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Genetically modified (GM) cotton: Its past, present and future: A review

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Abstract
A marked increase in global agricultural productivity and reduced inputs in terms of pesticide consumption since past two decades has become possible due to new biotechnological approaches like molecular genetic modification (GM crops) in agriculture. However, the future increases are hardly assured due to underinvestment in agricultural research, grown up population pressure and increased levels of resistance development in the insect-pests to the transgenic cotton. In this article, the triumph of Bt cotton after its introduction in 1996, the hurdles it encounters in terms of resistance problems to insects and secondary pest outbreaks; the possible strategies and future researchable areas for its sustainable cultivation have been discussed.

Keywords: Genetically modified cotton, past, present and future

1. Introduction
India is world's fifth largest country to have cultivated area under genetically modified (GM) crops, at 11.4 million hectares in 2017 [5]. But unlike other big growers, its entire GM crop area is under a single crop i.e., cotton incorporating genes from the *Bacillus thuringiensis* (Bt) a soil bacterium coding for resistance against *Heliothis* bollworms [5]. Several crop modifications achieved transgenic methods now being used worldwide. Perhaps the best known of these are crop plants into which a gene from Bt, a bacterium that has long been used as a biological pesticide because it produces a protein that is toxic to the larvae of certain kinds of insects. Among various crops, cotton is one such crop which enjoys the benefits of genetic engineering since the introduction of *Bt* gene. Insecticidal action of the bacterium was first noted by a Japanese scientist Ishiwata in the year 1901 [110]. The Bt was just a beginning at that time in USA. Since 1950s, the protein crystals and spores of several Bt strains have been used as microbial insecticides [20]. The Bt has been used as an insecticide for control of stored grain pests since 1938 in France and from 1961 as a registered pesticide in the USA. Silent Spring (1962), a book authored by Rachel Carson was also in support of products from *B. thuringiensis* that can be used as a bio-control, alternative to the chemical insecticides for killing various insect-pests.
About 162 insect species have been reported as the pests of cotton in India [21], of which 4-5 are emerging/invasive pests in the recent decade. Endotoxins produced by Cry genes of *B. thuringiensis* were toxic to larvae of certain lepidopteran and coleopteran insects [49]. The chewing type mouthparts in lepidopteran insects promote the ingestion of Bt toxins that solubilise the crystals in the midgut with alkaline pH 9 to 12. Activation of crystal proteins involves cleavage of toxins that vary from species to species of the insect [72]. As the insect larvae ingest Bt toxin it leads to stop them feed further and a long starvation causes their midgut paralysis, altered permeability and disintegrated epithelium with ultimate insect death within 2-3 days after exposure that may vary with the insect species, age of the larvae and the content of toxin being ingested [30]. In view to the toxic effect of Bt protein, the genetically modified (GM) insect-resistant cotton (*Gossypium hirsutum* Linn.) was first commercially developed by Monsanto in 1996 using cry gene of *B. thuringiensis* bacterium [75]. Transgenic or the Bt crops expressing the insecticidal proteins of Bt have been commercially available in the U.S. since 1996 and their adoption continues to expand rapidly in the United States and other parts of the world [45]. Since the commercialization of Bt cotton several different Cry genes from various bacterium strains have been identified. The cry genes- *Cry1*, *Cry2*, *Cry9* and *Cry13* proteins are effective against lepidopterous pests, *Cry3*, *Cry7* and *Cry8* proteins against coleopterans, while *Cry4*, *Cry10* and *Cry11* proteins affect the dipterous pests. Besides

cotton, Bt genes have been transferred to a large number of plant species including as cotton, potato, rice, eggplant, oilseed rape, chickpea and so on [112].

The transgenic cotton has played as an important tool in developing an IPM strategy [56, 61, 105], especially for lepidopteran larvae [57, 91, 92, 104]. Major Bt cotton growing nations are the USA [58], China [99], India [50], South Africa [38], Mexico [93], Argentina [76] and Pakistan [8] and experienced several benefits like reduced broad-spectrum insecticides, improved target pest control, reduced input cost, enhanced yield and good biological control. However, the targeted pests became resistant to Bollgard I (BG I) in several nations and to overcome this problem, Monsanto again released Bt cotton with double genes- Cry1Ac and Cry2Ab (Bollgard II).

2. Transgenic cotton: A global status

The transgenic cotton was cultivated over 11.4 and 189.8 million hectare area in 2017, respectively in India and the World [40]. GM cotton is the first transgenic non-food crop that has provided a specific, safe and effective tool against lepidopterous pests [15, 47, 62, 82] over chemical insecticides which have adverse effects on the natural fauna, pollinators and other non target invertebrates [47]. Cotton plant (Bollgard I) was genetically modified to incorporate gene conferring Cry1Ac protein (containing delta-endotoxins) derived from a natural soil bacterium *B. thuringiensis* (Bt) var. kurstaki [63]. A rapid adoption of GM crops has been reported globally (up to 26 countries), and more than 18 million farmers have grown these crops on more than 1.9 billion hectares. This crop has reflected substantial economic, health, environmental and social benefits to the growers by increasing their crop productivity and conserving the biodiversity [40]. In 2011, Paraguay approved GM cotton for the commercial production and planted about 12,000 hectares in 2015–2016. In India, cotton productivity increased through transgenic crop on 11.2 million hectares (96% of total cotton area). However, in Pakistan it was cultivated over 2.9 million hectares (97% of total 3 mha cotton area) [40]. In USA, Bt cotton is being planted since 1996 and it is estimated that 96 per cent of total cotton area (4.78 million ha) is under cultivation of Bt cotton. Brazil cultivated it as the third most major crop on an area of 1.01 million ha in 2017 [111].

3. First generation bt cotton (Bollgard-I)

The first transgenic cotton (BG I) containing lepidopteran pest resistant gene Cry1Ac was approved for commercial utilization in Australia and the USA in 1996, and in India it was commercially approved by GEAC on 26th March 2002.This was the beginning of a new era in cotton cultivation and since then plenty of Bt cotton hybrids from private sector have been approved every year by GEAC. Comparison of Bt vs non Bt has been done in several studies and reported that Bt cotton has been highly effective in controlling bollworms. There were lower (95%) rosette blooms reported on the transgenic lines - 62 Bt and 65 Bt than on the non-transgenic lines [24, 101]. The active larvae of pink bollworm (PBW), *Pectinophora gossypiella* Sunders recovered from incubated bolls and per cent seed damage was also reduced from 97 to 99 per cent in the transgenic over the non-transgenic lines. Benedict *et al.* (1996) reported natural bollworm incidence where there were less larvae (198 larvae/60 plants) on Bt cotton lines containing either Cry1Ac or Cry1Ab. Also there were low flower bud and square injury (20.60 and 11.77%, respectively) in such Bt lines. They reported 1460 kg/ha average yield in all Bt cotton lines over non Bt Coker 312 (1050 kg/ha). Harris *et al.* [35] in their studies revealed transgenic cotton to provide an excellent control of bollworm (BW), *Helicoverpa zea* in Mississippi during 1992-95. Bachelar *et al.* [10] compared the performance of Bollgard® cotton, NuCOTN 33B and other commercial conventionally protected (with pyrethroids) varieties for boll damage and revealed Bollgard® cotton to sustain about 50 per cent less damage by BW (2.30%) over the non Bt conventional cotton and pyrethroid protected conventional cotton (4.62%). Almost similar observations were recorded by Wier *et al.* [100] for Bt cotton over non Bt cotton during 1995, 1996 and 1997 respectively, in Mississippi state. In a field experiment, the non pyrethroid treated Bt cotton sustained a yield loss of 6.6 to 31.7 per cent over pyrethroid treated Bt cotton cultivars [68]. The insects were collected from Bt cotton fields (with Cry1Ac) over 44 generations and it was observed that in comparison with a susceptible strain, the larval resistance of the Bt-selected populations to Cry1Ac toxin was increased 106-fold [46].

