



A Critical Analysis of Development and Biosafety Issues of Genetically Modified Bt. Plants

Dr. Vijay Laxmi Hada

Assistant Professor, Under Affiliated College, University of Rajasthan, Rajasthan (India)

E-mail: vijayahada@gmail.com

ABSTRACT:

The purpose of this article is to explore potential solutions to the GMO controversy as well as concerns regarding Bt plants and their economic impacts of the introduction of biotechnology on the development and biosafety issues of genetically modified plants. Bt crops are genetically modified plants that produce a toxin similar to that of the *Bacillus thuringiensis* bacteria in plant cells. This means that the crops are protected from pests by the *Bacillus thuringiensis* bacterium. Specific proteins, referred to as "cry proteins" are secreted by the *Bacillus thuringiensis* bacteria and are poisonous to insects. Cotton, brinjal, potato, corn, etc. are a few examples of Bt crops. The poisonous cry protein found in the transgenic plants crystallizes in the insect's digestive tract, eventually leading to its death. Evaluation and development of transgenic plants will help farmers with crop quality, pest and disease resistance and weed management by herbicide resistance properties. These techniques have been used to develop many transgenic field and vegetable crops: wheat, corn, rice, tobacco, cotton, soybean, cabbage etc. Concerns about the effects of Bt crops on the sustainability of soil biodiversity and ecosystem services on agricultural land have been raised up by environmental groups. Potential interactions with non-target organisms are a major concern for the risk evaluation because Bt crops contain insecticidal proteins.

Keywords- Transgenic, Herbicides, Biodiversity, Ecosystem, Insecticide, *Bacillus thuringiensis*.

Introduction :

Developing plants that are resistant to pests has been one of the main objectives of biotechnology in agriculture. The use of genetic engineering to modify plant cells to express genes with particular characteristics is a novel method of controlling agricultural pests, such as insects, weeds, and diseases. Numerous plant species have been effectively transformed since 1983 through the use of several biochemical systems (Peferoen & Mellaert 1991). The term "transgenic" refers to the process of introducing a foreign gene sequence into the nuclear genome (DNA) of another organism. Various molecular techniques have been employed in the development of transgenic crops. The *Agrobacterium tumefaciens* system is the most widely used method for generating transgenic plants (Gasser & Fraley 1989, Steinbiss & Davidson 1989, Peferoen & Mellaert 1991). *Agrobacterium tumefaciens* are a soil bacterium. Many dicotyledonous and gymnosperm plants become infected with *Agrobacterium tumefaciens* when they sustain wounds (Steinbiss & Davidson 1989, Fosket 1994). According to Grierson and Covey 1988, Fosket 1994, this approach involves the introduction of a functional gene (herbicide tolerance, insect resistance) into the host DNA by a vector system. This method is primarily restricted to dicotyledonous plants, yet it functions incredibly effectively. Direct DNA transfer techniques have also been used to successfully modify genes in monocotyledonous plants, such as rice, wheat, and maize (Fromm et al. 1990, Fujimoto et al. 1993, Vasil et al. 1993, 1994). One another simple technique consists of protoplast incubation with plasmid DNA in the presence of polyethylene glycol (PEG) (Steinbiss & Davidson 1989, Vasil 1994). In addition to the methods previously mentioned, these strategies have proven to be highly helpful in the evaluation and production of transgenic plants that will help growers with crop quality, resistance to pests and diseases, and protection from weed-control herbicides. Numerous transgenic field and vegetable crops, including wheat, corn, rice, tobacco, cotton, soybean, cabbage, tomatoes, and potatoes have been developed using these techniques (Fromm et al. 1990, Beegle & Yamamoto 1992, Warren et al. 1992, Benedict et al. 1993, Fujimoto et al. 1993, Murry et al. 1993, Perlak et al. 1993, Vasil et al. 1993). A commonly employed gene for plant transformation is the one that codes for the dendotoxin generated by *Bacillus thuringiensis* Berliner, or Bt. In order to functionally express the dendotoxin obtained from Bt corn, potato, cabbage, tomato, soybean, cotton, tobacco, and rice have been transformed (Kumar & Sharma 1994). When the bacillus was identified from diseased *Bombyx mori* L. larvae in 1901, Japanese bacteriologist S. Ishiwata made the first discovery of Bt (Beegle & Yamamoto 1992, Knowles 1994). He explained the pathological effects it had on silkworm larvae. According to Beegle and Yamamoto (1992), Ishiwata observed that the pathogenicity had to be associated with some kind of toxin. Ernst Berliner identified a related microbe as *Bacillus thuringiensis* ten years later (Beegle & Yamamoto 1992). He was the first to characterize the bacterial species as a rod-shaped, spore-forming, gram-positive bacterium that was present in soil (Beegle & Yamamoto 1992). Funding for the study employing Bt as an *Ostrinia nubilalis* control agent was discontinued by North America. Edward Steinhaus sparked interest in the application and commercial development of Bt as a microbial control agent of lepidopteron pests in the early 1950s with a number of studies he published (Beegle & Yamamoto 1992). However, Bt product compositions

