



# Vulnerability of transgenic cotton to Hemipterans

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## Abstract

The Bt plants selected for this study were tested for the Bt gene integration which was confirmed by PCR analysis. The plants were grown without applying any pesticides. The data obtained showed that the incidence of sucking pests was drastically high in Bt cotton than in their non Bt counterparts. This may be due to the non availability of lepidopterans for sucking pests as a competitor. The farmers had to pay extra money for the seeds as well as the pesticides. This study reveals the possibility of Bt cotton, drastically affecting the biodiversity of certain types of insect pests.

## Keywords

Bt cotton, PCR, Hemipterans , Lepidopterans, pest incidence.

## Introduction

Economic crops such as wheat, rice, cotton, potato, canola and tobacco are presently raised from seeds endowed with genetically modified features like improved nutritional quality and better resistance to herbicides, viruses, insects and certain diseases (Ali *et al.*, 2008). Six biotechnologically derived crops - canola, corn, cotton, papaya, squash and soybean have already been introduced into the commercial arena (Christou *et al.*, 2006).

The transgenic Bt cotton plant expresses a crystal (cry) toxin called cry 1 Ac, originally derived from the soil bacterium, *Bacillus thuringiensis* which is a natural enemy of cotton bollworm and the endotoxins produced by bacteria are proved to be effective against lepidopteran insects (Prasad *et al.*, 2009).

The key documented benefits of Bt cotton are, 70 percent reduction in insecticide application in Bt fields resulting in a substantial saving in insecticide costs and an 80 – 87 percent increase in cotton yield. Thus, dramatic reduction in pesticide application in Bt cotton fields has also been reported in China and the proportion of farmers with pesticide poisoning has been reduced from 47 to 20 percent (Christou *et al.*, 2006)

Unlike metabolic inhibitors, endotoxins produced by transgenic crops (Bt transgenes) cause 100 percent mortality to the lepidopteran larvae at comparatively low concentrations. Endotoxins are not toxic to vertebrates and are highly target specific to lepidopteran hosts (Tuli *et al.*, 2000). The potential ecological benefits of transgenic plants are, reduced environmental impact from pesticides, increased yield, soil conservation and phytoremediation, since documented evidences show that transgenic plants can sequester heavy metals from soils and detoxify pollutants (Lovei, 2001).

Eventhough Bt cotton imparts many benefits, it has a few drawbacks that often lead the Bt technology into controversy. Bt cotton is not reachable to lepidopteran pests like the cotton bollworm (*Helicoverpa armigera* Hubner.) whereas it does not afford any protection against sucking pests of cotton especially, the hemipterans.

## Materials and methods

### Polymerase Chain reaction

Genomic DNA extraction was carried out from tender leaves of both Bt and non Bt cotton plants collected randomly. The primers used were specific to cauliflower

mosaic virus 35 S promoter region and NOS terminator respectively. Primers were synthesised by Helini Biomolecules, Chennai, Tamilnadu.

DNA was amplified in an Eppendorf thermocycler, programmed for a first denaturation step of 2 minutes at 94 °C followed by 35 cycles at 94 °C for 1 minute, at 63 °C for 1 minute and at 72 °C for 1 minute. A final extension at 72 °C was carried out for 10 minutes. The PCR products were then stored at 4 °C until electrophoresis was done. PCR products were separated on a 2% agarose gel prepared with 1x TAE buffer. The stained gel was visualized under ultraviolet trans illuminator (Daud *et al.*, 2009).

**Methodology**

The source of this study is the Bt and non Bt bunny cotton planted in the laboratory field (5 plots each 3m×3m) in May 2011. Imidacloprid treated seeds for sucking pests were used. The plants were grown in unprotected condition. No sprays were used after planting. The incidence of hemipterans and lepidopterans were recorded once in 10 days interval. The pests such as whitefly *Bemisia tabaci* Gennadius, mealybug and cotton boll worm were recorded randomly from leaves each one from top, bottom and middle of the plant. The data obtained was subjected to statistical analysis (Prasad *et al.*, 2009).

