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Resistance development in bollworms against Bt proteins deployed in genetically modified cotton

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Abstract
In this review, illustrated by examples, there were rare instances where dominant resistant alleles were found. Reason of resistance in fields may include different concentration of Bt protein used in different regions, this low concentration causes less binding of toxins to insect midgut. Mostly resistance development was observed under laboratory conditions against Cry proteins which are inserted in transgenic cotton. Under laboratory conditions, cadherin was found to be responsible for development of resistance in bollworm. Resistance developed in the boll worms was related to recessive alleles. Different strategies are used to delay the resistance. Development of pyramided cotton, stacking genes, refuge strategies can be helpful to show enhanced effects against resistance developed target pests. Such strategies can only delay resistance development for short time but cannot completely eliminate the resistance development. Further research is needed to know the reason for development of resistance in insects against expressed Bt proteins in transgenic cotton.

Keywords: Boll worms, transgenic cotton, resistance development, GMO's, Bt crop

1. Introduction
Genetically modified (GM) crops were developed to show the resistance against major insect pests. First generation plants containing insecticidal proteins from showed better resistance against target insects and second generation Bt plants are also containing novel approaches to control pests [1]. Transgenic cotton was found to be successful in terms of high productivity in Punjab, Pakistan [2]. Field trials of transgenic cotton showed less pest damage and more yield [3]. GM cotton expressing Cry1Ac and Cry2A was found to show resistance against target insects against *Helicoverpa armigera* under field conditions in Pakistan [4]. Bt cotton integrated with insecticides spray was also found to show more resistance against spotted boll worm as compared to control [5]. Some issues of Bt crop including non-target effects against insects pests, biological agents and development of resistance in insects are required to be observed under laboratory, semi-field and field conditions. The environmental effects of Bt crops can result in passing on DNA from transgenic crops to the environment which can be uptaken by other organisms [6]. Bt crops can result in effects on non-target organisms. The population of non-target pests can be helpful in determining non-target effects of Bt crop [7]. Increase in attack of secondary insects such as mites, aphids and bugs were observed in Bt cotton in China due to less pesticides [8]. However, resistance development in the target insects is major issue regarding Bt cotton. This paper will discuss the probable reasons for resistance development in boll worms against Bt cotton and strategies to reduce this developed resistance.

2. Development of resistance in insects against GM crops
The evolutionary capacity, insect ecology and cultivar performance will be helpful to consider the biochemical and genetic adoption of insects to GM crops [9]. *Alabama argillacea* was tested against transgenic cotton to sort out resistance development and management, feeding and dispersal behavior was found different on Bt and non-Bt cotton for neonate larvae of *Alabama argillacea* [10]. No field evidence has been documented for increased resistance, in Arizona 5 year field trials of *P. gossypiella* were conducted; 6 year trails of *O. nubilalis* were conducted in USA and in Northern China 3 year trials of *H. armigera* were conducted [11]. In another experiment it was described that resistance was surprisingly absent from the insects in case of GM crops [12].

Table 1: Different cotton cultivars or genotypes showing resistance to the target insects

Expressed gene/ Bt cotton Cultivars	Bt protein active against the insect species	References
MON62, MON65, MON81, MON82, MON84, MON249	*Heliothis virescens, Helicoverpa zea*	[13]
Cry1Ac	*Helicoverpa zea, Heliothis virescens*	[14]
GK-12, GK-2, R108, and NuCOTN 33^{B}	*Helicoverpa armigera*	[15]
NuCOTN33^{B}, Delta and Pineland 5415	*Pectinophora gossypiella*	[16]
Cry1Ac	*Helicoverpa zea, Spodoptera frugiperda*	[17]
Cry1Ac	*Helicoverpa armigera*	[18]
Cry1Ac	*Earias vitella*	[19]
KMG-1, KMG-2, KMG-3, MS-1, MS-2, NIAB-78, CRIS-134	*Earias vitella, Spodoptera exigua*	[20]
Cry1Ac/Cry1Ab and Cry1Ac gene	*Helicoverpa armigera*	[21]
Cry1Ac	*Helicoverpa armigera*	[22]
Vip3A and VipCot	*Helicoverpa zea, Heliothis virescens*	[23]