were unable to economically compete with broad spectrum insecticides that were less expensive. The first commercial several subspecies of *Bacillus thuringiensis* have been isolated; a few include *kurstaki*, *aizawai*, *sotto*, *entomocidus*, *berliner*, *thuringiensis*, *tenebrionis*, *israeliensis*, and *morrisoni* (Hofte & Whiteley 1989). *Bacillus thuringiensis* generates a parasporal crystal inclusion body during sporulation. The genes known as cry genes are responsible for producing this crystal protein. Hooft and Whiteley (1989) grouped the proteins expressed by these genes based on their structural similarity and insecticidal selectivity. Following are the four primary subclasses: According to Hofte & Whiteley 1989, Gill et al. 1992, Yamamoto & Powell 1993, Knowles 1994, Crickmore 1995, Cry1 proteins are specific to lepidopterans and have a molecular weight of about 130 kDa. Cry2 proteins are specific to dipterans and lepidopteron and have a molecular weight of 70 kDa. Cry3 proteins are specific to coleopterans and have a molecular weight of 70 kDa. Finally, Cry4 proteins are specific to dipterans and include 70-130 kDa size proteins. The presence of particular toxin-binding sites (receptors) in the gut of various insects and differences in the larval gut that impact the solubilization and processing efficiency of the pro toxin are the two factors that determine the activity of Bt in the host organism's gut (Hofte & Whiteley 1989). The protein enters the insect's system as a protoxin, which is then broken down into a structural and active fragment by protease enzymes in an alkaline gut (Hofte & Whiteley 1989, Gill et al. 1992, Knowles 1994, Kumar & Sharma 1994). According to Hofte and Whiteley (1989) and Kumar & Sharma (1994), the active fragment is poisonous and attaches to certain receptors in the target gut epithelial lining, causing the cells to swell and burst. When the cells rupture, the gut epithelium sloughs off, causing the insect to stop feeding and, if a lethal amount has been consumed, to eventually die.

Problem analysis :

The ubiquitous bacterium *Bacillus thuringiensis* (Bt) has been instrumental in the production of genetically engineered crops and agriculture in a very unique way. For almost a century, these bacteria have been a valuable aid in pest control due to their inherent insecticidal properties. It is generally acknowledged and authorized for use in organic applications as a natural biopesticide, but the introduction of Bt genes into important crops has generated greater controversy. Since humans started cultivating crops, we have been fighting a constant battle to keep pests out of our food. According to estimates from the Food and Agricultural Organization, pests, disease, and environmental protection cause 20–40% of the world's crop yields to be lost each year

Objectives :

The objectives of this study are:

1. To review changes in price for welfare of consumer in reference to introduction of Bt technology.
2. To draw the implications policies for transgenic Bt plants in India.
3. To provide suggestions to farmers regarding the adoption of Bt plants.
4. To analysis the biosafety aspects of Bt plants.

Methodology and methods :

Literature review for undertaking the study will be done by referring various national and International journals, published articles in various official standard books and referring to various websites on the internet.

Review of literature :

