Chapter 7

Transgenic Bt Cotton: Effects on Target and Non-Target Insect Diversity

Muhammad Arshad, Rashad Rasool Khan,
Asad Aslam and Waseem Akbar

Additional information is available at the end of the chapter

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Abstract

Occurrence of diversity in ecosystem sustains particular characteristic of a biological community and also ensures stability of the community. Transgenic crops may affect insect biodiversity by unintended impacts on non-target arthropod population. For example, transgenic GM cotton specific to target lepidopterous pests can change the cotton pest spectrum and may induce the growth of new harmful pest species having no pest status. The change in species composition may influence IPM approach in cotton crop. The results of authors' research studies as well as global impact indicate that GM cotton is highly specific to target pests and has no unintended impact on non-target insect population. GM cotton provides significant season-long field control of target pests (*Helicoverpa armigera*, *Earias* spp. and *Pectinophora gossypiella*), with no significant control of *Spodoptera* species. The decreased insecticide use in GM cotton has a positive impact on beneficial insect populations and can increase the stability of rare species. Bt cotton has no resistance against non-target sucking insect pests. As GM cotton has no adverse effects on the non-target insect population and can reduce the use of broad-spectrum insecticides, it can become an important tool of IPM program in cotton agro-ecosystem of Pakistan.

Keywords: target insects, non-target insects, diversity, GM cotton, Pakistan

1. Introduction

1.1. Transgenic Bt cotton

Cotton plant has been genetically modified to incorporate gene conferring insecticidal protein (Cry1Ac) derived from the naturally occurring soil bacterium *Bacillus thuringiensis* (Bt) var.

Kurstaki. Genes that express the delta-endotoxins are called "cry genes [1]. In lepidopterans, the chewing mouthparts promote the ingestion of Bt toxins and the crystals are solubilized in the midgut having alkaline environment (pH 9 to 12). The crucial step in the activation of crystal proteins is the cleavage of toxins which may vary in different insect species [2]. Larvae stop feeding after Bt toxin ingestion due to the onset of paralysis in midgut, altered permeability and disintegration of the epithelium that leads starvation to death of the insect within 2–3 days after exposure. The larval death may vary depending on insect species, larval age and the amount of toxin ingested [3].

Monsanto developed and commercialized the first insect-resistant transgenic GM cotton expressing Cry1Ac gene (Bollgard® I) in 1996 [4]. GM cotton, the first transgenic non-food crop, has provided a specific, safe and effective tool for the control of lepidopterous pests [5–8] as compared to insecticides (pyrethroids and carbamates) that adversely affect non-target arthropods and other invertebrates [9].

Transgenic Bt cotton has provided an important tool for developing an integrated pest management (IPM) strategy [10, 11], especially for lepidopterous larvae in cotton [12–15]. GM cotton expressing Cry genes is cultivated on 33.1 million ha in different cotton growing countries including United States [16, 17], China [18–20], India [21–26], South Africa [27–29], Mexico [30], Argentina [31, 32] and Pakistan [33–43] and experienced many benefits like reduced use of broad-spectrum insecticides, improved control of target pests, reduced production cost, increased yield and better opportunity for biological control.

The targeted pests have developed the resistance against Bollgard I in most of the countries. To overcome this issue, Monsanto has released Bt cotton containing two Bt genes Cry1Ac and Cry2Ab (Bollgard II). However, there are some other alternative means to minimize the development of resistance in target pests including: a) planting of refuge crop that does not contain Bt based product for susceptible target insect pests, b) consistent and high level of expression of Bt proteins in all plant structures, c) monitoring for shift in baseline susceptibility of target pests to Bt based products, d) use of other IPM control strategies (sowing time, new chemistry insecticides etc.) [43].

1.2. Global status of GM cotton

It is estimated that there is a rapid adoption of GM crops globally (up to 30 countries), and almost 18 million farmers have been grown these crops on more than 2 billion ha. GM crops have reflected substantial economic, health, environmental and social benefits to farmers by increasing crop productivity and conserving biodiversity [44].

GM cotton is being planted in USA since 1996 and it is estimated that 93% of total cotton area (3.98 million ha) is under cultivation of Biotech cotton. Biotech cotton is the third most important GM crop in Brazil and estimated to occupy 1.01 million ha in 2016/17. In India, farmers increased the cotton productivity by planting GM cotton on 11.2 million ha representing 96% of cotton area. Paraguay approved GM Cotton in 2011 for commercial production, and keeping in view the benefit of this technology, about 12,000 ha was planted up to 2015–2016. In Pakistan GM cotton is being cultivated on 2.9 million ha (97%) of the total 3 million ha of cotton area [44].

