Role of transgenic Bt-crops in promoting biological control and integrated pest management

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ABSTRACT: Since their commercial introduction in 1996 in the USA, the insect resistant transgenic Bt crops, notably Bt-cotton and Bt-corn, have given effective control of target pests and found overwhelming adoption in several countries. As of 2017, these Bt crops were cultivated in 14 countries on 100 m ha, including 11.4 m ha of Bt-cotton in India, which comprised 53% of 189.8 m ha of all GM crops grown in 24 countries. Such extensive cultivation of Bt crops, incorporated with genes derived from the soil bacterium, Bacillus thuringiensis (Bt), modified to express host-specific insecticidal crystalline (Cry) proteins, has resulted in higher crop yields by 22%, increased farmers’ profit by 68% and reduced chemical insecticide applications by 37%, thereby providing social, economic, health and environmental benefits. The reduced chemical sprays have contributed to conservation of parasitoids and predators leading to enhanced biological control in crop systems. Feeding tests carried out with predators like ladybird beetles and green lacewing and also with hymenopteran parasitoids have demonstrated Bt proteins to be safe to these natural enemies. The value of Bt crops in promoting biological control and integrated pest management is discussed.

KEY WORDS: Bt-cotton, Bt-corn, biosafety, IPM, parasitoids, predators

(Article chronicle: Received: 17-05-2019; Revised: 12-02-2020; Accepted: 16-02-2020)

INTRODUCTION

A new era in insect pest management began with the development of insect-resistant transgenic crops, incorporated with genes derived from the soil bacterium, Bacillus thuringiensis. Berliner, modified to express host-specific insecticidal crystalline (Cry) proteins. These are popularly called Bt crops. The year 1996 is regarded as a turning point as three Bt-crops received regulatory approvals in the USA for commercial cultivation. These were Bt-corn incorporated with the modified cry1Ab gene for protection against the notorious European corn borer, Ostrinia nubilalis (Hübner), Bt-potato with cry3Ab against the hardy Colorado potato beetle, Leptinotarsa decemlineata (Say), and Bt-cotton with cry1Ac against the dreaded cotton bollworm complex which included the tobacco budworm, Heliothis virescens (F.) and bollworm, Helicoverpa zea (Boddie) (Perlak et al., 1990; 1991). Following their success, these crops were subsequently introduced into several other countries either with only the insect resistant Bt-gene(s) or stacked with herbicide tolerant gene, EPSP, also derived from a bacterium. Later, an improved version of Bt-cotton (Bollgard II®) stacked with two Bt genes, cry1Ac along with cry2Ab2, was approved in Australia and the USA in 2002 and similarly Bt-corn stacked with two Bt genes, cry1Ab for protection against European Corn Borer and cry3Bb against the corn root worms, Diabrotica spp., was approved in the USA in 2003. India entered the transgenic world in March 2002 with the regulatory approval of Bt-cotton containing cry1Ac for control of cotton bollworms. Later, four other versions of Bt-cotton and also Bollgard II were approved in India between 2006 and 2009. The most recently approved Bt products include Bt-rice for control of rice stem borer in China in November 2009, ‘Smart Stax’ maize (corn) incorporated with 8 genes coding for resistance to two types of insect pests (European corn borer and root worm) and two types of herbicides (two genes for each) in the USA in March 2010, and Bt-brinjal for control of Fruit-and-shoot borer in Bangladesh in 2013 (Manjunath and Mohan, 2015).

Adoption of Bt crops

As of 2017, these Bt crops were cultivated in 14 countries on about 100 m ha (23.3 m ha with Bt alone and 77.7 m ha with Bt stacked with herbicide tolerance), including 11.4 m ha of Bt-cotton in India, which comprised 53% of 189.8 m ha of all GM crops grown by about 17 m farmers in 24 countries (19 developing, 5 industrialized) (ISAAA, 2017).
Prior to the introduction of Bt-cotton in India in 2002 by MAHYCO (Maharashtra Hybrid Seed Company Limited) in collaboration with Monsanto, the annual losses caused in cotton crops by bollworms, especially *Helicoverpa armigera* (Hübner), the others being *Pectinophora gossypiella* (Saunders), *Earias vitella* (Fabricius) and *E. insulana* (Boisduval), were estimated at about US$ 300 million despite repeated spraying of chemical insecticides (6 to 16 times or more for each crop). It was estimated that insecticides valued at $700 million were used on all crops annually in India, of which about 50% were used on the cotton crop alone. However, these were unable to control bollworms as these pests had developed resistance to all classes of insecticides due to their overuse or abuse. But, since dependable alternative methods were not available, farmers had no option except to ‘spray’ and ‘pray.’ Under the circumstances, Bt-cotton brought in a ray of hope to cotton farmers (Barwale *et al.*, 2004; Manjunath, 2005).