Two Bt cotton varieties (DP 33B and DP 35B) in Mexico provided good control of BW over the conventional variety (DP-5690) and in addition formers needed one less insecticide spray than the non-Bt cotton [17]. In efficacy of Bt cotton to PBW, the live PBW larvae and exit holes were found only in DP-50 and not in Bt cotton lines (MONS-1 and MONS-2), however no significant difference was there among varieties in number of PBW hits on carpel wall. Also the lepidopteron pressure during the season was low and therefore no significant differences in yield were seen among the varieties [83]. Henneberry *et al.* [37] revealed per cent bolls with larval entrance holes in the carpel walls with no difference in Bt (80 to 100%) and non-Bt cotton (65 to 100%). Per cent cotton bolls with live PBW larvae were less in Bt cotton (70%). Moreover, the Bt cotton bolls expressed higher resistance to PBW infestation up to 180 days (second fruiting cycle) showing reduced content of Bt toxin protein in the later stages of crop growth.

Henneberry and Jech [36] reported average entrance holes fed by PBW as 19.6 and 14.4 per boll on Bt cotton variety- NuCOTN33B and non-Bt cotton variety- DPL 5415, respectively with artificial infestation. They also reported that there were no larval exit holes found in NuCOTN33B but 1.8 exit holes per boll in DPL 5415. The infestation by this insect was 17.2 and 10.5 per cent in open mature and immature green bolls, respectively on DPL 5415, and 0.0 and 1.7 per cent, respectively, on NuCOTN33B. Similarly, the larval survival was reported as 21.5 per cent in DPL 5415 but <0.1 per cent in NuCOTN33B. Extremely low larval survival in NuCOTN33B from >11000 entrance holes indicated a high level of PBW susceptibility to this variety. Wu *et al.* [102] reported two Bt cotton cultivars- GK-1 and GK12 as highly resistant to ABW throughout cotton season. There control efficacy of GK-1 and GK-12 was 88.71-95.45, 92.75-97.65 and 93.33 per cent to the second, third and fourth generation ABW, respectively. A damage rate of 6.53 per cent to cotton squares was recorded to the Bt cotton cultivars with a yield increase of 97.05-393.77 per cent. The maximum damage by first and second instar ABW larvae was to the squares (41.91%) and flowers (45.16%), however there were no significant differences noticed in feeding behaviour of ABW on Bt-cotton and non Bt crop.

4. Second generation bt cotton (BG II)

The first generation Bt cotton (BG I) could not provide a complete crop safety during entire crop season from BW population which build up in the later stages of the crop. It contained single gene (Cry1Ac) that was highly resistant to the major insect pests- American bollworm, ABW (*Helicoverpa armigera* Hubner), spotted bollworms, SBW (*Earias vittella* Fabricius, *E. insulana* Biosdual and pink bollworm, PBW (*Pectinophora gossypiella* Saunders) in India but susceptible to other lepidopteran pests. The resistance will also develop, if the target lepidopterans regularly feed upon Cry1Ac Bt toxin for years. Keeping in view this, second generation Bt cotton (BG II) that contained double genes (Cry1Ac+Cry2Ab) was introduced which also provided enhanced mortality of tobacco budworm (*Spodoptera litura* Fabricius), fall armyworm (*Spodoptera frugiperda* J.E. Smith), beet armyworm (*Spodoptera exigua* Hubner), cabbage looper (*Trichoplusia ni* Hubner) and soybean looper (*Pseudoplusia includens* Walker) in addition to the BW complex [85]. Two types of adaptive responses - reducing the selective advantage of the resistance allele or modifying the mating system so fewer resistance alleles are passed on to future generations [2].

Bt technology has become the most convenient tool of insect management in case of cotton crop. The background of BG II in terms of its bio-efficacy and variability in Cry protein expression, and the dynamics of bollworms and *S. litura* were considered in priority as insecticide resistance management (IRM) tool in India. Since 2007 plenty of BG-II Bt hybrids expressing dual genes have been under cultivation. The knowledge on exact advantage of these hybrids over BG-I hybrids as well as non-Bt cultivars in terms of avoidable sprays, horizon of bio-efficacy and IGR action with respect to Indian Bt hybrids was scanty.

The initial evaluation reports of Bollgard II in Australia showed variable expression of Cry genes not only for a single gene but also for double genes-a pyramid case. The Bollgard II (Cry2Ab) gave more gene expression in seed (43.9 µg/g), leaves (23.8 µg/g), whole plant (8.87 µg/g) and pollen (<0.25 µg/g) during 1998. This gene was absent in Ingard while trends in cry1Ac were equal both in Ingard and Bollgard II with slightly more concentration in the later one. Further, the concentration of Cry2Ab protein in leaf samples from Bollgard II at different sampling dates in the season was maximum at 55 DAS in 1998 (40 µg/g) and 1999 (14.3 µg/g). However, such expression reached to 16 or 11 µg/g of tissue by 108 DAS [4].

Bt cotton was planted on 24.1 million hectares in 2017, which shows a decrease by 8% from 2016. The 8% increase in total Bt cotton area globally was mainly due to an improved global market value and the high adoption rate of insect resistant cotton in 2017 [3].

In evaluation of BGs I and II performance with artificial BW infestation, Gore *et al.* (2002) revealed that BW larvae injured a total of 6.4 fruiting forms per 10 plants in BG II, 11.5 fruiting forms on BG I while 25.0 fruiting forms on non-Bt cotton. The mean fruiting forms injury was 0.8 per BW larvae in BG II, 3.5 in BG I and 6.6 in non-Bt cotton. Chitkowski *et al.* [19] also evaluated the efficacy of BG II (Cry1Ac+Cry2Ab genes) against BW and revealed that there were large number of *H. armigera* and soybean looper, *Pseudoplusia* larvae in BG II cotton but it did not reach the treatment threshold of 3/ 100 plants on any of the sampling dates. In contrary, it was on 1 or 2 sampling dates in BG I cotton (Cry1Ac) and several times in conventional cultivar. There were negligible fruiting bodies damaged by corn earworm (*Helicoverpa zea* Boddie) in BG II, whereas Bollgard I had 4.3 per 3 m of row. In overall, the damaged fruiting bodies in BG were higher than in BG II on all the sampling dates, but there were significant differences noticed in only one location. On the contrary, damage to fruiting bodies in conventional cotton was significantly higher than in either BG I or II.

