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Transgenic cotton- its adoption, threats and challenges ahead: A review

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Abstract

A global adoption of transgenic cotton resulted in around 42 per cent rise in area in 2017-18 since last two decades. The bollworms (BW) were the most serious problem on non-transgenic cotton causing around 20-66 per cent yield loss in India. However, its adoption involved several benefits such as low insecticidal load, control of BW and more productivity but the farmers due to higher seed rates have been using illegal and low tolerance *Bt* cultivars that not even resulted into a heavy crop damage but also resistance in secondary pests that are not susceptible to the expressed toxin have become an increasing concern in some agro-ecosystems where *Bt* cotton is grown. The success of transgenic cotton is still an unanswered task in the developing countries.

Keywords: Transgenic cotton, adoption, threats, challenges.

1. Introduction

A rich history of cotton (*Gossypium hirsutum* Linn.) and its production are directly linked to the expanding human civilization [28]. Cotton seed is the second most important seed oil at Global level which is used for culinary purposes, and the oil cake residue is a protein rich feed fed to the ruminant livestock [48]. Presently, this crop is grown in more than 70 countries across the world over an area of 31.8 million hectares with production of 24,963 million kilogram bales and India commands highest share (36%) in terms of area and under its cultivation besides lower yield than rest of the top growing countries [40]. The cotton pests showed their time to time epidemic appearance and resulted into quantitative and qualitative crop losses in cotton growing states such as Maharashtra, Punjab, Karnataka, Gujrat, Haryana, Rajasthan, Madhya Pradesh, Andhra Pradesh and Telangana. A severe incidence by these pests on cotton had threatened the cotton growers in some states like Punjab and Haryana to give up this crop and divert towards cultivation of paddy which in turn have resulted in a continuous fall down of underground water table in cotton growing regions. However, introduction of transgenic cotton helped the farmers to get back from paddy to the cotton cultivation in several nations.

In China, the field studies have shown that farmers have reduced pesticide and labor costs by adopting *Bt* cotton; moreover, there is less exposure to toxic insecticides [71, 95]. Similarly, in Pakistan, eight *Bt* cotton varieties were approved few preliminary studies to compare the performance of existing *Bt* varieties with the recommended non-*Bt* varieties [4]. Cotton is Pakistan's main cash crop and is known as "White Gold" and it is the fourth largest cotton producing nation [1, 3] after China, the USA, and India. Transgenic technology is definitely a major factor to boost the agricultural productivity, especially in developing countries with additional positive effects on human health and the environment due to reduced pesticide loads [69]. However, the higher technology price of *Bt* cotton seed inhibits its wide adoption [72].

Insect pests and damage on cotton

Cotton plant is a specific one that seems to be designed by nature for attracting various insect pests. More than 1300 plant feeding insects are found in cotton systems world-wide [57], however, limited are the common inhabitants and only fewer have economic importance. Estimated, about 100 insect and mite species attack cotton crop in the USA of which only 20 per cent commonly cause damage under uncontrolled conditions while rest of 80 per cent being sporadic or secondary pests cause problem in some years either due to climate change or non-judicious use of insecticides or disruptions of natural control fauna [91].

The pest species vary with one production area to the next. About half a decade back, earlier to the intervention of transgenic technology, non-transgenic cotton (non-*Bt* cotton) was found

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susceptible to a variety of insect pests. Among the most destructive are the bollworms [American bollworm (ABW), *Helicoverpa armigera* Hubner; spotted bollworms (SBWs), *Earias vitella* Fabricius and *E. insulana* Boisduval; pink bollworm (PBW), *Pectinophora gossypiella* Saunders), foliage feeders (tobacco caterpillar, *Spodoptera litura* Fabricius; grey weevil, *Myllocerus maculosus* Desb.), sucking pests (whitefly, *Bemisia tabaci* Gennadius; green jassid, *Amrasca biguttula biguttula* Ishida; mealybug, *Phenococcus solenopsis* Tinsley; thrips, *Thrips tabaci* Linnman)], red cotton bug, *Dysdercus koengii* Fabricius and dusky cotton bug, *Oxycaranu shayalinipennis* Costa) occupy pest status and contribute to the lower yields in cotton crop [2]. Of these pests, the bollworms such as *H. armigera*, *E. vitella*, *E. insulana* and *P. gossypiella* are the global pests of non transgenic cotton, whereas the sucking pests - *A. bigutulla bigutulla*, *B. tabaci* and *A. gossypii* have started appearing as major pests on transgenic cotton besides their regular appearance on non transgenic cotton also. A few pests like boll weevil (*Anthonomus grandis*) are found in the USA only.