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1.3. Status of GM cotton in Pakistan

Adoption of Bt-cotton in Pakistan was not fast than that of the other major cotton growing countries. The cultivation of Bt-cotton in Pakistan started upon the release of Bt-cotton candidate lines (IR-NIBGE-2, IR-FH-901, IR-CIM-443 and IR-CIM-448, developed by NIBGE Faisalabad) in 2003–2004 for testing their performance in various localities of Pakistan. Later on these varieties started capturing area each year. In 2005–2006, area under these varieties was 0.20 million ha, of which 0.093 million ha was in the cotton belt of Punjab Province [45]. In 2009, Ministry of Food and Agriculture made a positive development for the introduction of Bt cotton varieties in the country to maximize cotton production and for this purpose a letter of intent was signed with Monsanto company, but process was delayed. During the meantime, these cotton varieties including IR-NIBGE-2 (approved as IR-NIBGE-1524), IR-FH-901 (approved as IR-NIBGE-901), IR-CIM-448 (approved as IR-NIBGE-3701) and Bt-121 acquired >40% of the total cultivated area of cotton in both the province (Sindh and Punjab). Later on these cotton varieties along with some new varieties were approved by the Punjab Seed Council (PSC) on March 31, 2010 to counteract the cultivation of adulterated and unapproved Bt cotton seed (**Table 1**).

Later on some more Bt cotton varieties were approved for commercialization but all these varieties contain a single Cry1Ac toxic gene. In 2014, Bt cotton was grown an area of 2.9 million ha indicating an adoption rate of 88% in the country. Of the approved 32 Bt cotton varieties, half were developed by private seed companies and half by public sector research institutes. It was estimated that about 700,000 resource poor and small farmers were benefited from Bt cotton cultivation. The economic benefits achieved from Bt cotton cultivation was US$1615 million for 2010–2013 [46]. However, the productivity of cotton in Pakistan is low (0.5 tons/ha) as compared to other Bt cotton growing countries. The agricultural productivity can be enhanced by increased adoption of Bt cotton, which would considerably

Sr. #	Variety/lines	Center of release	Year of cultivation and approval
1	IR-NIBGE-3701	NIBGE Faisalabad, Pakistan	Released for testing at farmer fields in 2003–2004 but approved in 2010 for Punjab, and in 2011 for Sindh
2	IR-NIBGE-901	NIBGE Faisalabad, Pakistan	Released for testing at farmer fields in 2003–2004 but approved in 2011 for Sindh
3	NS-121	Neelum Seed, Multan, Pakistan	Released in 2006, approved in 2010
4	MNH-886	Cotton Research Institute, Multan, Pakistan	Approved in 2012
5	FH-142	Cotton Research Institute, AARI Faisalabad, Pakistan	Approved in 2013
6	IUB-2013	Islamia University Bahawalpur, Pakistan	Approved in 2014

Table 1. The most popular Bt-cotton varieties (covered at least 10% area in any province) of Pakistan.

reduce insecticide applications, better quality of cotton, increased farm income, less exposure of insecticides to farmers and farm laborers and ultimate impact on food security efforts in the country.

1.4. GM cotton and insecticide use

Farmers rely heavily on the use of insecticides to control insect pests in cotton crop [47, 48]. This dependence on insecticides escalated the production cost. GM cotton containing Bt genes resulted in reduced application of pesticides for controlling the insect pests [8, 21, 49–51]. Insecticide application in Bt cotton has reduced up to 14 applications in China [52], 5-6 in Australia [53], 7 in South Africa [54] and 2.5 in India [55]. The introduction of Bt cotton in Southeast Asia significantly reduced the insecticide applications by 72%, increased yield of 11.4% and an estimated profit of US $126.02/ha [56]. The reduced insecticide use may increase the predator abundance and can affect the arthropod communities overall in Bt cotton field [57–61].

2. Diversity of insects on cotton crop

Cotton crop hosts a rich diversity of insect pests, predators and parasitoids. About 145 insect and mite pests have been reported in the cotton crop in Pakistan [97]. Cotton insect pests cause 35–40% yield loss [62]. The insect pest complex on cotton is divided into two groups: chewing insect pests and sucking insect pests. Among the chewing insect pests, cotton bollworm complex (*Helicoverpa armigera* (Hubner), *Pectinophora gossypiella* (Saunders) and *Earias Spp.*) are the most destructive ones in Pakistan and causes 30–40% yield reduction [63], because of damage to flowers, squares and bolls [64, 65]. Among sucking insect pests i.e., whitefly, jassid, thrips, aphid and cotton mealy bug are important [66, 67].