The untreated conventional, BG I and II cotton cultivars had same Heliothine egg deposition on the terminal portions and terminals with heliothine eggs varied from 8.1 to 8.7 per cent in conventional and Bollgard II cultivars, respectively. But, as with larval ratings, the per cent squares suffering BW damage low less in BG II than in BG I. Similarly, the per cent reduction in BW infested squares for BG I and II lines was 10-fold and 19-fold, respectively, while per cent squares sustaining damage were reduced by 6 fold by B I and 16-fold by BG II genotypes over the conventional cotton varieties [41]. Strickland and Annells [86] revealed excellent efficacy of BG II to the lepidopteran pests based on very less larvae over the conventional and INGARD cotton. The lepidopteran pest surviving in BG II was low of the cluster caterpillar, *S. litura*, which might be due to the poor control by Bt proteins. BG II gave higher yield (5.44 bales/ha) than conventional (1.60 bales/ha) and INGARD (4.83 bales/ha) using similar insecticides. Udikeri [96] found new generation Bt cotton MRC-7201 (BG-II) with Cry1Ac+Cry2Ab genes to be best against BWs. The incidence of *H. armigera* was 0.13 larva/plant in MRC-7201 (BG-II) with 5.05 per cent damage which was on par with MRC-6322 Bt that revealed BG-II as superior than all BG-I cultivars under unprotected conditions. Bheemanna *et al.* [16] reported no larval population of *H. armigera* on BG II (MRC-7201) over the BG-I (MRC-6322) that had 0.12 and 0.19 larvae/plant in the later season. Similarly, BG-II had no *S. litura* larvae, whereas BG-I recorded 4.34 and 5.41 larvae per meter row in both the locations. Also no defoliation was recorded in BG II due to *S. litura*, but it ranged from 20 to 35 per cent in BG-I and non Bt.

5. Present scenario of Bt cotton

In case of cotton crop usually the growers rely heavily on the use of chemical insecticides to control various pests on cotton crop [66, 81]. Their mere dependence on the insecticides has escalated the production cost in cotton. But the Bt cotton resulted in lesser applications of insecticides to control various insect pests [13, 18, 47, 55, 76]. Bt cotton is adopted by Indian farmers with such an enthusiasm that by 2012 there were 1128 Bt cotton hybrids available in the market. The insecticide applications were reduced up to 7 numbers in South Africa [43], 14 in China [74], 5-6 in Australia [29] and 2.5 in India [12] for controlling different pests on Bt cotton. In Southeast Asia, the Bt cotton significantly reduced the insecticide usage by 72 per cent thereby increasing the yield up to 11.4 per cent with estimated profit of US $126.02/ha [39]. Also the reduced insecticide use has increased the predatory fauna and affected the arthropod population in Bt cotton field [34]. At present, the transgenic crops are currently grown in 28 countries and adoption is predicted to continue its steady growth [40]. Although there is high dependency on Bt hybrids for cotton cultivation but there are few concerns emerged with adoption of Bt technology which are highlighted below.

6. Resistance breakdown, possible causes and future research

The most important strategy for delaying the development of resistance to Bt crops is to increases the chances of survival of susceptible insects on ''refuges'' of same crop plants that do not produce Bt toxins [33, 87, 88, 97]. The primary resistance mitigation measure for Bt crops has been the use of refuges to provide a source of large numbers of Bt-susceptible insects to counter any resistant insects and the IRM refuge strategy has largely been successful in delaying insect resistance [99]. However, an increase in area under Bt cotton and lack of proper regulations for growing refugia and IRM strategies leads to problems like development of resistant pest populations in cotton. The Bt cotton hybrids in India exhibits traits in hemizygous form as compared to Bt cotton varieties planted in the United States, China and Australia. There is great variation in expression of Cry1Ac in boll tissues ranged between 1.25 to 10.00 μg g^{-1} (dry weight basis) in India [51, 71] as compared to 5 μg g^{-1} in boll rinds of Bt cotton varieties in United States and Australia. Keeping all this in view United States started large scale laboratory studies in United states in 1997-98 to check the resistance of Bt Cry1Ac to Pink boll worm and their field collections yielded a highly resistant strain of PBW to Cry1Ac [70]. In China the Bt cotton (Cry1Ac) was reported to become tolerant to *H. armigera* in field studies during 2007 [54]. In 2008 high infestation of PBW in a Bt cotton with Cry1Ac was recorded in Gujrat, and in 2010 in Maharashtra and Madhya Pradesh [23, 27]. Bambawale *et al.* [11] emphasised that inadequate planting of refuges in Bt cotton fields may be one of the major cause of field resistance of Bt cotton to PBW in India. Field data from India and China showed substantial survival of susceptible pink bollworm larvae on approved Bt cotton varieties/hybrids that produce Cry1Ac [23, 69, 98], which indicates a failure to meet out the recommended high dose of cry proteins. Although the area planted to Bollgard II has dramatically increased since 2009, refuge management remains a critical component of managing resistance to all proteins in Bollgard II cotton. Kranthi [52] reported 40-80% incidence of PBW in BG II cotton hybrids in Amreli and Bhavnagar districts of Gujrat. Fradrick *et al.* [28] reported multi-toxin resistance enables PBW survival on pyramid Bt cotton containing Cry 1Ac and Cry 2 Ab. Jeffrey *et al.* [108] reported multi-toxin resistance enables pink bollworm survival on pyramid Bt cotton (BG II) containing Cry 1Ac and Cry 2 Ab genes. There was high risk of resistance to pink bollworm to Cry2Ab in India where the refuges of non-Bt host plants are scarce, resistance to Cry1Ac was widespread, and the exposure to Cry2Ab was extensive [109]. The North India *i.e.* Punjab, Haryana and Rajasthan, was assumed to be free from PBW incidence in Bt varieties/ hybrids until 2017 but in Kharif-2018 the incidence of PBW was observed in both BG I and II cotton hybrids/varieties up to varying extents in Haryana as well as Punjab (unpublished data). Also it has been highlighted [105] that the success of transgenic cotton is still being an unanswered task in the developing nations.