Some BWs are native pests which have expanded their ranges with the growth of cotton production, while the whitefly and PBW are exotic invaders. Although the levels of crop losses due to pests may appear small but their economic impact can be enormous. Several factors have contributed to the reductions of such losses over the past two decades of which transgenic cotton is one of the important aspects for control of bollworms.

Transgenic cotton and the insecticidal load

Prior to release of *Bt* cotton, the growers have been using several insecticidal sprays. Although, *Bt* cotton, in the early years, gained popularity through significant reduction of insecticide sprays against BW pests but, the non judicious application of insecticides against regularly appearing sucking and other foliage pests on transgenic cotton has alleviated the adverse impacts associated with such insecticides [49].

Patil [68] reported that there was no difference in number of interventions made on *Bt* and non-*Bt* hybrids for sucking pests management. It required 2-3 sprays in addition to seed treatment.

With *Bt* cotton it has been experienced that reduction in usage of insecticides lead to increased population of sucking insect pests [63]. Similarly, the reduced applications of insecticides may allow for a higher diversity and density of beneficial arthropods [66].

Traditionally, in India, cotton crop has shown highest insecticide consumption than on any other crop. However, *Bt* cotton drastically reduced the numbers of insecticide applications for controlling the key pests on cotton like ABW, PBW and SBW, and substantially contributed to stop the cost of production. The market share for cotton insecticides as a percentage of total insecticides declined steeply over the years from 46 per cent in 2001 to 26 per cent in 2006 and to 20 per cent in 2011.

The quantities of insecticide used to control BW reduced by 96 per cent from 5748 metric tons of active ingredients in 2001 to as low as 222 metric tons in 2011. Thus, insecticide use for the control of BW complex dropped at the same time to 95 per cent of total cotton area in 2014 was benefiting from controlling BW with *Bt* cotton [16].

The modifications in *Bt* cotton are not only associated with the reduction of insecticide application but also ineffectiveness of *Bt* cotton against the secondary pests. *Bt* cotton plants are genetically engineered to produce insecticidal toxins from the *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) a gram-positive bacteria. Cry toxins have specific activities against insects of orders - Lepidoptera (butterflies and moths), Diptera (mosquitoes and flies), Coleoptera (beetles), hymenoptera (bees, ants, wasps and sawflies) and invertebrates like nematodes [75]. The Cry toxins are ineffective against sucking insect pests like jassid, aphids, whitefly and mites, piercing insects such as leaf bugs (*Lygus lucorum* and *Adelphocoris suturalis*) and root-dwelling pests [19].

The negative environmental impacts such as a reduction in biodiversity, insect resistance to insecticides, negative effects on non target species (e.g. natural enemies) and the development of secondary pests have also been attributed to the use of insecticides [33, 61, 86].

Kouser and Qaim [51] and Krishna and Qaim [53] were first to study the changes in insecticide use through *Bt* cotton adoption In India. They reported *Bt* cotton to reduce 50 per cent pesticide applications, with the largest reductions of 70 per cent occurring in the most toxic types of chemicals.

Accumulative decline in pesticide usage for the period 1996 to 2011 was estimated at 473 million kg of active ingredient with a saving of 8.9 per cent. In year 2011, there was 37 million kg reduction in pesticide usage which was equivalent to 8.5 per cent pesticide saving. Decline in pesticide usage reduced direct exposure of farm labor to the pesticide harmful effects of pesticides on non-target organisms, and reduced amounts of pesticide residues in food and food products. The additional benefits to farmers would be to control insect pests which have become resistant to commonly used pesticides, and reduction in crop protection costs [45].

The basic reasons that attracted the attention of cotton growers towards transgenic cotton over the non-transgenic one was reduced insecticidal load against BW pests besides its clear benefits on environment and the farmer health [46].