The area planted with Bt-cotton in India was about 0.03 million in 2002, the first year. It increased consistently from year to year to reach 11.4 million hectares in 2017 equivalent of 93% of the total cotton area of 12.24 m ha grown in the country (ISAAA, 2017). It is a remarkable growth rate in 15 years. Similarly, the number of farmers adopting this technology also increased from about 20,000 in 2002 to over 7.5 million in 2017, reflecting their confidence in this technology. Bt-brinjal for control of fruit-and-shoot borer, *Leucinodes orbonalis* Guenée, has completed all the regulatory trials, but its final approval is pending with the Ministry of Environment and Forests. Thus, Bt-cotton continues to be the only GM crop approved in India since 2002. Since the last few years, bollworms, more particularly *P. gossypiella*, has shown resistance to Bt-cotton. One of the reasons for this is non-compliance of insect resistance management practices like refuge planting as recommended by the regulatory authorities. Nevertheless, insect resistance is a natural phenomenon and it is only a question of time. Therefore, we should always be proactively ready with new products to replace the older one as and when need arises. The development of Bollgard II, Bollgard III, etc., is a progressive step in this direction.

**Regulation of Bt/transgenic crops**

In every country, the prescribed bio-safety requirements are to be fulfilled before a transgenic crop/product is approved for commercialization. All the transgenic crops, including Bt crops, that have been commercialized so far have undergone and passed comprehensive biosafety and agronomic trials with regard to potential for food toxicity, food allergenicity, cross pollination and effect on non-target beneficial organisms including biological control agents. Proactive measures have also been recommended to prevent or delay the development of pest resistance to Bt proteins expressed *in-planta*.

In India, the major responsibility for regulation of Genetically Modified Organisms (GMOs) is with the Ministry of Science and Technology (MoST) and Ministry of Environment and Forests (MoEF), Govt of India. Other ministries and also other institutions are involved as and when required. For example, the Ministry of Agriculture, Indian Council of Agricultural Research (ICAR), Indian Agricultural Research Institute (IARI), Agricultural Universities, State Departments of Agriculture and Horticulture, etc. may be involved for issues related to GMOs. The Department of Biotechnology (DBT) is the nodal agency for biotech research and promotion and it functions under MoST. Two important committees, namely Institutional Bio-Safety Committee (IBSC) and Review Committee on Genetic Manipulation (RCGM), work under the purview of DBT. Another major committee, namely Genetic Engineering Appraisal Committee (GEAC), is constituted under MoEF. These committees are represented by experts drawn from various fields and organizations across the country and are responsible to ensure that proactive safety studies are carried out on GM products before they are approved for commercialization.

In the USA, the regulation of genetically modified organisms (GMOs) is overseen by three federal agencies: United States Dept. of Agriculture (USDA), Environmental Protection Agency (EPA) and Federal Drug Administration (FDA). Similar regulatory bodies exist in other countries also. No other crop or food was/is subjected to as much stringent scrutiny as are GM crops/products before they are openly cultivated or marketed (Manjunath, 2005).

The ICGEB (International Centre for Genetic Engineering and Biotechnology) has provided a list of several websites that provide a comprehensive array of biosafety-related information (*ICGEB*: [http://www.icgeb.org/~bsafesrv/databases/general.html](http://www.icgeb.org/~bsafesrv/databases/general.html)). In India, the biosafety data pertaining to Bt-cotton and other GM crops can be viewed in the website of IGMORIS (Indian GMO Research Information System), created by the Department of Biotechnology, Govt. of India (IGMORIS: [http://igmoris.nic.in/major_developments1.asp](http://igmoris.nic.in/major_developments1.asp)).

Globally, as of now, over two thousand studies dealing with the safety of GMOs, published in scientific journals, are available. Almost every major scientific body and regulatory agency in the world has reviewed such research data and strongly vouched that the food and feed derived from GM
crops are safe (Entine, 2013; Wendel, 2013). The GM products are a result of thorough research and evaluation and are ‘Substantially Equivalent’ to their non-GM counter parts. Therefore, the safety of GM crops / foods is on par with those that are conventionally produced.