More than 90 per cent of cotton growers did not use refuges packet across Maharashtra, Andhra Pradesh and Punjab in India [60]. To overcome the problem of resistance development in Bt cotton we need to be strict in making guidelines and regulations of growing refuges along with the cotton crop, though it is not a permanent solution to the insect resistance but will definitely delay the development of resistance. It is mandatory to grow refuges of non Bt cotton in or near Bt cotton fields in some parts of United States to delay pest resistance (USEPA, 2002). Mallet and Porter [59] emphasised that seed mixtures have several advantages relative to block refuges and also these mixtures will increase the dominance of resistance by increasing survival of heterozygous larvae relative to homozygous susceptible larvae. In 2010, the regulations were modified to include refuges planted with mixtures of Bt and non-Bt seeds that yield a random array of Bt and non-Bt plants side-by side within fields in United States. Keeping in view this, Indian governments has also taken the decision to use seed mixtures of Bt and non-Bt plants side-by side from the *kharif*-2019 onwards. Also mass Release of sterile moth of Pink boll worm and plantings of refuges of non Bt cotton will help to suppress the resistance to two decades in Arizona [89,90]. Another way to combat insect resistance is gene pyramiding of Bt cotton genes, which helps in delaying resistance development. In respect of this Bollgard III cotton released by Monsanto which adds one more protein *i.e.*Vip3A to Cry1AC and Cry2AB found in Bollgard II, to create a triple-mode-of action [26]. Bayers Crop Sciences Twin Link cotton offers two Bt proteins Cry1Ab and Cry2Ae and Twin Link Plus offers three Bt proteins (Cry1Ab, Cry2Ae and Vip3Aa19), whereas, Wide Strike III of Dow Agro Sciences has contains Cry1Ac, Cry1F and a Vip3A for durability and improved insect resistance management [26]. Despite legal regulations of GEAC, BG I cotton was brought to India illegally in 2000 in Gujarat and also illegal smuggling and cultivation of BG III cotton seeds (5.5 lakh packets) into the Vidarbha district was reported [107]. Monsanto was all set to release BG III (with an additional gene *vip3*) in India, however, it withdrew its decision considering the anti-GM atmosphere. It entered illegally through traders in Yavatmal from Andhra Pradesh apart from Gujarat [107]. Although these technologies are new and not registered for commercial cultivation in India but surely these will help in enhancement of resistance durability and sustainable cotton production. Strict regulation in plantings of refuges, mass release of male sterile moths and gene pyramiding are some of the areas which when integrated will result in insect resistant management in cotton.

7. New emerging pests

Since 2002, with the adoption of Bt cotton, the changes in complex of various insect-pests on Bt cotton are evident in India due to its large scale cultivation. Mealy and mirid bugs emerged as potential threat to the Bt cotton and with the cultivation of Bt cotton mealy bug has became havoc in cotton since 2006. During 2007- 08, the pest caused serious damage in Punjab with nearly 0.2 million bales yield loss (Worth Rs 1.59 billion). It also caused similar situation in Haryana state followed by Rajasthan, Sourashtra (Gujarat), Maharashtra, Andhra Pradesh, and once again the farmers were forced to rely on heavy insecticidal usage once to control mealy bugs on this crop. For the sustainability of Bt technology, there needs longer-term monitoring of possible secondary effects and farmers' behavior in maintaining refuges [77]. The conventional taxonomy and molecular analyses has confirmed the presence of mealy bug, *Phenacoccus solenopsis* (Tinsely) throughout the country. Other mealy bug species, *Phenacoccus solani* and *Maconellicoccus hirsutus* (Pseudococcidae: Homoptera) also appear in the isolated patches. A green mirid, *Creontiades biseratense* (Distant) started appearing since 2005 in Karnataka and causes heavy shedding of squares and small sized bolls in Bt cotton. Apart from this, *Campylomma livida* (Reuter) was reported from Maharastra. Besides this, *S. litura* is also potential threat to the Bt cotton. The major reasons which have triggered the outbreak of secondary pest species on Bt cotton was reduced applications of broad-spectrum insecticides, natural enemy populations and interspecific competition with the target pests [105].

8. Impact on diversity of pests and predators

The increased populations of the natural enemies on the transgenic cotton may be due to lesser applications of chemical insecticides [94]. The transgenic cotton may act as a refuge for the predatory fauna in the large scale crop production and there the non-Bt cotton may be sprayed with insecticides [6]. The absence of hosts or direct toxic effects of Bt toxin resulted into the reduced parasitoid activity on Bt cotton [67]. Some adverse effects of Bt toxin on the survival and development of predatory fauna have been reported [73]. There were no significant differences reported in the abundance of insect predators between unsprayed Bt and non Bt cotton fields [65]. Although, the transgenic cotton is more effective to the target pests only without any adverse effects on the predatory fauna [1] but the behavioural change of non-target organisms or by the removal of their prey/hosts make the chances the insect predators and parasitoids being indirectly influenced [84]. Several studies have shown no direct effects of Bt toxin on green lace wing predator, *Chrysoperla carnea* Stephens [79]. The adverse effects 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 [78]. A few indirect effects on the predatory insects have been reported through unhealthy prey/hosts but at the same time the increased parasitism of unhealthy prey/host due to Bt toxin their population may be increased [78]. The transgenic cotton can affect field predatory fauna through removal of eggs, larvae and pupae of lepidopterous pests which serve as their food sources [103]. The Bt toxic effects on predatory fauna might be either due to ingestion of Bt toxin fed lepidopterous larvae or the consumption of intoxicated non-target prey might have picked up the Bt toxin [25]. A rich diversity of insect predatory fauna and the parasitoids found on cotton play a significant role for regulation of the pest population [64]. A few field reports have indicated no significant difference of populations of predatory fauna on Bt and non-Bt cotton and where the differences noticed indicated their populations significantly higher on Bt than the non-Bt cotton due to lower insecticide use in the Bt fields [53]. Dhillon and Sharma [22] reported no significant influence of *Bt* cotton on the abundance of different natural enemies like chrysopids, ladybird beetles and spiders. Several field studies have indicated no direct adverse effects of transgenic crops on the insect predatory fauna [7]. Singh [105] has also talked about the eco-toxicological impact of Bt cotton on predatory fauna.

The transgenic cotton acts specifically against the target pests without any direct adverse effect on non-target pest population. The BG I provided a significant season long field control of the most target pests like American bollworm, *Helicoverpa armigera*, spotted bollworms, *Earias spp.* and pink bollworm, *Pectinophora gossypiella*) with effect on tobacco caterpillar, *Spodoptera* species. Genetically modified or Bt cotton is no longer resistant to pink bollworm- a major pest in Maharashtra, prompting the state government to write to the Union government to seek its intervention [42]. The reports of BW, *H. zea* larvae feeding in white flowers of BG cotton have been relatively common since the commercialization of this technology in 1996 [32]. Also the reduced insecticidal applications on Bt cotton has positive impact on populations of beneficial insects and can enhance the stability of rare species. The transgenic cotton is not resistant to the non-target sucking insect pests. As this cotton has no adverse effects on the non-target insect population and need lesser applications of broad-spectrum insecticides, therefore it can be an important tool of IPM program in cotton agro-ecosystem [18, 105].