3. Efficacy of GM cotton against bollworms

Bt cotton showed a substantial resistance against boll worms (please see table 1). *Heliothis virescens* was found to be susceptible to the six Bt cotton cultivars as compared to control lines. Less feeding was observed in laboratory experiments. While under greenhouse and field conditions lower damage were observed. For *H. zea* less damage for buds and bolls were observed on transgenic cotton cultivars as compared to control [13]. Effect of Bt cotton expressing Cry1Ac on survival and development for larvae and pupae in *H. zea* and *H. virescens* [14]. Comparative study of insecticidal activity of Bt protein of four transgenic cotton lines including GK-12, GK-2, R108, and NuCOTN 33^{B}, among which NuCOTN 33^{B} was developed by Monsanto company while other three lines were developed by China, GK-12 and NuCOTN 33^{B} showed higher resistance against *H. armigera* under laboratory and field conditions [15]. In another experiment, NuCOTN 33^{B}, Delta and Pineland 5415 were found to show better control of pink bollworm as compared to non-Bt cotton in artificially infested studies [16]. *H. zea* and *S. frugiperda* were tested against Cry 1Ac. Larval survival of *H. zea* and development *S. frugiperda* were found to be correlated to the Bt toxin concentration in different plant parts [17]. Cry1Ac expressing Bt cotton showed promising resistance against the *H. armigera* larvae survival. Under field conditions lower larval densities were found in Bt cotton as compared to the control varieties [18]. Cry1Ac against *Earias vittela* was tested from different regions in India. Variable toxicity was found in different areas when collected in 2001-02 [19]. Seven transgenic cotton varieties and genotypes were tested for field performance against insect pests infestation under laboratory and field conditions. Response of target insects varied from different cotton cultivars and genotypes [20]. Bt transgenic cotton carrying *Cry1Ac/Cry1Ab* fused gene and C*ry1Ac* in GK19 and BG 1560 were investigated in under laboratory and field conditions in China. Population dynamics of larval densities showed that Bt toxin varied in the Bt cotton plants with time and growth. The field studies revealed that population dynamics were decreased in Bt cotton fields [21]. Cry1Ac expressing cotton showed promising control of *H. armigera* in cotton planting region Punjab, Pakistan and results found that larval densities were quite lower in Bt cotton fields as compared to control [22]. Bt cotton expressing Vip3A or Vip3A+Cry1Ab showed promising effects against the *H. zea* and *H. virescens* under field conditions during 2005-07. Bt lines were having less fruiting damage and lower larval survival as compared to control [23]. Double Bt toxins were found to be more effective in bollworms control as compared to the single toxin Bt cotton. It was found that population of *Spodoptera exigua* and *Pseudophusia includes* were significantly lower in Bollgard II as compared to Bollgard, population of *Spodoptera furgiperla* and *Estigmene acrea* were also lower in Bollgard II as compared to Bollgard but not significantly difference was found, and dual toxins expressing cotton was more successful in case of *Heliothis virescens* [24].

Table 2: Development of resistance in boll worms against Bt protein.

Bt protein	Insect Species	Effects found		Reference
		Laboratory studies	Field studies	
Cry 1Ac	*Pectinophora gossypiella*	Yes	-	[25]
Cry 1Ac	*Helicoverpa armigera*	Yes	-	[26]
Cry 1Ac	*Pectinophora gossypiella*	-	No	[27]
Bt cotton	*Pectinophora gossypiella*	-	Yes	[28]
Cry1Ac	*Pectinophora gossypiella*	No	-	[29]
Cry1Ac	*Pectinophora gossypiella*	Yes	-	[30]
Cry1Ac	*Helicoverpa armigera*	Yes	-	[31]
Cry1Ac	*Heliothis virescens*	Yes	-	[32]
Cry1Ac	*Pectinophora gossypiella*	Yes	-	[33]
Cry1Ac	*Pectinophora gossypiella*	Yes	Yes	[34]
Cry1Ac	*Helicoverpa armigera*	Yes	Yes	[35]
Cry 2Ab	*Helicoverpa armigera*	-	No	[35]