The regulatory studies carried out in various countries have indicated that Bt crops and other transgenic crops have not caused any scientifically proven adverse effect on humans, animals, other non-target beneficial organisms including parasitoids and predators, or the environment anywhere in the world. However, there are a few organizations and individuals who opposed the Bt technology even when it was in the experimental stage and made unsubstantiated allegations against their safety and benefits. Such doubts and allegations have been scientifically clarified (Manjunath, 2011; Manjunath and Mohan, 2015), but the opponents are continuing their tirade against these crops, thereby creating confusion and fear among farmers and public. However, millions of farmers all over the world have readily adopted this technology and derived benefits for more than two decades and they are asking for more!

Safety and regulation of Bt and Bt-crops

Bacillus thuringiensis is a common soil bacterium found throughout the world. The insecticidal crystalline Bt proteins used in commercialized Bt-crops are highly host-specific and require certain specific conditions for them to be effective against the target pests. In the first place, these proteins must be ingested to be insecticidal. This happens when the larvae feed on Bt plant tissues. Once ingested, the mode of action of Bt proteins is complex and involves solubilization, proteolytic stability, binding to the midgut epithelium, formation of ion channels in the midgut cells, and finally lysis of these cells leading to starvation and death of the concerned insect (English and Slatin, 1992). Only a few insect groups have the appropriate mid-gut characteristics and binding sites for a particular Bt Cry protein to be active. For example, Cry1-type proteins control various Lepidoptera, Cry2-type proteins affect certain Lepidoptera and Diptera, and Cry3 proteins control certain Coleoptera (Head, 2005). Such proteins are not toxic to humans, animals or other non-target organisms including parasitoids and predators as they lack the required specific conditions.

In fact, the insecticidal property of Bt was discovered way back in 1901, well before the advent of Bt transgenic technology. Bt formulations consisting of bacteria and Bt proteins in a crystalline form were the choice biopesticides on fruits and vegetables since 1938 all over the world including India. Before such products were approved for registration, the Bt organism and the Bt proteins they produce underwent and satisfied a battery of safety tests.

Thus, Bt proteins have undergone safety tests twice - the first time during registration of Bt formulations and the second time while testing transgenic Bt crops. A large body of scientific literature/data are available globally that demonstrate the safety of Bt proteins to humans and other non-target species as well as to the environment whether expressed in the GM plants or sprayed on crops (McClintock et al., 1995; WHO, 1999; Betz et al., 2000; EPA 2001; Sanahuja et al., 2011). Therefore, any allegations or fears about safety of Bt and Bt-crops have no scientific basis.

Safety of natural enemies

Parasitoids and predators constitute one of the most valuable beneficial fauna in crop systems and, therefore, their safety is given due importance while evaluating Bt crops. This aspect has been reviewed by Head (2005).

There are two potential ways of natural enemies being exposed to Bt proteins: direct feeding on pollen, nectar or other plant tissues of Bt crops, or secondary exposure through feeding on prey species that have themselves fed upon Bt plants.

It has been found that Bt protein expression in Bt crops is highest in actively growing green tissues, lower in older vegetative tissues and reproductive tissues, and lowest or absent in the phloem (Head et al., 2001; Raps et al., 2001). This suggests that regulatory testing should focus upon those natural enemies that opportunistically feed on pollen or vegetative tissues of crops. Furthermore, direct routes of exposure generally lead to relatively greater exposure to the Bt proteins in Bt crops than secondary exposure as the level of Bt protein that is present in herbivores that have fed on Bt plants is far lower than the level of Bt protein present in the plant tissues, presumably because of dilution effects (Head et al., 2001; Dutton et al., 2002). Further, some insects, particularly phloem feeders like aphids, ingest only minimal amounts of Bt protein because little or no Bt protein is present in the parts of the plant where they are feeding (Head et al., 2001; Raps et al., 2001). Thus, predators feeding on these different prey species will be exposed to very little Bt protein. Keeping these in mind, several regulatory tests were carried out with some representative predators and parasitoids.

Testing of predators

The predators tested in the laboratory for currently registered lepidopteran-active Bt proteins (e.g., Cry1Ab,
Cry1F, Cry1Ac and Cry2Ab), as a part of the regulatory packages for Bt crops, have included ladybird beetles (Coleoptera) and the green lacewing, Chrysoperla carnea Stephens (Neuroptera: Chrysopidae). These groups were selected because of their abundance and importance in cropping systems, especially corn and cotton ecosystems (Betz et al., 2000; EPA, 2001).