Farmers consider insecticides as a main sole to manage the insect pests in cotton crop. Most of the insecticides used, are broad-spectrum, which disturb the insect biodiversity, damage the beneficial insect fauna, hazardous to human health and environment, as well as leading to insect pests resurgence and outbreaks of secondary pests [68]. The insecticide application to cotton crop is the most intensive and the crop is to be considered as the largest insecticide consumer throughout the world [69]. It is estimated that in Pakistan, farmers spend US$300 million on pesticides annually, of which more than 80% is used on cotton, especially for bollworms.

2.1. Impact of GM cotton on target insect pests

Among the target insect pests of Bt cotton *Helicoverpa armigera* Hubner, *Earias* spp., *Spodoptera* spp. (Lepidoptera: Noctuidae), *Pectinophora gossypiella* Saunder (Lepidoptera: Gelechidae) and *Spodoptera* spp. are more serious pests of cotton in Pakistan. They damage the cotton plant by feeding on squares, flowers and bolls and in severe damage caused significant yield reduction [70].

2.1.1. Cotton bollworm (Helicoverpa Armigera)

Commonly known as cotton bollworm (CBW) is one of the damaging pests of cotton and many other field crops worldwide [8, 71–73]. In India, this pest causes an estimate crop loss of US $350 million annually and farmers have to spray 15–20 times. Farmers in Pakistan also rely heavily on the use of chemical to control this pest and this indiscriminate use of insecticide particularly pyrethroids has developed resistance in this pest against insecticides [74, 75].

Our research studies have shown that transgenic Bt cotton offers great potential to significantly reduce the pesticide application for the control of major lepidopterous pest, *H. armigera* in Pakistan. The bollworm larval densities in Bt cotton remained below the threshold level; hence, no insecticide application is needed in Bt cotton. The results have shown no ovipositional differences between Bt and conventional cotton, as female moths cannot differentiate between Bt and non-Bt cotton for oviposition [76].

Transgenic Bt cotton varieties have lethal effect against *H. armigera* [77–81] and proved to be very effective in controlling this pest, causing 80–90% mortality in Australia [70], more than 90% in China [82] and 40–50% in India [83]. However, some studies have showed inadequate control of *H. armigera* with Bt cotton [84]. Some studies have showed no oviposition difference of *H. armigera* between transgenic Bt and non-Bt cotton [85, 86]. While, other reported greater number of eggs in Bt cotton than conventional cotton because of better leaf canopy due to lower damage [48]. It is also observed that there is a variation in Bt cotton resistance throughout the growing season and has shown the higher resistance to *H. armigera* at the last 10 days of May (94.5%) and July (83.3%), which decreased in August (22.7%) [84]. Similarly, some other field research studies conducted in Pakistan [87] and somewhere else [88–91] have showed significantly lower population of *H. armigera* in Bt cotton as compared to non-Bt cotton.

2.1.2. Pink bollworm (Pectinophora gossypiella)

It is the most important pest throughout the world, wherever the cotton is grown [92, 93] and almost difficult to control this pest because of its cryptic feeding habit. Bt cotton containing Cry1Ac can effectively control this pest [94–96]. Our research results indicated a lower density of rosette flowers and larvae in Bt cotton as compared to conventional cotton [97]. The study indicated that some larvae survived in Bt cotton, late in the season (end of September and October). It may be due to the decreased Bt toxin expression at lateral stage of plant [98]. However, it is admired that Bt cotton effectively suppressed the larval density in early season to an extent that pest could not cause an economic damage in the late season. Our results and those of other investigators support the efficacy of Bt cotton for pink bollworm control [99–101].

2.1.3. Spotted bollworm (Earias spp.)

It is an important pest of cotton in Indo-Pak subcontinent and cause damage to fruiting bodies and shedding of squares, flowers and bolls [102, 103]. Although, the primary target of transgenic Bt cotton is to control cotton bollworm, *H. armigera* but it also has a significant impact on other bollworm species, including *Earias insulana* & *E. vittella*. It occurs as an early to mid-season pest in cotton and hence transgenic Bt cotton can effectively control this pest during early-mid phase of the crop, when toxin expression is high. Baseline susceptibility data has shown that Cry1Ac was highly toxic to spotted bollworm with LC_{50} ranged from 0.006 to 0.105 μg/ml of diet and 0.88 ng/cm^2 for leaf-dip bioassays [104].