9. Insecticide resistance management (IRM)

The US Environmental Protection Agency's (USEPA) Office of Pesticide Programs (OPP) has worked on the development and analysis of IRM strategies and has mandated specific IRM requirements for Bt crops since 1995[31]. As plant incorporated protection system of toxin administering Bt crystal proteins offers a great deal of selection pressure to bollworms. Thus resistance development in bollworms such as *H. armigera* and *H. zea* (Boddie) has been well adopted and hence refugia have been incorporated as IRM strategy by EPA of UAS which our country too adopted. The key IRM strategy used to delay the resistance development followed worldwide is maintaining refugia (20% protected or 5% unprotected non Bt cotton) at the beginning of era of Bt cottons and now growing dual gene cultivars. Use of insect resistant BTK lines or equally efficacious commercial BTK cultivars in production agriculture can reduce insecticide applications against various insect pests while increasing farm profit and opportunities for the use of biological control [14].

The transgenic plants expressing insecticidal proteins from Bt first commercialized in 1996, but according to some scientists, regulators and environmentalists their widespread use could inevitably lead to resistance development and the loss of public health particularly, the susceptibility of various insect pests to Bt proteins. A decade later, Bt corn and cotton were grown over more than 80 million hectares area worldwide. Despite dire predictions to the contrary, resistance to Bt crop has yet to be documented, indicating that resistance management strategies have been effective so far. However, the current strategies to delay the resistance development remain far from ideal. A decade with no resistance provided a timely opportunity to the researchers, regulators and industrialists to reassess the risk of resistance and the most effective ways to preserve Bt and other novel insect-resistant crops [80]. Arshad *et al.* [8] presented few alternative means to minimize the resistance development in target pests i.e., i) planting of refuge crop that does not contain Bt based product for susceptible target insect pests, ii) expression of Bt proteins at consistent and high levels in all the plant structures, iii) monitoring for shift in baseline susceptibility of target pests to Bt based products, and iv) using other IPM control strategies.

10. Future perspectives

An increasing confidence in the benefits of Bt crops and the availability of new Bt varieties conferred the protection against additional crop pests [44]. In recent years, unusually high level of pink bollworm infestation on dual Bt cotton (BG II) has been observed in south and central India [48]. Complete failure of Bt cotton has been experienced in India due to emergence of pink bollworm which developed resistance against Bt cotton. Such incidences cause great concerns among the scientists as presently there is no other way than insecticidal sprays to manage the PBW. Also, the lack of resistance observed in the field to current Bt crops attests to the potential sustainability of this technology, but caution continues to be warranted because at least one insect species, the diamondback moth, has evolved resistance in the field to foliar Bt sprays, and the selection pressure for resistance in other insect species to Bt cotton will only increase its adoption levels.

The second-generation insecticidal transgenic cotton should not be seen as a panacea to manage insect pest problem and Bt resistance but rather as a changing knowledge related to the ecology and genetics of resistance to Bt. Any highly effective strategy for a given crop is based on the best available models and will not remain static in the face of our rapidly changing knowledge. The continuous use of Bt crops too will result in development of the selection pressure on various pests from these crops. Success of the application of Bt crop in cotton is unquestionable but the panic of developing resistance in pests against Bt protein will always be there. Although selection pressure on pests can be minimized by using multifaceted IPM strategies [106] by which a particular Bt variety can last for many years in cultivation. However, there is need of continuous search for resistance genes and their interaction with insect pests. Monitoring for changes in resistant-allele frequencies in field populations may increasingly play a critical role in the future insecticide resistance management. Economic threshold level of various pests also needs to be re-determined continuously in scenario of changing pest status. There is need to re-access the chemicals used in cotton crop with the emergence of new pests on Bt cotton [105]. It should be estimated that presently chemical used on Bt cotton crop are still decreased or not which was the reason for adaption of Bt cotton. GM technology should be last resort to tackle insect-pests damage in any crop when there is not enough variation in the germplasm.

As said by Dr. M. S. Swaminathan in an interview to The Hindu, 16 August 2017 [4] that in most cases normal Mendelian breeding is sufficient in tackling various biotic and abiotic challenges and genetic engineering technology is not necessary. There is need to devise a way to get the GM technology's benefits without its associated risks and it should be used where there is no other way to address the challenge. Moreover, there needs monitoring and management of resistance to Cry proteins in the first and second generation genotypes for sustainability of Bt technology, develop an IPM for BG-II cotton with emphasis on sucking pest management with biorationals to achieve pesticide free cultivation of cotton and conduct studies on causes and remedies to contain the problems associated with expression of toxin [86].

11. References

1. Akhurst RJ, James W, Bird LJ, Beard C. Resistance to the Cry1Ac delta-endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). J Econ Ent. 2003; 96:1290-1299.
2. Andow DA, Ives RA. Monitoring and adaptive resistance management. Ecol App. 2002; 12:1378-1390.
3. Anonymous. Global transgenic crop area continues to grow; exceeds 50 million hectares. Biotech Glo Upd. 2002; 4:1-5.
4. Anonymous. http://www.thehindu.com/opinion/interview/why-cant-thegovernment-pro vide-a-higher-income-for-farmers/article19498056-ece. 2017.
5. Anonymous. India's genetically modified crop area fifth largest in world. The Indian Express. Tuesday, 11 September 2018. https://indianexpress. com/article/india/indias-genetically-modified-crop-area-fifth-largest-in-world-5255662/, 2018
6. Armstrong JS, Leser J, Kraemer G. An inventory of the key predators of cotton pests on Bt and non-Bt cotton in West Texas. In: Proceedings of Beltwide Cotton Conference, San Antonio, USA; 2000, 1030-1033.
7. Arshad M, Khan HAA, Abdul-ur-Rehman M, Saeed NA. Incidence of insect predators and parasitoids on transgenic Bt cotton in comparison to non-Bt cotton varieties. Pak J Zool. 2015; 47:823-829.
8. Arshad M, Zain-ul-Abdin, Gogi MD, Arif MJ, Khan RR. Seasonal pattern of infestation by spotted bollworm, *Earias insulana* (Boisd.) and pink bollworm, *Pectinophora gossypiella* (Saund.) in field plots of transgenic Bt and non-Bt cottons. Pak J Zool. 2015; 47:177-186.
9. Arshad M, Khan RR, Aslam A, Akbar W. Transgenic Bt Cotton: Effects on Target and Non-Target Insect Diversity. 2018, 155-174. http://dx.doi.org/10.5772/intechopen. 73182,2018
10. Bacheler JS, Mott DW, Morrison DE. Efficacy of grower managed Bt cotton in North Carolina. Proc. Beltwide Cotton Conf., New Orleans, LA, USA, 1997, 858-861.
11. Bambawale OM, Singh A, Sharma OP, Bhosle BB, Lavekar RC, Dhandapani A. Performance of Bt cotton (MECH-162) under integrated pest management in farmers' participatory field trial in Nanded district, Central India. Cur Sci, 2004; 86:1628-1633.
12. Barwale RB, Gadwal VR, Usha Z, Brent Z. Prospects for Btcotton technology in India. A gBiofor. 2004; 4:23-26.
13. Benedict JH, Altman DW. Commercialization of transgenic cotton expressing insecticidal crystal protein. In: Jenkins J, Saha S, editors. Genetic Improvement Cotton: Emerging Technologies. Enield, New Hampshire, USA: Science Publications. 2001, 137-201.
14. Benedict JH, Sachs ES, Altman DW, Deaton WR, Kohel RJ, Ring DR *et al*. Field performance of cotton expressing transgenic Cry1Ac insecticidal proteins for resistance to *Heliothis virescence* and *Helicoverpa zea* (Lepidoptera: Noctuidae). J Econ Ent, 1996; 89: 230-238.
15. Bez FS, Hammond BG, Fuchs RL. Safety and advantages of Bacillus thuringiensis protected plants to control insect pests. Reg Toxicol and Pharmacol. 2000; 32:156-173.
16. Bheemanna M, Patil BV, Hanchinal SG, Hosamani AC, Bansi AB. Comparative performance and economics of Bollgard-II cotton under irrigated condition. J Cotton Res and Develop, 2008; 22(1):118-121.
17. Burd T, Bradley JR, Duyn JW, Van DJW, Dugger P. Performance of selected Bt cotton genotypes against bollworm in North Carolina. Proc. Beltwide Cotton Conf., Orlando, Florida, USA. 1999, 931-934.
18. Carriere Y, Dennehy TJ, Pedersen B, Haller S, Ellers-Kirk C, Antilla L *et al*. Large-scale management of insect resistance to transgenic cotton in Arizona: Can transgenic insecticidal crops be sustained? J Econ Ent. 2001; 94:315-325.
19. Chitkowski RL, Turnipseed SG, Sullivan MJ, Bridges WG. Field and Laboratory evaluations of transgenic cottons expressing one or two *Bacillus thuringiensis* var.