The species of ladybird beetles selected for studies included the convergent ladybird beetle, Hippodamia convergens Guerin-Meneville (Coleoptera: Coccinellidae) and the pink-spotted ladybird beetle, Coleomegilla maculata De Geer (Coleoptera: Coccinellidae). The other predators tested included Geocoris spp. (Heteroptera: Lygaeidae), Orius insidiosus Say (Heteroptera, Anthocoridae) and the American hoverfly, Eupeodes americanus Wiedemann (Diptera, Syrphidae), which are also abundant in crop systems (Candolfi et al., 2004; Hagerty et al., 2005; Jun-Ce Tian et al., 2015).

These tests have used a variety of designs, with differing degrees of realism in terms of the route and level of Bt exposure. Since it is known that many predators feed on some amount of pollen at some point in their life cycle, many of these studies have involved feeding predatory insect species with pollen from Bt crops and comparable control lines. None of these studies has found any adverse impact of Bt pollen on the survival or development of various insect predators (Pilcher et al., 1997; Al Deeb et al., 2001; Jun-Ce Tian et al., 2015).

However, Hilbeck et al. (1998 a, b; 1999) conducted a number of laboratory studies wherein they fed the predatory lacewing C. carnea with lepidopteran larvae that had fed on Bt corn. They recorded higher mortality and slower development of lacewings than those fed on comparable controls. This example has been exploited by the opponents of Bt technology to allege that Bt crops are detrimental to biological control agents! Subsequent studies by other researchers indicated that these results were due to the predator feeding on nutritionally poorer prey rather than any toxic effect of the Bt protein (Dutton et al., 2002; Romeis et al., 2004). Such a situation should have little relevance to the field because other prey sources that are not affected by Bt crops will be more available and probably preferred under natural conditions (Head, 2005).

Numerous field studies also have focused on generalist predators, particularly C. maculata, C. carnea, O. insidiosus, and guilds of carabids because of their abundance in cornfields and their perceived importance. No adverse effects have been seen for any of these species in these studies or in the broader, community-level studies of Bt corn (Pilcher et al., 1997; Lozzarella, 1999; Candolfi et al., 2004) and Bt cotton (Xia et al., 1999; Hagerty et al., 2005).

In contrast, the insecticidal sprays had clear adverse impact on almost all common predators, particularly those foraging above ground, both in conventional corn crops (Candolfi et al., 2004) and cotton crops (Xia et al., 1999; Hagerty et al., 2005; Head, 2005; Wu and Guo, 2005). Thus, Bt crops go a long way in conserving predators and promoting biological control/integrated pest management.

**Testing of insect parasitoids**

The possibility of direct exposure of adult parasitoids to Bt proteins in fields is very limited. The only route is through feeding on pollen or nectar but, as already mentioned, the amount of Bt protein present therein is very negligible. Egg parasitoids like Trichogramma will not be exposed to Bt protein at all. However, secondary exposure to Bt proteins may occur when the parasitoid larvae feed on their hosts that have already fed on Bt plants. The studies indicated that in such cases, the larval development of the parasitoids may be adversely impacted. For example, when reared on Bt susceptible insects that had fed on Bt corn, the larval development and mortality of the parasitoid, Parallorhogas pyralophagus Marsh (Hymenoptera: Braconidae) was adversely affected, but the fitness of emerging adults was not impacted (Bernal et al., 2002).

Jun-Ce Tian et al. (2015) found no adverse effect on the development of the parasitoid, Aphidius colemani Viereck (Hymenoptera: Braconidae), when reared on the green peach aphid, Myzus persicae (Sulzer) (Hemiptera: Aphididae) that fed on Bt broccoli expressing Cry1Ab or Cry1C. In fact, they found no trace of Bt protein in the aphids, reflecting the host specificity of these proteins.

Overall, as with predatory species, studies conducted have revealed no toxic effects of Cry1, Cry2 or Cry3 proteins against parasitoids for Bt protein concentrations at or much greater than maximum possible exposure under natural conditions (Betz et al., 2000; EPA, 2001).

It was generally observed that the level of parasitoid populations of the target pests found in Bt crops is lower than that found in unsprayed non-Bt crops. For example, the population of Macrocercus cingulum Brischke (Hymenoptera: Braconidae), a parasitoid of European corn borer, was found to be lower in Bt corn than in conventional corn (Candolfi et al., 2004). Similarly, a few specialist parasitoids that parasitize cotton bollworms in cotton have been found to be rarer in Bt cotton than in non-Bt cotton (Xia...
et al., 1999). This again has been cited by the critics of this technology to criticize that Bt crops have suppressed natural enemies. Here, the fact is that since the target pests have been effectively controlled, their numbers have dwindled and, therefore, being host-dependent, the natural enemies, as expected, have moved away from there to other fields where hosts may be available. This would happen with any other control measures. Bt in no way has suppressed them. It is pure commonsense. On the other hand, the insecticidal sprays used in conventional corn (Candolfi et al., 2004) and cotton (Xia et al., 1999; Hagerty et al., 2005; Wu and Guo, 2005) have clear adverse impacts on these same parasitoid species.