Johnson and Gould (1992), examined the parasitism of *Heliothis virescens* (F.) on transgenic tobacco and found that low levels of Bt protein expression in transgenic tobacco appear to be compatible with parasitoids for *H. virescens* suppression. Heinz et al. (1994) found that feeding on *H. virescens* larvae infected with a recombinant nuclear polyhedrosis virus did not negatively impact two predators, *C. carnea* and *Ostrinia insidious*. Melin and Cozzi (1990) found that two different Bt spray products had no effect on either adult *C. carnea* larvae or adult larvae. Following the application of Bt microbial sprays and bait formulations on tobacco, beetle populations were observed; populations of two species, *Hippodamia convergens* Guerin meneville, and *C. maculata*, were unaffected by the treatments (Melin & Cozzi 1990). Sims (1995) examined the impact of transgenic cotton expressing the Bt Cry1Ac protein on a number of natural enemies, such as *H. convergens*, *C. carnea*, and *Nasonia vitripennis* (Walker), and found no negative effects. In contrast to these findings, a laboratory study conducted by Giroux et al. (1994) found that M-One, a Bt microbial spray used to suppress the Colorado potato beetle, *Leptinotarsa decemlineata*, lowered the predation rate of *C. maculata*. This study indicates that Bt can have an impact on predators and that there may be a valid reason to think that predator behavior and feeding efficiency in the field may be impacted by high Bt protein expression in corn pollen. Apart from the natural adversaries, a number of secondary lepidopteron pests such as the black cutworm (*Agrotis ipsilon*), Hufnagel stalk borer (*Papaipema nebris*), Geunee armyworm (*Pseudaletia unipuncta*), Haworth and corn earworm (*Helicoverpa zea*) may feed on transgenic Bt maize and may cause economic harm. The environmental effects of depending too much on transgenic products have been examined by Hollander (1991). These effects can be divided into two categories: those that arise directly from the introduction of certain agents into the environment, and those that compound over time. Studies have indicated that when Bt microbial sprays are used in large-scale pest control operations, non-target Lepidopteron species may be ecologically impacted (Miller 1990). The community of non-target organisms that would most likely be impacted is identified as the first step in the risk assessment analysis of transgenic Bt corn. Studies evaluating the effects of microbiological Bt sprays or transgenic organisms on nontarget natural enemies are scarce.

Types of Bt crops :

There are following types of Bt crops were produced in India.

Bt Cotton :

The Bt gene is genetically engineered into the Bt cotton to protect the plants from bollworm, a major pest of cotton. The worms that reside on the Bt cotton leaves become drowsy and inactive, which lessens their damaging effects on the plants. Ingesting the deadly proteins generated by the Bt crops, worms are killed as they swallow the plant. Bt cotton is the first genetically modified plant to be commercially commercialized in India. Toxins produced by the plant in response to the Bt gene being inserted into the plant's DNA were used to develop a transgenic variety of cotton. As the caterpillar feeds on the plants, the toxins generated by the plant would paralyze the guts of caterpillar. This has been developed by MAHYCO (Maharashtra Hybrid Seeds Company) in collaboration with American company, Monsanto.

Bt Brinjal

Bt brinjal is also produced by genetic transformation of a crystal protein gene *cry 1 Ac* from the bacterium *Bacillus thuringiensis*. In order to offer resistance against lepidopteron insects, Bt brinjal was developed. The proteins generated by Bt genes attach to the receptors on the insect's membrane, causing the membranes to become porous. The insect dies as a result of this disturbance to its digestive system.

Bt Corn

An insect-resistant cultivar Bt corn is a genetically modified crop introduced by the Bt gene. Bt corn has not been linked to any known harmful impacts on human health. If non-target insects have a close relationship to the target pest, like the monarch butterfly, then Bt corn may have an unfavorable effect on them.

Use of Bt technique as a biological pest control

Bt technique benefits align well with the fundamental objective of integrated pest control, which is to maintain naturally occurring beneficial organisms while keeping pest numbers below the point of economic destruction (Pedigo 1995). According to Hollander (1991), society can gain greatly from the ability to replace harmful pesticides with safer, more effective, and biodegradable alternatives. These benefits are becoming increasingly important factors when growers decide what methods to use in controlling insect pests. A wide range of commercial Bt products are used to control pests in forests and agriculture. *Bt israelensis* is applied as a larvicide for 7 mosquito species. *Bt tenebrionis* is effective against coleopteran larvae that feed on leaves, such as *Leptinotarsa decemlineata*, which is the Colorado potato beetle. However, more than two-thirds of Bt-based products use strains from the kurstaki subspecies, which are effective against more than 55 lepidopteron species, including *Ostrinia nubilalis* (Adang 1991, Beegle & Yamamoto 1992). Commercial products which show activity against *Ostrinia nubilalis* include Dipel, Javelin, Larvin, M-Peril, and MVP (ESA 1994). Chemical pesticides are frequently applied in the Corn Belt's various regions to Plant resistance and cultural control are two other management strategies that can be employed, although their effectiveness varies greatly and is dependent on a number of variables. Although it might be challenging to detect and assess the economic impact, biological control is another aspect that keeps corn borer populations under control. Control is lost when larvae start feeding beneath the leaf sheath and pierce through to the stalk. However, a new strategy using Bt to more effectively control *Ostrinia nubilalis* has been made possible by biotechnology, which kills larvae before they enter the stalk. Numerous investigations have documented these insects' susceptibility to various strains of Bt protein under laboratory conditions (Burgess 1981, Hofte et al. 1988, MacIntosh et al. 1990, Stone & Sims 1993, Ebor et al. 1994), and in field conditions (Miller 1990, Ali & Young 1993, Bartels & Hutchison 1995, Johnson et al. 1995). The impact of transgenic plants (potatoes) on secondary lepidopteran insects has been the subject of study (Ebor et al. 1994). They observed a significant impact on the potato pests *Ostrinia nubilalis* and *Phthorimea operculelia* (Zeller).