kurstaki Berliner proteins for management of Noctuid (Lepdoptera) pests. J Econ Ent, 2003; 96:755-762.

20. Crook NE, Jarrett P. Viral and bacterial pathogens of insects. J App Bacterio. 1991; 70:91-96.
21. Dhaliwal GS, Arora R, Dhawan AK. Crop losses due to insect pests in Indian Agriculture, An Update. Ind J Ecol, 2004; 31:1-7.
22. Dhillon MK, Sharma HC. Comparative studies on the effects of Bt-transgenic and non-transgenic cotton on arthropod diversity, seed cotton yield and bollworms control. J Env Biol. 2013; 34:67-73.
23. Dhurua S, Gujar GT. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Manag Sci. 2011; 67:898-903.
24. Douglas FW, Hollis MF, Randy WD, Robert EB. Yield, yield components and fibre properties of insect resistant cotton lines containing *Bacillus thuringiensis* toxin gene. Crop Sci, 1994; 34:38-41.
25. Duton A, Obrist L, Dalessandro M, Diener L, Muller M, Romeis J *et al*. Tracking Bt-toxin in transgenic maize to assess the risks on non-target arthropods. Bulletin OILB/ SROP, 2004; 27:57-63.
26. Edpuganti SL. Resistance Development in Pink Bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) to Bt Cotton and Resistance Management Strategies. Int J Pure and App Biosci. 2018; 6(1):1296-1302.
27. Fabrick JA, Ponnuraj J, Singh A, Tanwar RK, Unnithan GC, Yelich AJ. Alternative splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to Bt cotton in India. PLoS ONE. 2014; 9(5):97900.
28. Fradrick AJ, Gopalan C Unnithan, Alex J Yelich, Ben DeGain, Luke Masson, Jie Zhang *et al*. Multi-Toxin Resistance Enables Pink Bollworm Survival on Pyramided Bt Cotton. Sci Rep. 2015; doi: 10.1038/srep16554.
29. Fit GP. Deployment and impact of transgenic Bt cotton in Australia. In: The Economic and Environmental Impacts of Agbiotech: A Global Perspective. Kalaizandonakes NG, editor. Kluwer: New York. 2003, 141-164.
30. Gill SS, Cowles EA, Pietrantonio PV. The mode of action of Bacillus thuringiensis endotoxins. An Review of Ent. 1992; 37:615-636.
31. Glaser JA, Matten SR. Sustainability of insect resistance management strategies for transgenic Bt corn. Biotech Adv. 2003; 22:45-69.
32. Gore J, Leonard BR, Chorch GE, Cook DR. Behaviour of bollworm (Lepidoptera: Noctuidae) larvae on genetically engineered cotton. J Econ Ent. 2002; 95:763-769.
33. Gould F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Ann Rev Ent. 1998; 43:701-726.
34. Hagerty AM, Kilpatrick AL, Turnipseed SG, Sullivan MJ, Bridges WC. Predaceous arthropods and lepidopteran pests on conventional, Bollgard, and Bollgard II cotton under untreated and disrupted conditions. Env Ent. 2005; 34:105-114.
35. Harris FA, Furr RE, Calhoun DS. Cotton insect management in transgenic Bt cotton in the Mississippi Delta, 1992-1995. Proc. Beltwide Cotton Conference, 1996; 2:854-858.
36. Henneberry TJ, Jech LF. Seasonal pink bollworm, Pectinophora gossypiella (Saunders), infestation of transgenic and non-transgenic cottons. Southwest Ento, 2000; 25:273-286.
37. Henneberry TJ, Forlow L, Torre T, Fiaulconer S, Hill SS. Pink bollworm egg infestations and larval survival in NuCOTN 33b and Deltapine cotton in Arizona, 2000. In: 2000 Arizona cotton Report. http:// cals. Arizona edu. /pubs/crops/az 1170/az 11707c. pdf.
38. Hofs JL, Fok M, Vaissayre M. Impact of Bt cotton adoption on pesticide use by smallholders: A 2-year survey in Makhatinilats (South Africa). Crop Prot. 2006; 25:984-988.
39. Hubbell BJ, Marra MC, Carlson GA. Estimating the demand for a new technology: Bt cotton and insecticide policies. American J Agri Econ. 2000; 82:118-132.
40. ISAAA. Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years. ISAAA Brief No. 53. ISAAA: Ithaca, NY, 2017.
41. Jackson RE, Bradley JR, Van DJW. Field performance of transgenic cotton expressing one or two *Bacillus thuringiensis* endotoxins against bollworm, *Helicoverpa zea* (Boddie). The J Cotton Sci. 2003; 7:57-64.
42. Jain B. Bt cotton falling to pest, Maharashtra tensed. The Times of India. 5 July, 2017. https://timesofindia.indiatimes.com/city/mumbai/bt-cotton-falling-to-pest-maharashtra-tensed/articleshow/59449010.cms.
43. James C. Global status of commercialized transgenic crops: 2002. ISAA Brief NO.27, International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY, USA, 2002. http://www.isaaa.org.
44. James C. Global status of commercialized transgenic crops: 2003. ISAAA Brief No. 30, International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY, USA, 2003.
45. James C. Preview: Global Status of Commercialized Biotech/GM Crops: 2004. ISAA Brief No. 32, International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA, 2004.
46. KM W, Guo YY. Changes in susceptibility to conventional insecticides of a Cry1Acselected population of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Pest Manag Sci. 2004; 60:680-684.
47. KM W, Guo YY. The evolution of cotton pest management practices in China. Annual Rev Ent. 2005; 50:31-52.
48. Komarlingam MS. An area-wide approach to pink bollworm management on Bt cotton in India – a dire necessity with community participation. Curr Sci. 2017; 112(10):1988-1989.
49. Koziel MG, Carozzi NB, Currier TC, Warren GW, Evola SV. The insecticidal crystal proteins of Bacillus thuringiensis: past, present and future uses. Biotech and Genc Engi Rev, 1993; 11:171-228.
50. Kranthi KR. Impact of Bt cotton in India. Cotton Stat and News, 2013; 36:1-4.
51. Kranthi KR, Dhawad CS, Naidu S, Mate K, Patil E, Bharose AA. Temporal and intraplant variability of Cry1Ac expression in Bt cotton and its influence on the survival of the cotton bollworm *Helicoverpa armigera* (Hübner). Curr Sci. 2005; 89:291-297
52. Kranthi KR. Pink bollworm strikes Bt cotton. Cotton Stat News. 2015; 35:1-6.