Bt cotton containing Cry1Ac proved to be effective against this pest and significantly control the larval population [78, 105, 106]. Another research study conducted in Pakistan investigated the infestation trend of spotted bollworm in different plant parts of transgenic Bt and conventional cotton cultivars and reported minimum infestation of 3.36% in transgenic variety, "IR-FH-901" as compared to conventional variety, "FH-900" with 10.5% infestation [65].

2.1.4. Armyworm (Spodoptera spp.)

Commonly known as beet armyworm and fall armyworm is a multivoltine, polyphagous pest and can cause significant damage to cotton crop in case of severe infestation. Bt cotton with Cry1Ac proved not to be effective against armyworm, *Spodoptera* spp. [65, 105, 107–110]; hence, no significant differences in larval density between Bt and non-Bt conventional cotton [111, 112] and insecticide applications are needed to control this pest in Bt cotton. In Pakistan Bt cotton varieties proved to be less affective against armyworm and less mortality (13.3–53.3%) noted on different Bt cotton varieties containing CriAc. Some other field studies have shown that there were no significant differences in larval density among Bt and non-Bt cotton [112, 113]. As Bt cotton varieties expressing single toxin gene (Cry1Ac) have no resistance against armyworm, *Spodoptera* species, to overcome this problem a Bollgard® II cotton was developed that contain Cry1Ac and Cry2Ab, which provide the adequate control of armyworm and cotton bollworms [114–123].

2.2. Impact of GM cotton on non-target insect pests

The potential impact of GM crops on non- target organisms is a strategic concern among farmers, policy makers and scientist working on the development of GM crops as an ideal pest control tactic. Non-target organisms include all organisms except for the pest to be controlled. Examples of non-target organisms would be birds, reptiles, mammals, fish and other insects. A number of studies have shown that Bt toxin is highly selective and has no adverse effects on non-target insect fauna in cotton [124–127].

2.2.1. Impact of GM cotton on non-target major sucking insect pests

Among the non-target, sucking insect pests of GM cotton, whitefly, jassid, thrips, aphid and cotton mealy bug are the most important in Pakistan. These are very destructive pests during seedling and vegetative phase of cotton as they suck the sap of the plant, make it weak and in case of severe infestation wilting and shedding of leaves occur.

The field research study indicated that transgenic Bt cotton proved to be very effective against certain chewing lepidopterous pests and reduced the insecticide applications [37]. But at the same time, non-target sucking insect pests may become the significant insect pests, because the reduced use of insecticides in Bt cotton can increase the sucking insect

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pest complex [90]. Most of the research studies have showed the higher population of sucking insect pests mainly; jassid, whitefly, aphid and thrips in transgenic Bt cotton [85]. Some other research studies conducted in Pakistan [63] and India [48] have found no significant differences in sucking insect pests; whitefly, jassid and thrips population among transgenic Bt and non-Bt cotton. As Bt cotton has no resistance against sucking insect pests and requires continuous use of pesticides and other control tactics for effective management of these insect pests [84, 105, 128].

Seed treatment provided the better protection against early-season sucking pests in transgenic cotton. As, there is no direct impact of Bt toxin on the non-target insect species but the ingestion of Bt toxin may prolong the development time during which herbivores are more exposed to parasitoids and predators [129]. It is suggested that Bt cotton along with pesticide applications could provide protection against target and non-target insect pests. But for the long term implementation of Bt cotton as a component of IPM, it is important that such varieties should be transformed with Bt genes that have also the resistance against non-target sucking pests to reduce the number of pesticide applications.

2.2.2. Impact of GM cotton on non-target natural enemies

Cotton crop hosts a rich diversity of insect predators and parasitoids, which have the significant role in regulating the pest population [130, 131]. Most of the field studies have shown no significant effects of Bt crops on natural enemies [40, 42, 60, 124, 129, 132–134]. Some reported the reduced activity of parasitoids in Bt cotton due to the absence of hosts or direct toxic effects of Bt toxin [86, 135, 136].

Bt cotton may act as a refuge for insect predators and spiders in large scale cotton production, where non-Bt cotton may be sprayed with insecticides [58]. Although Bt cotton is effective against target pests and have no direct influence on natural enemies [80] but there are the options that natural enemy population may be indirectly influenced by the behavioral change of non-target organisms or by the removal of their prey/hosts [124, 126, 137]. Some laboratory studies have reported indirect effects on natural enemies' population through unhealthy prey/hosts but at the same time population may be increased because of increased parasitism of unhealthy prey/host due to Bt toxin [124, 137–140].