Use of Bt technique in Developing resistant varieties

The first report on the production of transgenic corn expressing a developed insecticidal crystal protein derived from Bt was published in 1993 by Koziel et al. Corn plants were engineered to contain the cry1 Ab gene, which confers highly excellent protection against *Ostrinia nubilalis*, derived from the Bt subspecies kurstaki HD-1 strain. The cry1 Ab gene has been successfully transcribed utilizing the 35S promoter of the cauliflower mosaic virus (CaMV) or a combination of the pollen-specific promoter and the corn phosphoenolpyruvate carboxylase (PEPC) promoter (Koziel et al. 1993). The following tests were carried out to confirm the presence and function of the proteins when there was enough leaf material available: the B-glucuronidase (GUS) histochemical assay, transgenic PCR analysis, growth in the presence of PPT (phosphinothricin, a selectable identifying marker), ELISA (enzyme linked immunosorbent assays) for the Cry1Ab protein, and insect bioassays using *Ostrinia nubilalis* larvae (Koziel et al. 1993). Other advantages of transgenic Bt corn include that it offers protection to the entire plant during the growing season, so growers should be able to cut down on scouting. Moreover, there are no added labors or application expenditures. Transgenic Bt corn eliminates a number of the drawbacks of Bt microbiological sprays, but it also introduces a few possible new ones, such as insect resistance issues, growers disregarding pests in their fields, and unidentified impacts on non-target insects. The Environmental Protection Agency (EPA) has accepted the registration of transgenic Bt corn, and it will

be introduced into agricultural areas in 1996 (Dean Christensen, Ciba Seeds, personal communication). There are questions about whether sufficient short- and long-term studies have been conducted to ascertain the ecological effects of Bt toxins on the ecosystem (Raybould & Gray, 1994). One component of the cost-benefit analysis that is used to decide whether or not to release a novel material, transgenic plants into the environment is risk assessment (Jepson et al. 1994).

Biosafety issues related to Bt plants

According to the Environmental Protection Agency (EPA), all Bt endotoxins expressed in genetically modified plants satisfies the fundamental legal safety requirement of "responsible certainty of no harm". Consuming foods derived from and manufactured using genetically modified Bt crops in accordance with the projected exposures. To ensure that these fundamental requirements are met, the EPA conducts three separate investigations before coming to this determination. It describes its approach in assessing Bt crop safety as follows: In order to reasonably assure that the combined exposure to these proteins won't cause any harm, a number of different kinds of data are needed for the Bt plant-incorporated protectants. Starting with the information that shows the Bt protein functions like a dietary protein, it does not exhibit any oral toxicity at high levels, and it does not structurally like any known food allergen or protein toxin. A review of the scientific literature provides some, but not all, of the EPA's assessment's arguments. First, it seems that some Bt proteins are getting into the bloodstream of mammals. They could then spread to different organs and wreak harm. Second, the EPA assumes that Bt proteins break down very rapidly due to their highly acidic conditions in the human stomach and that these fragments are harmless. A variety of unusual but common gastrointestinal disorders that are known to impede the breakdown of Bt endotoxins are not given much consideration by the agency. Third, research on the allergenicity the ability of Bt toxin fragments to cause allergies or poisonous characteristics has received little to no attention. Smaller turned Bt proteins made in genetically modified plants have the potential to attach to intestinal lining cells in human gut and influence several physiological processes. The extensive and rigorous study EPA scientists perform in tracking the breakdown products of chemical pesticides in the food chain stands in contrast to this lenient approach to evaluating the risk of Bt endotoxins. The fact that the Bt toxins expressed by GM Bt plants differ from those employed in the acute oral toxicity assays mandated by the EPA raises additional valid concerns.