53. Kumar KR, Chandrasehar G, Ayyappan S. Assessment of arthropod communities in transgenic and conventional cotton in Kancheepuram District, Tamil Nadu. J Ecobiol. 2007; 19:201-207.
54. Li G, Wu K, Gould F, Wang J, Miao J. Increasing tolerance to Cry1Ac cotton from cotton bollworm, Helicoverpa armigera, was confirmed in Bt cotton farming area of China. Ecol Ento. 2007; 32:366-375.
55. Liu S, Liu D, Jia T. Studies on the chemical treatment of bollworm resistant cotton in the Shaanxi cotton growing area. China Cotton. 2002; 29:20-24.
56. Liu YB, Tabashnik BE, Dennehy TJ, Patin AL, Sims MA, Meyer SK, Carriere Y. Effects of Bt cotton and Cry1Ac toxin on survival and development of pink bollworm (Lepidoptera: Gelechiidae). J Econ Ent. 2001; 94:237-1242.
57. Lu Y, Wu K, Jiang Y, Guo Y, Desneux N. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nat. 2012; 487:362-365.
58. Lutrell RG, Jackson RE. *Helicoverpa zea* and Bt cotton in the United States. GM Crops & Food: Biotechnology in Agriculture and the Food Chain. 2012; 3:213-227. DOI: 10.4161/ gmcr.20742.
59. Mallet J, Porter P. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Pro Royal Soc Biol Sci, 1992. DOI: 10.1098/rspb.
60. Mayee CD, Choudhary B. Adoption and Uptake Pathways of Biotech Cotton among Farmers in Selected Cotton Growing Villages of Maharashtra, Andhra Pradesh and Punjab in India. Executive Summary of the Project Undertaken by Indian Society for Cotton Improvement, 2013. http://www.cicr.org.in/isci/5-2/Paper_5.pdf),
61. Mellet MA, Schoeman AS, Broodryk SW, Hofs JL. Bollworm Helicoverpa armigera (Hubner), (Lepidoptera: Noctuidae) occurrences in Bt and non-Bt-cotton fields, marble hall, Mpumalanga, South Africa. African Ent. 2004; 12:107-115.
62. Mendelsohn M, Kough J, Vaituzis Z, Mathews K. Are Bt crops safe? Nature Biotech. 2003; 21:1003-1009.
63. Naglaa AA. The story behind Bt cotton: Where does Sudan stand? GM Crops & Food. 2014; 5:241-243. DOI: 10.1080/21645698.2014.997119.
64. Naranjo SE. Long-term assessment of the effects of transgenic Bt cotton on the function of the natural enemy community. Env Ento. 2005; 34:1211-1223.
65. Naranjo SE, Ellsworth PC. Arthropod communities and transgenic cotton in the Western United States: implications for biological control. In: Proceedings of 1st International Symposium on Biological Control of Arthropods: Honolulu, Hawaii; 2003, 284-291.
66. Nasreen A, Cheema GM, Ashfaq M, Saleem MA. Survival of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammitidae) after exposure to different insecticides: Laboratory studies. Pak J Zool. 2004; 36:79-82.
67. Ning X, Song Q, Kong X, Chen H, Meng J, He Y *et al*. Preliminary research on the regularity of population luctuations of major insects and natural enemies in the field of Bt transgenic cotton in the Xinjiang region. China Cotton, 2001; 28:12-13.
68. Obando RA, Gonzalea GJ, Olivas GJM, Magana MJE, Martinez GA, Dugger P. Bollgard Gene cotton as an alternative for IPM in Delicias, Chihuahua, Mexico. Proc. Beltwide Cotton Conf., Orlando, Florida, USA. 1999; 2:967-968.
69. Ojha A, Sree KS, Sachdev B, Rashmi MA, Ravi KC, Suresh PJ. Analysis of resistance to Cry1Ac in field-collected pink bollworm, Pectinophora gossypiella (Lepidoptera: Gelechiidae), populations. GM Crops Food. 2014; 5:280-286.
70. Patin AL, Dennehy TJ, Sims MA, Tabashnik BE, Liu YB, Antilla L. Status of pink bollworm susceptibility to Bt in Arizona, in Proceedings – 1999 Beltwide Cotton Conferences, Orlando, FL, 3–7 January 1999, ed. by Dugger P and Richter DA. National Cotton Council of America, Memphis, TN, 1999, 991-996.
71. Pawade V, Thakare SM, Thakare AS, Ghodaki BS. In-season variation in cry1ac expression in various plant parts of different Bt cotton hybrids in India. International J Env Sci, 2015; 5:675-680.
72. Perlak FJ, Oppenhuizen M, Gustafson K, Voth R, Sivasupramaniam S, Heering D *et al*. Development and commercial use of Bollgard® cotton in the USA - early promises versus today's reality. The Pl Journal. 2001; 27:489-501.
73. Ponsard S, Gutierrez AP, Mills NJ. Effect of Bt-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four heteropteran predators. Env Ent. 2002; 31:1197-1205.
74. Pray CE, Huang JK, RFH, Rozelle S. Five years of Bt cotton in China- the benefits continue. The Pl Journal. 2002; 31:423-430.
75. Purcell JP, Perlak FJ. Global impact of insect-resistant (Bt) cotton. AgBiof. 2004; 7:27-30.
76. Qaim M. Bt cotton in India: Field trial results and economic projections. World Development. 2003; 31:2115-2127.
77. Qaim M, De-Janvry A. Bt cotton and pesticide use in Argentina: Economic and environmental effects. Env and Develop Econ. 2005; 10:179-200.
78. Ren L, Yang Y, Qin Q, Yu Y. Reciprocal effects of the transgenic cotton and parasitoids on the development of cotton bollworm. Jiangsu J Agri Sci. 2004; 20:80-83.
79. Romeis J, Duton A, Bigler F. Bacillus thuringiensis toxin (Cry1Ab) has no direct effect on larvae of the green lacewing Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae). J Insect Physiol. 2004; 50:175-183.
80. Sarah LB, Jian ZZ, Richard TR, Anthony MS. Insect resistance management in GM crops: past, present and future. Nat Biotech. 2005; 23:57-62.
81. Sharma HC, Pampapathy G. Influence of transgenic cotton on the relative abundance and damage by target and non-target insect pests under different protection regimes in India. Crop Prot. 2006; 25:800-813.
82. Shelton AM, Zhao JZ, Roush RT. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annual Revof Ent. 2002; 47:845-881.
83. Sieglaff DH, Ellsworth PC, Silvertooth JC, Hamilton. Preliminary evaluation of the next generation of Bt cotton, 1999. http: // cals. arizona. edu / pubs / crops / az1123 / az11237c. pdf.
84. Sisterson MS, Biggs RW, Olson C, Carriere Y, Dennehy T J, Tabashnik BE. Arthropod abundance and diversity in Bt and non-Bt cotton fields. Env Ent. 2004; 33:921-929.
85. Somashekara R. evaluation of first and second generation