Transgenic cotton and the secondary pests

The major reasons which have triggered the outbreak of secondary pest species on *Bt* cotton might be either the reduced applications of broad-spectrum insecticides, natural enemy populations and interspecific competition with the target pests. The transgenic cotton is effective only against BW complex while the other foliage and the sucking pests have regular appearance on transgenic like the non-transgenic cotton, and thus cotton growers have been using various insecticides against non-BW pests on transgenic cotton.

Occurrence of secondary pests is clearly linked with profitability, which in turn is affected by other important factors such as seed quality, resistance development, farm size, regional, social and institutional variability and farmers' knowledge, skills and wealth. For instance, the early adopters are similar in terms of managerial performance to late adopters or small-scale farmers may introduce a bias to the results [18].

In field plots, the population of non-target pests was higher on *Bt* cotton over non-*Bt* cotton, also the population of sucking pests - *A. gossypii*, *A. biguttula biguttula* and *B. tabaci* was less on bivalent cotton (SGK321) which contained Cry1Ac + CpTI over the univalent cotton (GK321) containing Cry1Ac, whereas, the population of *T. tabaci* and *Lygus lucorum* was high [81].

Venkateshalu [85] used *Bt* cotton hybrids - Mahyco (MECH) and Rasi (RCH) and reported *Bt* cotton hybrids- MECH-184

Bt as superior based on lower jassid and aphid followed by MECH-162 *Bt* while MECH-12 *Bt* was highly susceptible to these sucking pests. Similarly, RCH-2 *Bt* performed was found superior to the sucking pests followed by RCH-20 *Bt* and RCH-144.

Bhavyarani [6] reported recommended package of practice (RPP) without involving *Bt* and non-*Bt* cotton hybrids module as superior based on low incidence of jassid (1.16/leaf), thrips (0.89/leaf), red cotton bug (1.70 bugs/plant) and dusky cotton bug (0.57 bugs/plant). Both the *Bt* (9.04) and non-*Bt* (8.86/leaf) cotton were good based on aphid bio intensive pest management (BIPM). She also reported higher population of natural fauna in BIPM treatment - RCH-2 *Bt* (2.93/plant) and RCH-2 non-*Bt* (2.39/ plant).

There were no significant differences noticed in population density of jassid, aphid and thrips on *Bt* and non-*Bt* cotton in Punjab, Pakistan, provided with application of an suitable insecticide against these pests on *Bt* cotton [65].

Dhillon [24] studied the effect of four *Bt* cotton hybrids and their non-*Bt* counterparts for the management of BWs and their effects over non target insects and revealed no significant differences in populations of cotton jassids and whiteflies on *Bt* and non-*Bt* cotton. The per cent plants infested with aphids in *Bt* and non-*Bt* cotton were similar. Similarly, no significant differences in populations of ash weevils, red, green and dusky cotton bugs were observed on *Bt* and non-*Bt* cottons.

Udikeri [83] while assessing the impact of *Bt* cotton on dynamics of aphid in RCH 2 *Bt* and non-*Bt* cotton hybrids, reported aphid population range as 8.58 /leaf (34 ISW)-42.15/leaf (50 ISW) with mean as 23.82/ leaf in RCH 2 *Bt* and 6.22-37.08/leaf (46 ISW) with mean 21.37/ leaf in RCH 2 non-*Bt* cotton, respectively, indicating no significant variation.

The reduction in insecticide use and the ineffectiveness of *Bt*-cotton against the sucking and foliage pests has led to a reversal of the ecological role of cotton [58, 59].

The total reliance, and in few cases, the indiscriminate use of insecticides has resulted into the negative environmental and ecological impacts like emergence of secondary pests besides reduction in biodiversity, insect resistance to insecticides and adverse effects on the non-targets such as predatory fauna and insect pollinators [73].

Insect resistance and *Bt* cotton

In few reports, the insects like jassid, aphid, whitefly and pink bollworm have been found to develop several times resistance to the transgenic cotton in India and the world [19, 65, 81].