**Results**

**Polymerase chain reaction**

In this experiment, control reaction is done using plant specific primer designed to amplify a region around 700bp length from plant chloroplast gene. PCR results confirmed the integration of Bt gene in the plants.

**Pest incidence**

From the observation (Table 1) it was very clear that hemipterans attacked the Bt cotton more than their non Bt counter parts. In our observations in the cotton plantation made in May 2011, the number of Hemipteran pests and the damage caused by them were more in transgenic cotton than in non transgenics. The hemipteran pest incidence in Bt was significantly larger than that in non Bt. The lepidopteran pest incidence in Bt was significantly lesser than that in non Bt.

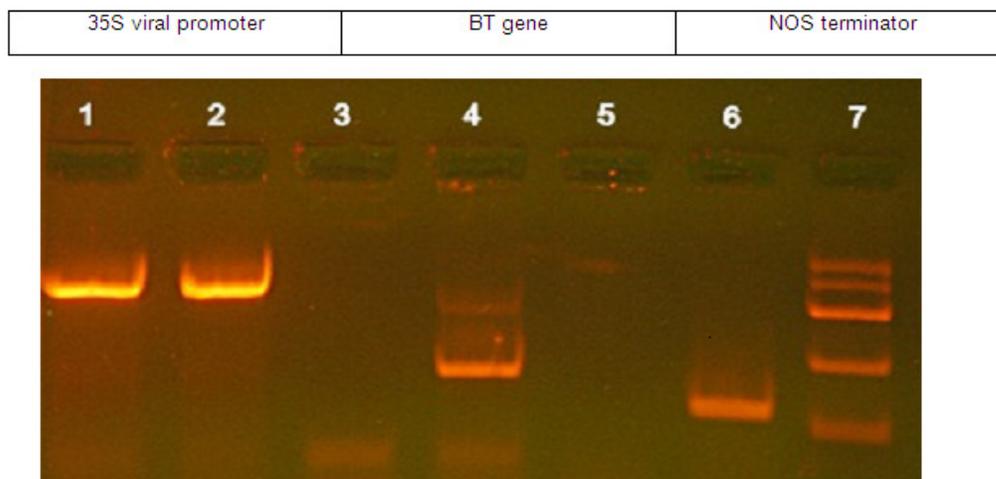
For the first 30 – 35 days after germination, there were no or lesser number of pests in Bt cotton as the seeds were pretreated with imidocloprid. But when the crop was about 80 – 90 days old, moderate to heavy infestation of aphids and white flies was observed, prominently in Bt than in non Bt crop.

**Discussion**

The attack by sucking pests on Bt cotton has already been reported by various authors. Reed *et al.* (2000) observed that transgenic plants were infested by a number of sucking pests. Bambawale (2004) reported that the incidence of sucking pests was more or less similar in both Bt and non Bt hybrids. However this result was contradicted by Radhika *et al.* (2004). Abro *et al.*, 2004 and Cui and Xiu, 2000 reported that the incidence of sucking pests was high in Bt hybrids than their non Bt counterparts.

In 2007, in the Malwa belt of rural Punjab, a large area of Bt cotton fields was attacked by mealy bug pests (Goswami, 2007). Mealy bugs, being small sap sucking insects caused severe economic damage to a wide range of vegetables, horticultural and field crops.

