, Mette MF, Matzke AJM (2000) Transgene silencing by the host genome defense: implications for the evolution of epigenetic control mechanisms in plants and vertebrates. Plant Mol Biol 43:401–415

Mendelsohn M, Kough J, Vaituzis Z, Matthewset K (2003) Are Bt crops safe? Nat Biotechnol 21:1003–1009

Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2004) Are Bt crops safe? AgBioForum 7(1&2):30

Messeguer J (2003) Gene flow assessment in transgenic plants. Plant Cell Tissue Organ 73:201–212

Metcalfe DD (2003) Introduction: what are the issues in addressing the allergenic potential of genetically modified foods? Environ Health Perspect 11:1110–1113

Murray SR, Butler RC, Hardacre AK, Timmerman-Vaughan (2007) Use of quantitative real-time PCR to estimate maize endogenous DNA degradation after cooking and extrusion or in food products. J Agric Food Chem 55:2231–2239

Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK (1996) Identification of a Brazil nut allergen in transgenic soybeans. N Engl J Med 334:688–692

Noteborn HPJM, Kuiper HA (1994) Safety assessment strategies for genetically modified plant products case study: *Bacillus thuringiensis*-toxin tomato. In: Jones DD (ed) proceeding of 3rd international symposium on the biosafety results of field tests of genetically modified plants and microorganisms. University of California, Division of Agriculture and Natural Resources, Oakland, CA, pp 199–207

Oosterhuis DM, Jernstedt J (1999) Morphology and anatomy of cotton plant. In: Smith CW, Cothren JT (eds) Cotton: origin, history, technology and production. Willie, Darvers, pp 170–206

Pakistan National Biosafety Rules (2005)

Palm CJ, Donegan KK, Harris D, Seidler RJ (1994) Quantification in soil of *Bacillus thuringiensis* var *kurstaki* d-endotoxin from transgenic plants. Mol Ecol 3:145–151

Paparini A, Romano-Spica V (2004) Public health issues related with the consumption of food obtained from genetically modified organisms. Biotechnol Annu Rev 10:85–122

Paparini A, Romano-Spica V (2006) Gene transfer and cauliflower mosaic virus promoter 35S activity in mammalian cells. J Environ Sci Health 41:437–449

Perlak FJ, Oppenhuizen M, Gustafson K, Voth R, Sivasupramaniam S, Heering D, Carey B, Ihrig RA, Roberts JK (2001) Development and commercial use of Bollgard® cotton in the USA: early promises versus today's reality. Plant J 27(6):489–501

Pilcher CD, Rice ME, Obrycki JJ (2005) Impact of transgenic *Bacillus thuringiensis* corn and crop phenology on five nontarget arthropods. Environ Entomol 34:1302–1316

Poehlman JM (1994) Breeding field crops, 3rd edn. Iowa State University Press, Ames

Prado JR, Segers G, Voelker T, Carson D, Dobert R, Phillips J, Cook K, Cornejo C, Monken J, Grapes L (2014) Genetically engineered crops: from idea to product. Annu Rev Plant Biol 65:769–790

Pray C, Naseem A (2007) Supplying crop biotechnology to the poor: opportunities and constraints. J Dev Stud 43(1):192–217

Prescott VE, Hogan SP (2006) Genetically modified plants and food hypersensitivity diseases: usage and implications of experimental models for risk assessment. Pharmacol Therap 111:374–383. http://www.isaaa.org/resources/publications/pocketk/6/default.asp

Prescott VE, Campbell PM, Moore A, Mattes J, Rothenberg ME, Foster PS, Higgins TJV, Hogan SP (2005) Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. J Agric Food Chem 53:9023–9030

Pusztai A (2001) Genetically modified foods: are they a risk to human/animal health? Action Bioscience. http://www.ask-force.org/web/Pusztai/Pusztai-GM-Foods-Risk-Human-Animal-Health-2001.pdf

Pusztai A, Bardocz S, Ewen SWB (2003) Genetically modified foods: potential human health effects. In: D'Mello JPF (ed) Food safety: contaminants and toxins. CAB International, Wallingford, pp 347–372

Rahman M, Rashid H, Shahid AA, Bashir K, Husnain T, Riazuddin S (2007) Insect resistance and risk assessment studies of advanced generations of basmati rice expressing two genes of *Bacillus thuringiensis*. Electron J Biotechnol 10(2):241–251