Kung [56] was first to report the resistance to OP compounds in *A. gossypii* and subsequent studies were resistance to the carbamates [29] and pyrethroids [96]. In the early 1980s, OP compounds were replaced the for aphid control. Aphid evolved 126 folds resistance for deltamethrin while 412 fold for fenvalerate by year 1986 [89]. The high levels of resistance were reported from Xinjang (766 fold) and Shandong (1,835 fold) in the cotton aphid during 1995-96 [14]. Hollingsworth [38] carried out studies with 16 populations of *A. gossypii* from Hawaii and the comparisons of LC50 indicated 3.6 fold resistance to endosulfan, 390 fold resistance to fenvalerate, 9.2fold resistance to methomyl and >2,000-fold resistance to the oxydemeton-methyl.

The LC50 for imidacloprid susceptible strain of *B. tabaci* was worked out as 1.7 ppm and a 16 ppm concentration was determined as diagnostic for imidacloprid resistance. All the 10 strains collected from Almeria (Spain) revealed low insect mortality at diagnostic dose than the susceptible strains. Intensive application of imidacloprid in Almeria was responsible for the occurrence of resistance in this locality [62].

The transgenic insect resistant technology with *Bt* genes was launched to reduce farmers' dependence on insecticide usage for managing Lepidopteran and the Coleopteran pests. The given the application of chemical insecticides causes considerable negative diseconomy in developing countries [84], which is an important direction.

Deguine [20] reported general resistance development in *A. gossypii* to most of the insecticides especially OPs on cotton in Cameroon since 1993. The clones of *A. gossypii* revealed resistance to the monocrotophos and dimethoate under laboratory toxicity tests.

A strain of *A. gossypii* from Southern France was resistant to several insecticides especially primicarb over a susceptible strain (S) and there was highest resistance factor of 1350 [21].

In the evaluation of the susceptibility of cotton jassid to insecticides at TNAU, Santhini and Utthamasamy [74] reported methyl demeton, dimethoate and phosalone effective to reduce jassid population, while methyl demeton significantly superior over rest of the chemicals with varied mortality with populations for Coimbature (70.00%), Annur (66.67%) and Udumalpet (62.33%). The third instar nymphal mortality in the range of 26.67-33.3 per cent in their studies for different locations showed the chemicals at the recommended doses to be ineffective revealing the jassid to pick up tolerance to commonly used insecticides at different locations.

Using leaf dip technique in bioassays, the degree of resistance was ascertained to acephate, endosulfan and cypermethrin among populations of tobacco aphid, *Myzus nicotianae* Blackman drawn from 3 eco-niches, - Guntur, Prakasam and East Godavari districts in Andhra Pradesh. The studies revealed acephate to have 281.3 and 36.21fold while endosulfan 750 and 532.8 fold resistance in populations from Guntur and Prakasam districts, respectively while cypermethrin had 485.5 and 535.8 fold resistance in the respective population over the East Godavari population [15].

The insecticide resistance acquired by jassid population was relatively less on cotton to endosulfan, monocrotophos and cypermethrin at Guntur district than those at Warangal and Kurnool districts in Andhra Pradesh. The studies also revealed Guntur and Kurnool populations to be resistant to the phosphomidon [13].

Jeyapradeepa [43] carried out the resistance monitoring studies for *A. devastans* from six different locations in Tamil Nadu, with the mean resistance as 26.73 for dimethoate, 18.14 for methyl-o- demeton and 19.48 per cent for Viagaidam, whereas the population from Oddanchatram and Viagaidam showed moderate level of resistance, and from Karuppayuani and Kullikulam susceptibility to all the insecticides tested.

Herron [34] in Australia reported the populations of *A. gossypii* to display a high to extreme resistance to OP, endosulfan and pyrethroids and it was linked with control failure that lead to the serious impact on the cotton industry.

Kalra [47] in studies on the toxicity of different insecticides, i.e., malathion, oxydemeton methyl, phosphomidon, dimethoate, thiomethoxam, endosulfan and monocrotophos against jassid in cotton recorded their LC50 values as 1.097, 0.126, 0.112, 0.178, 0.000447, 0.063 and 0.063 per cent, respectively.

Based on generation of baseline susceptibility response to five commonly used insecticides viz., dimethoate, methyl demeton, acephate and imidacloprid in *Amrasca devastans* was generated, where LC50 (ppm) values for dimethoate (seven generations) varied from 41.03 to 153.90; for methyl demeton from 50.32 to 205.92, for acephate (six generations) from 46.02 to 114.79 while for imidacloprid was 0.00056 (one generation) [42].