Bt cotton can affect natural enemies in field by the removal of eggs, larvae and pupae of lepidopterous pests that serves as food sources [91]. Some studies showed the adverse effects of Bt toxin on the survival and development of some predators [109]. It may be due to the ingestion of Bt toxin during feeding on lepidopterous larvae or may be due to the consumption of intoxicated non-target prey that may pick up the Bt toxin [141]. While, most of the studies experienced no effect of Bt toxin on a main predator, *Chrysoperla carnea* [142] and reported no significant difference in abundance of insect predators between unsprayed Bt and non-Bt cotton fields [143]. The reduced insecticide use in Bt cotton can increase the predaceous arthropod population [144]. Some other field studies reported no significant difference of natural enemy populations between Bt and non-Bt cotton fields and where the differences were present, natural enemy populations were significantly higher in Bt than non-Bt cotton, mainly due to lower insecticide use in Bt cotton fields [145].

2.2.3. Impact of GM cotton on the overall abundance and insect diversity

Bt cotton can alters the insect diversity especially predators and parasitoids by reducing the abundance of *Helicoverpa* spp. and some other lepidopterous species [146–148]. A little numerical difference was found in the overall abundance and diversity of insect community in unsprayed Bt and conventional cotton fields [149], but another field study showed that species richness and total abundance reduced by 2.4–16.3 and 71.0–78.3%, respectively in Bt than non-Bt cotton fields [150]. Similarly, a three-year field studies have revealed no significant differences in species richness, evenness and diversity between unsprayed Bt and non Bt cotton, but plots receiving insecticides have slightly higher evenness.

The reduced insecticide use in Bt cotton may increase the minor insect pests' community, which are suppressed under intense insecticide applications [92]. The mirid bugs, which were minor insect pests in northern China, now have attained the status of main pests and population has increased 12-folds mainly due to the Bt cotton cultivation on large scale [151].

However, Shannon's index for total arthropod community and the neutral arthropod guild found significantly higher in Bt cotton fields than those in non-Bt cotton [152]. A comparison of Shannon-Weaver diversity indices in Bt and non-Bt cotton under sprayed and unsprayed conditions revealed that Bt cotton increased the diversity of arthropod communities and pest subcommunities; however, it decreased the diversity of natural enemy sub-communities [153]. A comparison of canopy and ground dwelling arthropod community revealed no significant difference in the abundance of total insect community between unsprayed Bt and non-Bt cotton [134]. In addition, the relative greater abundance of honey bees; *Apis mellifera*, *A. cerana*, *A. dorsata* and other pollinators in Bt than non-Bt cotton, indicate that Bt cotton may be a good source of nectar and pollen for insect pollinators [152]. Similarly, some other field studies have revealed that Bt cotton increased the stability of insect community, pest and natural enemy sub-communities and found no significant effects on the non-target insect diversity [154, 155].

3. Conclusions

A plenty of insects inhabit the cotton crop, including the target and non-target insects. Transgenic Bt cotton has resistance against major target insect pests; *H. armigera, Earias* spp. & *P. gossypiella* and significantly reduce the insecticide applications. This reduction in pesticide use has a positive impact on natural enemies and increased the stability of beneficial rare species. Bt cotton varieties with Cry1Ac toxin are ineffective against armyworm, *Spodoptera* spp. However, some inhibitory effects of Bt toxin on the growth of armyworm larvae are observed but there is a chance that this pest may become the major and alarming pest in Bt cotton field in Pakistan. Bt cotton has no resistance against sucking insect pests; jassid, whitefly, thrips, aphid & mealybug and insecticides are used to control these pests. To increase the stability of Bt based products as an important tool of IPM in cotton, it is crucial that such varieties should be transformed with *Bt* toxin genes, which also have other resistance traits against non-target insect pests to reduce the number of insecticide applications. There is also need to re-determine the economic threshold levels for sucking pests and bollworms in Bt cotton due to increased beneficial abundance and the change of pest status. The biotechnological efforts, in developing the transgenic Bt cotton varieties, should also focus on the sustainable temporal and intra-plant expression of Cry1Ac toxin in all plant parts.

Author details

Muhammad Arshad[1]*, Rashad Rasool Khan[1], Asad Aslam[1] and Waseem Akbar[2]

*Address all correspondence to: arshaduaf@gmail.com

1 Department of Entomology, University of Agriculture Faisalabad, Pakistan

2 Plant Protection Division, Nuclear Institute of Agriculture, Tando Jam, Hyderabad, Pakistan

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