Advantage of Bt crops :

The Bt gene offers a number of benefits when associated to crop pest management, such as:

1. Limited host range, little to nonexistent residual.
2. Safe to handle for cultivators and inoffensive to vertebrates.
3. Bt crops contribute to the protection of helpful insects.
4. They lessen the need for synthetic pesticides, which aids in the control of soil contamination.
5. It increases the agricultural output, which boosts the farmer's income. As a result, farm productivity rises.
6. It leads to the production of disease-free crops owing to the reduction of pesticides.
7. Its higher yields over a short period of time make it easy to feed a growing population.
8. A natural defense against European corn borer larvae and other insect pests is provided to the plant by the Bt proteins.

Disadvantage of Bt. crops :

1. Bt crops are more expensive than those farmed organically.
2. It may interfere with the normal flow of genes.
3. No noticeable effect immediately.
4. Crop productivity may decrease if pests develop resistance to the toxins these crops generate.
5. Timing of application of Bt. microbial insecticides is critical.
6. Because UV breaks down the protein, several applications of the Bt product might be required for greater efficacy.
7. Specificity may be viewed as a drawback because producers frequently need to control multiple pests. Accurate placement of the treatment is necessary to where the insect is feeding.

Ethical issues related to genetically modified organisms :

1. Unpredictable outcomes could arise from the introduction of genetically modified organisms into the ecosystem.
2. The issue of claiming patent rights has also emerged with genetically modified organisms, which are produced to fulfill food and medicine requirements.
3. The emerging and developing countries of the world, like Africa, India, and others, have widespread knowledge of their natural resources. Conversely, low biodiversity exists in developed nations. Therefore, developing countries should enact laws to stop the exploitation of their natural resources.
4. Under the Environment Protection Act of 1986, the government took the initiative to establish the Genetic Engineering Approval Committee, or GEAC. The development, usage, import, export, and storage of genetically modified organisms are governed by laws and regulations made by this body.

Result and Discussion :

Scientists that support biotechnology believe that in order for agriculture to succeed in the twenty-first century, artificial gene technologies are necessary. Genetically engineered crops are thought to be the answer to feeding the world's expanding population and expanding food supply. Recently, "Golden Rice" with enhanced vitamin A has become popular; this could help address the issue of malnutrition in underdeveloped nations. Herbicide-resistant crops reduce the need for herbicides and represent a significant advancement in the reduction of pesticide residues in the environment. Life Science firms acknowledge that GMOs with agronomic features reduce production costs per unit and, consequently, offer a more affordable global food supply. Some quality-enhanced crops contain unsaturated instead of saturated fatty acids and are therefore healthier for humans. Plants with characteristics that carry the gene(s) for resistance to abiotic stress (frost, drought, etc.), which are predicted in the near future, may pave the way for new developments in crop production. Crops with anti-cancer properties or other health-promoting traits, such as reducing "back cholesterol," may be generated in the future. There is currently little scientific proof that genetically modified organisms are harmful to human health. Four categories can be

used to classify objections against GMOs-

1. Concern about human health, 2. Environmental concern 3. Ethical issues and 4. Political issues. One significant issue is the deterioration of public confidence in scientists and technical specialists, who are trusted to assess whether new technology will have unanticipated and undesirable side effects. Some people view the ethical objection as interfering with their "core" identity, and thus believe that the transfer of genetic material across species that could not occur naturally should not be allowed (Buckwell 1999). A major contributing factor to the intense resistance to biotechnology products in the EU is the belief held by some consumers that the follow for the use of genetically modified organisms (GMOs) in food production stems from a small group of powerful transnational corporations. It is believed that this group may be able to influence the regulatory approval process for their own commercial gain (Buckwell 1999).

Conclusion :

The result of this study is consistent with the conclusion that this particular Bt toxin can be harmful to mammals. Every new Bt toxin that is sprayed on our food crops needs to be investigated to see if it has any negative effects on the human intestine. We should also pay close attention to Bt crops that express relatively high levels of multiple Bt toxins in the harvested portion of the crop that is consumed in different forms by humans, farm animals, and pets.

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