In the studies on sensitivity of an *A. gossypii* strain to some OPs using Potter tower bioassay technique, the comparison of this strain with a carbamate and OP susceptible strain showed 36.7 folds resistance to dimethoate while there was no difference observed for monocrotophos, methamidophos, and profenophos [67].

Worldwide no sign of field resistance in BWs to Cry 1Ac has been observed during last 10 years in the first generation *Bt* cotton (BG I). In second generation *Bt* cotton, the synergistic effect of two genes i.e. BG II (Cry1Ac+Cry2Ab) could further delay the resistance development in BWs to the insecticidal proteins [41].

Based on evaluation of toxicity of few commonly insecticides against *A. gossypii* there was a shift in the level of its susceptibility after three years. There was increase in LC50 and LC90 values of endosulfan, monocrotophos, dimethoate, acephate and cypermethrin as 1.63, 1.92, 2.84, 1.47 and 2.19, 4.24, 3.42, 5.92, 2.98, 4.13 folds, respectively. The comparison of LC90 values with recommended concentrations of test insecticides also revealed the aphid population of Guntur district to develop resistance to the test insecticides. Acetamiprid was found as highly toxic among various new insecticides followed by diafenthiuron and thiamethoxam recommended for management of *A. gossypii* [12].

A baseline susceptibility data was generated for 6 commonly used insecticides viz., thiomethoxam, imidacloprid, dimethoate, methyl demeton, acephate, and monocrotophos with range of LC50 values as 0.3412-1.0414, 0.4583-1.8055, 3.0096-10.6924, 12.598-49.2606, 1.4615-5.3284 and 1.1866-3.70567 for the field population of *A. gossypii*, respectively [70].

In studies on insecticide resistance in major cotton pests at various cotton regions in Andhra Pradesh, jassid population of Guntur district was resistant to endosulfan, monocrotophos, phosphomidon and cypermethrin [44].

Jhansi [44] monitored the insecticide resistance in major cotton pests in cotton growing districts of Andhra Pradesh and reported *A. gossypii* population of Guntur district to have resistance to endosulfan (1.94), dimethoate (3.66) and cypermethrin (2.5). Similarly, Warangal population of aphid also showed resistance to endosulfan, dimethoate, phosphomidon, carbaryl and cypermethrin. The white fly population of Guntur district also recorded the resistance to BHC, endosulfan, dimethoate, phosalone, acephate, monocrotophos, quinalphos, triazophos and carbaryl.

Bt cotton has shown resistance to the BW complex- *H. armigera, P. gossypiella, E.vitella* and *E. insulana* both under field as well as laboratory conditions [52] but no resistance to the sucking insect pests like whitefly, jassid and aphid [37, 77].

Wang [88] in studies on resistance of *A. gossypii* to fenvalerate, omethoate, imidacloprid, acetamiprid, carbosulfan, and endosulfan in four cotton and one non-cotton growing region at Shandong province in China during 1985, 1999, and 2004, the dose-response results indicated aphid as the highly resistant to fenvalerate, and the resistance ratios (RRs) increased from 30- 370-fold in 1985 to 370-2150-fold from different regions over the susceptible population (S). Aphid also exhibited strong resistance to imidacloprid and acetamiprid with RRs of 17- to 97-fold in 2004. The aphid resistance to omethoate varied greatly among the five geographical regions, and the RRs ranged from 5 to 80-fold. In contrast, the resistance to carbosulfan did not show significant increase from 1999 to 2004 in all the regions.

Kumar [55] studied on insecticide resistance in *A. gossypii* to 5 common insecticides on cotton, viz., monocrotophos, acephate, dimethoate, phosphomidon and triazophos during *kharif* 2005 and revealed a shift in the level of susceptibility to these insecticides. The LC50 and LC90 values were reported to increase as 121.50, 20.00, 9.61, 7.96, 2.38 and 7.68, 3.84, 1.66, 0.60, 0.46 folds to the respective insecticides.