Bt cotton genotypes for characterization of resistance to bollworms and tobacco caterpillar. M.Sc. Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India, 2009.
86. Strickland GR, Annells AJ. The seasonal dynamics of arthropods in conventional, INGARD® and Bollgard II® cotton genotypes in a winter production system at Kununurra, Bentley Delivery Centre WA 6983 @ State of Western Australia. 2005, 1-13.
87. Tabashnik BE. Evolution of resistance to Bacillus thuringiensis. Annual Rev Ent. 1994; 39:47-79.
88. Tabashnik BE. Delaying insect resistance to transgenic crops. Proc Natl Acad Sci USA 2008; 105:19029-19030.
89. Tabashnik BE, Morin S, Unnithan GC, Yelich AJ, Ellers-Kirk C, Harpold VS *et al*. Sustained susceptibility of pink bollworm to Bt cotton in the United States. GM Crops Food, 2012; 3(3):1-7.
90. Tabashnik BE, Sisterson MS, Ellsworth PC, Dennehy TJ, Antilla L, Liesner L *et al*. Suppressing resistance to Bt cotton with sterile insect releases. Nat Biotech. 2010; 28:1304-1307.
91. Tian JC, Yao J, Long LP, Romeis J, Shelton AM. Bt crops benefit natural enemies to control non-target pests. Scientific Reports. 2015; 5:16636. DOI: 10.1038/srep16636.
92. Torres JB, Ruberson JR. Canopy- and ground-dwelling predatory arthropods in commercial Bt and non-Bt cotton ields: Paterns and mechanisms. Env Ent. 2005; 34:1242-1256.
93. Traxler G, Godoy-Avila S, Falck-Zepeda J, Espinoza-Arellano JJ. Transgenic Cotton in Mexico: Economic and Environmental Impacts [Unpublished Report]. Auburn, AL: Department of Agricultural Economics, Auburn University, 2001
94. Turnipseed SG, Sullivan MJ. Consequences of natural enemy disruption with applications of “hard” insecticides prior to the bollworm light in conventional and Bt cotton. In: Proceedings of Beltwide Cotton Conference: Orlando, Florida, USA; 1999, 1110-1112.
95. US Environmental Protection Agency. (https://www.epa.gov/regulationbiotechnology-under-tsca-and-fifra/ insect-resistance-management-bt-plantincorporated), 2002.
96. Udikeri SS. Evaluation of new generation Bt cotton genotypes, Sustainability of Cry protein expression, computation of ETL, Effect on aphid predators and development of IPM module for Bt Cotton under rainfed conditions. Ph.D. Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India, 2006.
97. USEPA. Biopesticides registration action document: Bacillus thuringiensis plant-incorporated protectants. Available: http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm. 1998.
98. Wan P, Huang Y, Wu H, Huang M, Cong S, Tabashnik BE. Increased frequency of pink bollworm resistance to Bt toxin Cry1Ac in China. PLoS ONE. 2012; 7(1):e29975.
99. Wang G, Wu Y, Gao W, Fok M, Liang W. Impact of Bt cotton on the Farmer’s livelihood system in China. In: International Cotton Conference, Rationales and evolutions of cotton policies in main producing countries. ISSCRI International Conference. Montpellier, France, 2008.
100. Wier AT, Mullins JW, Mills JM. Bollgard cotton update and economic comparisons including new varieties, Proc. Belt wide cotton conference, San Diego, California, USA, 1998; 2:1039-1040.
101. Wilson FD, Flint HM, Deaton WR, Fischhoff DA, Perlak FJ, Armstrong TA *et al*. Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera : Gelechiidae) and other Insects. J Econ Ent, 1992; 85:1516-1521.
102. Wu KM, Geo YY, Wang WG. Field resistance evaluations of Bt transgenic cotton GK series to cotton bollworm. Acta Phyto Sinica. 2000; 27:317-321.
103. Wu K, Lin K, Miao J, Zhang Y. Field abundance of insect predators and insect pests on Delta-Endotoxin-producing transgenic cotton in northern China. In: 2nd International Symposium Biological Control of Arthropods: Davos, Switzerland. 2004, 362-368.
104. Wu K, Lu Y, Feng H, Jiang Y, Zhao J. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science. 2008; 321:1676-1678.
105. Singh S. Transgenic cotton its adoption, threats and challenges ahead: A review. J Ent and Zool Studies. 2018; 6(5):1989-1997.
106. Dhawan AK, Singh S, Kumar S. Integrated pest management (IPM) helps reduce pesticide load in cotton. J Agri Sci and Tech, 2009; 11:599-611.
107. Shrivastav S. BG-III cotton illegally grown in Yavatmal. The Times of India, 2017. https://timesofindia.indiatimes.com/city/nagpur/bg-iii-cotton-illegally-grown-in-yavatmal/articleshow/60986490.cms. 2017.
108. Jeffrey AF, Gopalan CU, Alex JY, Ben DG, Luke M, Jie Z *et al*. Multi-Toxin Resistance Enables Pink Bollworm Survival on Pyramided Bt Cotton. Scientific Reports 5, Article number: 16554. https://www.nature.com/articles/srep16554. 2015.
109. Tabashnik BE. ABCs of Insect Resistance to Bt. PLoS Genetics, 2015, 11: e1005646. https://doi.org/10.1371/journal.pgen.1005646.
110. Mohammed AI, Natalya G, Mathew J, Lee AB. *Bacillus thiringensis*: A genomics and proteomics perspective. Bioeng Bugs. 2010; 1(1):31-50.
111. Singh A. Bt cotton in India - Current Scenario. Cotton Stat and news. 2018; 16:1-8.
112. Singh S, Sharma DK. Impact of different transgenic Crops (Bt) on insect biocontrol agents. Int J Life Sci Res. 2015; 3(1):214-229.