Fig. 1 Polymerase chain reaction



Lane 1: Control Leaf sample with control rbcL gene  
 Lane 2: BT cotton leaf sample with control rbcL gene  
 Lane 3: Control leaf sample with 35S specific primer

Lane 4: BT cotton leaf sample with 35S specific primer  
 Lane 5: Control leaf sample with NOS specific primer  
 Lane 6: BT leaf sample with NOS specific primer

Lane 7: HELINI Quickref DNA ladder [100bp, 250bp, 500bp, 750bp, 1000bp]

**Table 1: Pest incidence in Bt and nonBt under unprotected condition**

Days	White fly (X)		Mealybug (Y)		Lepidopteran (Z)	
	Bt (X <sub>1</sub> )	NonBt (X <sub>2</sub> )	Bt (Y <sub>1</sub> )	NonBt (Y <sub>2</sub> )	Bt (Z <sub>1</sub> )	NonBt (Z <sub>2</sub> )
10	0	0	0	0	0	0
20	4	0	3	0	0	0
30	23	3	9	0	0	1
40	41	7	17	2	0	3
50	47	12	24	7	0	3
60	53	19	41	13	0	7
70	76	25	49	16	3	7
80	81	20	51	12	2	9
90	97	34	42	17	3	8
100	77	27	40	21	2	6
110	63	21	32	28	0	3
120	61	17	21	23	0	2

n=12, v=11

Mean  $\bar{X}_1=51.92$   $\bar{X}_2=15.42$   $\bar{Y}_1=27.42$   $\bar{Y}_2=11.58$   $\bar{Z}_1=0.83$   $\bar{Z}_2=4.08$

Sum

of Squares  $X_1=10264.92$   $X_2=1350.92$   $Y_1=3446.92$   $Y_2=1054.92$   $Z_1=17.67$   $Z_2=110.92$

Pooled

variance  $X=527.99$   $Y=204.63$   $Z=5.85$

Standard

error  $X=9.38$   $Y=5.84$   $Z=0.987$

T value  $X=3.89$   $Y=2.71$   $Z=3.29$

$t_{0.05(2,11)}=2.228$

Plate1 White fly infestation



Plate 2 Mealy bug infestation



White fly is highly polyphagous and has been recorded on a wide range of cultivated and wild plants. However, the magnitude of infestation and the nature of extent of injury varied with plant species, seasons and localities (Lanjar and Sahito, 2007). The white fly, which attacked the cotton plants, was identified as *Bemisia tabaci* (Genn.). Wilson *et al.*, (1992) reported that the number of *B. tabaci* Genn. on transgenic cotton plants was significantly higher than on non transgenics. This was probably due to the reduced leaf feeding damage by lepidopteran insects and not because of higher white fly susceptibility of Bt cotton. *B.tabaci* is a very small insect and its small size belies its ability to move

relatively large distances locally placing many hosts within communities at the risk of infestation.

The infestation of Bt cotton by hemipterans affected pest biodiversity as lepidopterans were completely eliminated. Several scenarios predict, “irreversible and catastrophic” harm to the biodiversity due to the use of GM crops (Conner *et al.*, 2003). Since Bt cotton only targets the boll worm, outbreak of sucking pests such as jassids and aphids predominated. This is inevitable in nature- when one pest is targeted and controlled in an artificial manner, other pests attack in greater numbers and nature's balance is biased and tilted.

With a sudden resurgence of hemipteran pests, applying synthetic pesticides became inevitable. Thus the pesticide cost added to the high cost of Bt cotton seeds. About 450 g of Bt cotton seeds (along with 100g of non Bt seed for refuge planting) was priced at Rs. 1600 (about \$32). Farmers normally used 2.5 kg/ha of seed. So the actual cost of Bt cotton seed was Rs 3500/ ha (about \$70), about \$50/ ha more than non Bt cotton (Zhang *et al.*, 2004). The high seed cost outweighed the advantages. The so called magic bullet Bt cotton had turned into a bitter pill for farmers (Goswami, 2007).

When Bt cotton was planted extensively, there was a possibility for drastic changes in the biodiversity pattern of hemipterans. Hemipterans were unduly favoured, while lepidopteran populations shrunk. Hemipterans were likely to attack normal populations where regular crops (Non Bt) were raised. In the long run, the skewed biodiversity could harm the ecosystem at large.

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