Wang [87] determined the imidacloprid resistance dynamics and cross-resistance in *N. lugens* on rice and revealed the resistance levels in Nanning (Guangxi), Haiyan (Zhejiang), and Nanjing and Tongzhou (Jiangsu) populations to increase in 2005 (200 to 799 fold) over the susceptible strain, but decreased in 2007 (135 to 233fold) with less insecticide application. Similarly, laboratory population was challenged with imidacloprid in successive generations and after 23 generations, the resistance ratio increased (200 to 1298 fold). The imidacloprid increased the resistance level even more with its continuous selection than has already been developed in the population, which decreased the resistance rapidly (759 to 114 fold) with stopping selection after 17 generations and became stable without any further decrease. They also obtained similar results with resistance ratio of 625-fold from field population collected from Tongzhou. Thus, the study is valuable for formulating resistance-management strategies against *N. lugens.*

Similarly, Wen [90] conducted studies on the imidacloprid resistance in field populations of brown plant hopper (BPH) in rice (AQ, NJ, GL and WJ) in China showing field populations BPH to develop moderate to high level of resistance to imidacloprid that was attributed mainly to the enhanced P450 monooxygenases detoxification and could be enhanced in the same growing season at repeated applications of insecticide. In the studies, the imidacloprid resistant hoppers also had not shown cross-resistance to all the neonicotinoid insecticides and the high levels of imidacloprid resistance in BPH were very unstable indicating selection of efficient substitute neonicotinoids and implementation of window control in the resistance management.

Kshirsagar [54] while monitoring the insecticide resistance against cotton jassid revealed moderate to high levels of resistance to the imidacloprid and acetamiprid over the dimethoate that was found as one of the highly effective insecticides tested. When compared to the susceptible strain of cotton jassid, the resistance ratio for imidacloprid, acetamiprid and dimethoate was 23.41, 19.08 and 5.21-fold, respectively.

Transgenic cotton and its eco-toxicological role

The monitoring of *Bt* cotton was failed to show any significant effects on predators, including *C. carnea* [92]. Several efforts were made to determine the effect of *Bt* crops on non target organisms and some negative effects have been reported [36]. The negative side effects on *C. carnea* described in the laboratory [35] have so far not been reflected in terms of reduced populations in the field.

The populations of predators and parasitoids may decline owing to prey or host depletion in highly resistant TPs, but their persistence is not necessarily threatened if other nearby crops support acceptable host or prey species, or if their host range includes species other than the target pests [26]. Field experiments with *Bt* cotton had shown little reduction in beneficial insect populations as a whole, but large scale commercial planting of highly resistant plants is bound to have repercussions for species specific to target pests [27]. Combined effect of natural enemies and sublethal exposure to *Bt* cotton expressing Cry1Ac on the survival of BW larvae (*Helicoverpa zea*). In the laboratory studies, the sublethal exposure was achieved by rearing larvae for 1-4 days on *Bt* cotton before transferring the survivors to untransformed cotton in the field. No difference in *H. zea* survival between transgenic and untransformed plants was observed when natural enemies were excluded. However, when natural enemies were present, larvae exposed to sublethal doses of *Bt* cotton survived at lower rates than larvae reared entirely on untransformed cotton [60].

Minor effect of *Bt* cotton on natural enemy population in comparison with the alternative use of broad-spectrum insecticides was observed which reduced the natural enemies population up to 48 per cent. Most of *Bt* cotton that express Cry1 protecting plants from lepidopteran pest damages and have high level of resistance to primary pest (target pest) especially *H. armigera* [80]. Transgenic cotton caused no harmful impact on chrysopids and coccinellids under field [22]. The impact of *Bt* cotton adoption on farmer pesticide poisoning was analyzed with the result no eco-toxicological effects from a broader perspective [50, 51, 64, 94].