Overall, Bt crops like Bt-cotton and Bt-corn truly contribute to conservation of parasitoids and predators and enhance biological control, especially of non-target pests like sucking pests that are not controlled by Bt.

Advantages of Bt crops

Bt-crops like Bt-cotton and Bt-corn offer several advantages (Manjunath, 2011):

- **Bt**-technology is made available in the seed itself. Farmers have to just sow the Bt seeds as they do with conventional seeds. The resulting plants have the in-built ability to produce Bt-proteins within their body (leaf, stem, buds, flower, bolls) and defend themselves against the target pests. No extra efforts or equipment are needed to use this technology.
- **Bt** protein is expressed in all parts of the plant (*i.e.*, constitutive expression). The newly hatched larvae feeding on any part of the plant - leaves, stem, flowers, squares, bolls - will ingest Bt-protein and die within one or two days, thereby preventing any potential serious damage to the crop.
- **Bt** is present within the plant almost throughout its life, providing pest control day and night. Therefore, there is no need to monitor the pest activities to initiate control measures.
- **Bt**-proteins, being host specific, affect only the target pests and are safe to non-target organisms including ladybird beetles, green lacewing and other beneficial predators as well as to hymenopteran and other parasitoids, honeybees, etc.
- Transgenic **Bt** technology is compatible with other control measures such as biological control, pheromones, botanical insecticides as well as chemicals that are recommended for integrated pest management. In fact, this technology can serve as a major component of IPM.
- **Bt**-crops help to avoid or minimize chemical sprays, thus contributing to cleaner environment, better health, and conservation of biological control agents and biodiversity.
- **Bt**-crops offer protection from target pests right from the early days of the crop, leading to a healthy crop, greater harvest and more profit.
- The **Bt**-farmers experience a far lesser tension and are certainly better off than the earlier scenario of ‘spray and pray.’

In other words, **Bt**-crops provide social, economic and environment benefits leading to conservation of biological control agents and biodiversity.

**DISCUSSION AND CONCLUSIONS**

Bio-safety and environmental safety are accorded the highest priority while regulating **Bt** and other Genetically Modified (GM) crops. Every country, including India, has developed stringent biosafety protocols and it has been made mandatory that every GM crop has to pass through all the prescribed tests before they are given regulatory clearance. All the **Bt**-crops that have been commercialized so far have undergone this arduous but necessary process in every country. For example, in India, **Bt**-cotton, prior to its approval in 2002, has undergone over 500 field trials and a large number of biosafety tests for 7-8 years, supervised by over 150 scientists from 9 national research organizations and 13 agricultural universities (Manjunath and Mohan, 2015). The biosafety tests carried out in all the countries have proved that **Bt** proteins are host-specific and are safe to humans and other non-target organisms including biological control agents and the environment.

As of 2017, **Bt** crops, mainly **Bt**-corn and **Bt**-cotton, were cultivated in 14 countries on about 100 m ha (23.3 m ha with **Bt** alone and 77.7 m ha with **Bt** stacked with herbicide tolerance), including 11.4 m ha of **Bt**-cotton in India, which comprised 53% of 189.8 m ha of all GM crops grown by about 17 m farmers in 24 countries (19 developing, 5 industrialized) (ISAAA, 2017). Such extensive cultivation of **Bt** crops has resulted in higher crop yields by 22% owing to effective control of target pests, increased farmers’ profit by 68% and reduced chemical insecticide applications by 37%, thereby providing social, economic, health and environmental benefits. The reduced chemical sprays have contributed to conservation of parasitoids and predators leading to enhanced biological control, especially of sucking and other non-target pests that are not controlled by **Bt**. Feeding tests carried out with ladybird beetles, green lacewing and other beneficial predators as well as with braconid and other hymenopteran species.
parasitoids have demonstrated $Bt$ proteins to be safe to these natural enemies.

No technology, however powerful, can solve all the problems and last forever. Every technology has its own strength and limitations and, therefore, the best way is to make use of them as per suitability as recommended in Integrated Pest Management (IPM). There is no need to be biased towards any technology. $Bt$-technology, being user-friendly, safe to environment and supportive of biological control, and compatible with all other plant protection measures, can be used as a major thrust in IPM. It is very powerful and can match the temporal efficacy of chemical pesticides, thus providing the much-needed strength and stability to IPM.

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