Rahman M, Shaheen T, Ashraf M, Zafar Y (2012) Bridging genomic and classical breeding approaches for improving crop productivity. In: Ashraf M et al (eds) Crop production for agricultural improvement, vol 1. Springer, The Netherlands, pp 19–41

Rahman M, Shaheen T, Irem S, Zafar Y (2015) Biosafety risk of genetically modified crops containing *Cry* genes. In: Lichtfouse E et al (eds) CO_2 sequestration, biofuels and depollution. Environmental chemistry for a sustainable World 5. Springer, The Netherlands, pp 307–334

Richards HA, Chung-Ting Han RG, Hopkins ML, Failla WW, Ward CN, Stewart CN Jr (2003) Safety assessment of recombinant green fluorescent protein orally administered to weaned rats nutrient interactions and toxicity. J Nutr 133:1909–1912

Romeis J, Bartsch D, Bigler F, Candolfi MP, Gielkens MM, Hartley SE, Hellmich RL, Huesing JE, Jepson PC, Layton R, Quemada H, Raybould A, Rose RI, Schiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD (2008) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nat Biotechnol 26:203–208

Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P (2011) Bacillus thuringiensis: a century of research, development and commercial applications. Plant Biotechnol J 9:283–300

Saxena D, Flores S, Stotzky G (1999) Insecticidal toxin in root exudates from Bt corn. Nature 402:480

Sayanova O, Smith MA, Lapinskas P, Stobart AK, Dobson G, Christie WW (1997) Expression of a borage desaturase cDNA containing an N-terminal cytochrome b5 domain results in the accumulation of high levels of delta6-desaturated fatty acids in transgenic tobacco. Proc Natl Acad Sci USA 94:4211–4216

Sims SR, Holden LR (1996) Insect bioassay for determining soil degradation of *Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) protein in corn tissue. Environ Entomol 25:659–664

Singh NP, McCoy MT, Tice RR, Shneider EL (1988) A simple technique for quantification of low level of DNA damage in individual cells. Exp Cell Res 715:184–194

Singh AK, Mehta AK, Sridhara S, Gaur SN, Singh BP, Sarma PU, Arora N (2006) Allergenicity assessment of transgenic mustard (*Brassica juncea*) expressing bacterial cod A gene. Allergy 61:491–497

Stotzky G (2000) Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. J Environ Qual 29:691–705

Subedi KD, Ma BL (2007) Dry matter and nitrogen partitioning patterns in Bt and non-Bt near-isoline maize hybrids. Crop Sci 47:1186–1192

Subramanian A, Qaim M (2010) The impact of Bt cotton on poor households in rural India. J Dev Stud 46:295–311

Tabashnik BE (2010) Communal benefits of transgenic corn. Science 330:189–190

Tapp H, Stotzky G (1998) Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. Soil Biol Biochem 30:471–476

Taylor SL, Hefle SL (2002) Genetically engineered foods: implications for food allergy. Curr Opin Allergy Clin Immunol 2:249–252

Tice RR, Agurell E, Andeson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicity testing. Environ Mol Mutagen 35:206–221

Tryphonas H, Arvanitakis G, Vavasour E, Bondy G (2003) Animal models to detect allergenicity to foods and genetically modified products: workshop summary. Environ Health Perspect 11:221–222

Wu G, Cu HR, Shu Q, Xia YW, Xiang YB, Gao MW, Cheng X, Altosaar (2000) Stripped stem borer (*Chilo suppressalis*) resistant transgenic rice with a cry1Ab gene from Bt (*Bacillus thuringiensis*) and its rapid screening. J Zhejiang Univ 19:15–18

Xu-Chongren MY, Chang S (2001) Research on biosafety of transgenic Bt cotton. College of life sciences. Beijing University, Beijing

Yanni SF, Whaen JK, Ma BL (2011) Field-grown Bt and non-Bt corn: yield, chemical composition, and decomposition. Agron J 103:486–493

Yonemochi C, Fujisaki H, Harada C, Kusama T, Hanazumi M (2002) Evaluation of transgenic event CBH 351 (StarLink) corn in broiler chicks. Anim Sci J 73:221–228

Zaman M (2015). Efficacy and impact of GM (Bt) cotton in ecosystem. Ph.D. thesis, QA University, Islamabad

Zaman M, Mirza MS, Irem S, Zafar Y, Rahman M (2015) A temporal expression of Cry1Ac protein in cotton plant and its impact on soil health. Int J Agric Biol 17:280–288