Many investigations in the recent years have examined the effect of *Bt* crops on natural enemies [10]. Based on data from 1990 to 2010 at 36 sites in six provinces of northern China, there was an marked increase in abundance of three types of generalist arthropod predators (ladybirds, lacewings and spiders) while an decreased abundance of aphid pests associated with widespread adoption of *Bt* cotton and reduced insecticide sprays in *Bt* cotton. In an evidence, the predators were reported to provide additional biocontrol services spilling over from *Bt* cotton fields onto neighboring crops (maize, peanut and soybean) [59]. Transgenic cotton did not affect immature parasitoid, *E. formosa* mortality but it affected development time up to adult for *E. formosa*. The parasitoid insect reached the adult stage faster on non-*Bt* over the *Bt* cotton. The effects of transgenic cotton on the parasitoid were complex but generally interpretable in terms of host whitefly quality variation among host plants used as food by the whiteflies during their development [5]. No significant influence of *Bt* cotton on abundance of natural enemies of crop pests viz., chrysopids, ladybird beetles was observed suggesting that there were no adverse effects of *Bt*-cotton on the natural fauna under field conditions [23]. Parasitization of *P. solenopsis* on *Bt* cotton by hymenopteran parasitoids viz., *Aenasius bambawaei, A. dactylopii, Hibiscus eytelweinii, Promuscidea pulchellus* and *P. unfasciativentris* ranged between 7.18 to 61.49% and 16.67 to 75.00%, respectively during year 2007-08 and 2008-09, with peak parasitization of 54.69 and 61.49%, respectively, during 44th and 1st meteorological week [7]. In a review, Singh and Sharma [79] reported several transgenic crops including cotton as the safe to various insect biocontrol agents like predators and parasitoids.

Refuge (non-*Bt* crop) and its impact on pests

The objective of cultivating non-*Bt* refugee around *Bt* cotton is either to reduce the selection pressure and or protect or delay the resistance development in the insect pests to the *Bt* cotton. The low frequency of ABW resistance to Cry1Ac was attributed due to the effectiveness of the implementation of Insect Resistance Management (IRM) strategies in combating resistance build up in the pest populations and the factors which checked such resistance build up in the target pest (s) might be their strict compliance to homozygous *Bt* genes in *Bt* varieties (unlike heterozygous *Bt* genes in *Bt*-hybrids of India) and the refuge strategy. Thus, restricting *Bt* cotton only to merely 30 per cent of area and the timely introduction of dual-gene *Bt* cotton also attributed towards low resistance build up in the target pest.

Gujar [32] studied the impact of structured strip row refugia (10 to 50%) in the *Bt* cotton crops JKCH1947*Bt* (Cry1Ac) and MRC7017BGII (Cry1Ac and Cry2Ab) on the pest complex and cotton yield in 2008 reported a negligible incidence by sucking pests but high incidence of spotted bollworm, *E. vittella*, and the leaf roller, *Sylepta derogata* on non-*Bt* cotton. A total cotton seed yield of *Bt* crop plus the refuge declined proportionately with the increase in proportion of non-*Bt* cotton. They also revealed significant reduction of total cotton production where plantation of 40 per cent non-*Bt* cotton as refuge but 30 per cent non-*Bt* cotton in JKCH1947*Bt* and 20 per cent non-*Bt* cotton in MRC7017*Bt* did not affect total seed cotton yield over 100 per cent *Bt* cotton.

A low compliance to planting refuge crops may favour a rapid evolution of resistance. There is a science behind planting *Bt* cotton with alternating rows of refugee cotton plants. As the insects evolve adapt to their growing conditions and eventually become resistant to the *Bt* toxins. A refuge crop is planted in the same field as the *Bt* crop, which allows some of the targeted insects to survive and produce offspring with some still susceptible to *Bt* cotton while others resistant to it. However, the *Bt*-resistant offspring mate with *Bt*-susceptible insects and produce *Bt*-susceptible offsprings, thus resulting in population reduction of *Bt*-resistant targeted insects in the fields [31].

Transgenic cotton: Threats and challenges ahead

It was Monsanto which introduced first generation *Bt* cotton called Bollgard (BG I) in 2002 and Bollgard II (BG II) in 2006, the latter of which is still the de facto GM cotton variety [76]. The impact of *Bt* cotton was analyzed in developing countries like South Africa [82], China [39], and India [30] that revealed significant decline in pest infestation, increased yield potential with higher profitability to the farmers after adopting *Bt* cotton. Genetically modified (GM) crops were first commercialized in the US, Canada, Mexico, Argentina, China and Australia in 1996 and in 2016 more than 1.8 crore farmers in 26 countries planted GM crops. Before introduction of transgenic cotton, the bollworms were an serious threat to the cotton crop resulting into reduction of 30-40 per cent yield in Pakistan and 20-66 per cent potential losses in India [8].