4. Development of resistance in boll worms against Bt proteins deployed in transgenic cotton

From table. 2, transgenic cotton in Arizona expressing Cry1Ac showed resistance against the pink boll worm. It was found that in laboratory only two strains were susceptible but in the field four strains were found susceptible to the Bt cotton [25]. Less resistance was found to the *H. armigera* first instar when used Cry1Ac expressing Bt cotton [26]. *P. gossypiella* did not show increased resistance development against Cry1Ac in the fields of Bt cotton [27]. In Arizona,

transgenic cotton was observed for the insect resistance at large scale for pink boll worm, because it has more genetic potential to develop resistance [28]. No effect of Cry 1Ac on *P. gossypiella* under laboratory conditions were observed [29]. It was found that there was resistance development in *P. gossypiella* against Cry 1Ac under laboratory conditions [30]. In another studies also found that *Helicoverpa armigera* showed the developed resistance under laboratory conditions [31]. *Heliothis virescens* was also found to develop resistance against Cry 1Ac under laboratory conditions [32]. *P. gossypiella* showed resistance against Cry 1Ac under laboratory conditions [33]. *P. gossypiella* showed resistance against Cry 1Ac under laboratory and field conditions [34]. *Helicoverpa armigera* showed development of resistance against Cry 1Ac under laboratory and field conditions [35]. *H. armigera* showed no resistance development against Cry 2Ab under field conditions [35]. Cadherin gene was found involved in resistance against Cry1Ac in *H. armigera* [36].

Table 3: Different strategies to delay resistance development in target insects in GM crops.

Expressed gene/ GM crop	Bt protein active against the insect species	Strategy followed	Effects	References
Cry1Ac/Cry1C (Broccoli)	*Plutella xylostella*	Pyramided genes	+ive	[37]
Bt Cotton	Beet army worm,	Refuge strategy	+ive	[28]
Cry 1Ac	Cabbage looper *Pectinophora gossypiella*	Higher Bt concentration	+ive	[38]
Cry1Ac and Cry2Aa	*Heliothis virescens*	Brush border membrane vesicles	-ive	[39]
Bt cotton	*Pectinophora gossypiella*	Sterile insect technique	+ive	[40]

5. Strategies to delay resistance in transgenic crops

From table 3, pyramided genes in GM plants were found to be helpful in delaying resistance development in insects such as *Plutella xylostella* which was less resistant to two pyramided Bt toxins Cry1Ac and Cry1C [37]. While refuge strategy was observed successful for beet army worm and cabbage looper [28]. It was found that dominance of resistance depends upon concentration of Bt toxin, if concentration of Bt protein Cry 1Ac will be higher in transgenic cotton, itcan result in recessive inheritance of resistance in pink boll worm [38]. Gene stacking of Cry1Ac and Cry2Aa under laboratory conditions showed possibility of cross resistance in case expressed in co-occurrence [39]. Sterile insect technique for pink boll worm was also found successful in transgenic cotton [40].

Transgenic cotton has different scenario as compared to other transgenic crops. In Bt cotton field population only recessive alleles were found to show resistance. Early detection of pink boll worm resistance development against Bt cotton can be helpful to develop strategies to reduce further development in Bt crops in future in China [41]. A study conducted on observing the resistance development besides the refuge crops in Bt cotton in Arizona found that distance of the Bt cotton and refuge should not be more than 1 mile. Beet armyworm, cabbage looper were found to have less chances to develop resistance against Bt cotton in Arizona as compared to pink boll worm [28]. Although research has been initiated to delay the resistance development in insects against Bt toxin but still lot of research is required to stop the resistance development in insects from practical point of view [42] [43].

Reports of molecular basis of resistance development in insects against GM crops were published, in which it was assumed that less Bt concentration caused more resistance in insects due to lower binding of toxin in insect midgut [44]. In another report, high concentration of Bt protein and small size of refuge area was found enough to reduce the level of resistance development in insects in GM crops [45]. However, in China zero refuge strategy for Bt cotton against boll worms can be acceptable on basis of its economic analysis, scientific data and simulation using bio-economic model [46].

6. Conclusion

It can be ascertained that resistance has been developed in bollworms against the Bt proteins which are deployed in Bt cotton. For Bt cotton, only few field evidences were observed. Resistance was observed in bollworms against Cry proteins (especially Cry 1Ac) at laboratory scale. However, recessive resistance was observed in case of experiments conducted under laboratory conditions. Some strategies can be helpful to delay resistance development in boll worms such different Bt toxins in plant, pyramided toxins strategy, refuge strategy in the Bt cotton. These strategies can delay resistance in bollworms but cannot stop the resistance development in future. A strategy should be developed through Integrated Resistance Management to reduce the resistance development in insects.

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