The transgenic or so called *Bt* cotton expressing the *Cry I Ac* toxin is derived from *B. thuringiensis* successfully controlled various lepidopteran pests, especially bollworm complex which was the major constraint in productivity of cotton [9]. The threats include out-crossing by pollen transfer to non-transgenic plants, food safety concerns, resistance development in the target pests and effects on predatory/beneficial fauna and the biodiversity [11, 25, 78, 93].

The global adoption of *Bt* cotton rose up dramatically from 0.76 million hectares area when introduced in 1996 to 7.85

million hectares in 2005 cotton-growing season and 54 per cent cotton in United States, 76 per cent in China, and 80 per cent in Australia was grown a single or the multiple *Bt* genes [8].

Maharashtra neighbour, Gujrat grows more cotton than any other state in India. A high cotton seed price compelled the growers to use illegal non-approved *Bt* cotton crop that caused huge damage to crops because of low tolerance to insect pests. Also the *Bt* cultivars are more sensitive to several factors like internal crop phenology, atmospheric changes (CO2 concentration), heat stress, nutrition, insect pests, boll distribution pattern, diseases and nematodes, removal of fruiting branch and/or floral bud, introduction of *Bt* gene, and terpenoids and tannin production in the plant body are responsible for changes in the efficiency of *Bt* gene and *Bt* cotton yield include [8], and therefore, their performance needs be investigated.

India, the world's biggest cotton producer, has the 5th largest area under GM crop cultivation, and *Bt* cotton seeds account for 40 per cent of the Rs.14,000 crore nation seeds market [76].

Any new technology has its benefits as well as the threats; benefits associated with the use of transgenic crops involve reduced conventional and broad-spectrum insecticidal sprays and the target pests, improved yield, low production costs, and more compatibility over the other biological control agents [3].

Future of *Bt* cotton for pest control

In several developed and developing nations, the *Bt* cotton has promised the increased income of the growers. The new *Bt*-strains are reported on a regular basis, especially new proteomics methods can be utilized to screen for novel toxins over a large scale. The *Bt* genes introduced to the cotton plants conferring insect resistance is major success in terms of protection levels afforded by expression of *Bt*-toxins.

The first generation (BG I) cotton crop (resistant only to the BW complex) has been extraordinarily successful with a few reports of insect pests evolving resistance and this first generation cotton has already been supplanted with BG II varieties (resistant to BW complex and the tobacco caterpillar) which possess more resilient traits generated through stacking and pyramiding resistance genes and further efforts for searching more effective and potent strains are not ending [17].

In the forthcoming years, BG III plants have been designed to prevent or delay the onset of resistance and provide there more durable levels of protection. The science of biotechnology would increase the chances to achieve the objectives for achieving the multi-mechanistic resistance in cotton. Evaluating the *Bt* cotton having insecticidal genes in the fields is a vital component of overall process for creating and deploying insect resistant *Bt* plants which are useful and sustainable [46].

2. Conclusions

Globally, the wide adoption of cultivation of transgenic cotton resulted in increase of 31.80 million hectares area during 2017-18 over increase of area from 0.76 million ha since 1996-97. Maximum area under *Bt* cotton is covered by countries such as Australia (80%) followed by China (76%) and the USA (>50%) with single or multiple *Bt* genes. Prior to its release, the BW complex was a major threat on non-transgenic cotton that resulted into 30-40 per cent yield loss in Pakistan and 20-66 per cent in India. Adoption of new technology in the form of transgenic cotton involved several benefits and challenges. The transgenic cotton has several advantages like as reduced insecticidal load, control of bollworm pests and more yield potential. In contrast, the farmers due to higher seed prices are using illegal non-recommended and low tolerance *Bt* cultivars that caused huge damage to the crop due to insect pests and bollworms (especially *P. gossypiella)* and sucking pests have resistance to such cultivars. Besides few other factors responsible for changing the efficiency of *Bt* gene and crop yield are internal crop phenology (genetics), CO2 concentration, nutrition, insect pests, boll distribution pattern, diseases and nematodes, removal of fruiting branch and/or floral bud, introduction of *Bt* gene, and terpenoids and tannin production in the plant body, etc. It is evident that due to lower insecticide use, secondary pests that are not susceptible to the expressed toxin have become an increasing concern in some agro-ecosystems where *Bt* cotton is grown. In nutshell, the success of transgenic cotton is still an unanswered task in the developing nations.

3. References

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