Short Communication

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VERTICAL FLOW OF *BT* GENES IN TRANSGENIC COTTON (*Gossypium hirsutum* L.)

S. Ramzan[1], Mahmood-ur-Rahman[1*], T. Shaheen[1], K. Hussain[1], M. Qasim[1], M. Asif[2] and S. A. Bukhari[3]

[1]Department of Bioinformatics and Biotechnology, [3]Department of Applied Chemistry and Biochemistry GC University – Faisalabad 38000, Pakistan; [2]Cotton Research Institute, Ayub Agricultural Research Institute, Jhang Road, Faisalabad, Pakistan
*Corresponding Author E-mail: mahmood1233@yahoo.com; mahmoodansari@gcuf.edu.pk

ABSTRACT

Vertical gene flow is a major ecological concern where transgenic crops are under cultivation. Rate of vertical gene flow is helpful to determine genetic contamination of Bt and non-Bt cultivars. Vertical gene flow was determined by analyzing the DNA isolated from leaf samples. The DNA was amplified by PCR using gene specific primers for various Bt genes, e.g. MON-531 event, *cry1Ac* and *cry2A* genes. Total 316 samples including Bt (76 samples) and *non-Bt* (240 samples) cotton were analyzed. Bt cotton samples, showed 22%, 15% and 9% gene flow from non Bt cotton for MON531 event, *cry1Ac* and *cry2A* respectively. Non-Bt cotton resulted in 20%, 10% and 10% gene flow from Bt cotton for MON531 event, *cry1Ac* and *cry2A* respectively. The results clearly showed that MON531 event has major share in vertical gene flow in transgenic cotton in Pakistan. Our results indicated the current position of genetic contamination due to transgenic cotton in Pakistan. The percentage of gene flow will increase every passing year. So, here we recommend to cultivate Bt cotton according to the recommendations of National Bisafety Committee (NBC) and use of proper measures to control vertical gene flow.

Keywords: Bt cotton, *cry1Ac* gene, *cry2A* gene, Genetic contamination, MON531 event, Vertical gene flow.

INTRODUCTION

Cotton is the first crop which is genetically engineered, transformed and is being grown commercially. Transgenic cotton transformed with genes derived from soil bacterium *Bacillus thuringiensis* (*Bt*), which express cry toxins and it is shown to be effective against many Lepidopteran insects (Stewart *et al.* 2001; Tabashnik *et al.* 2008). Genetically engineered crops may spread Bt genes from one individual to another and from one place to another and in this way act as a source for gene flow. It causes change in the frequency of genes or alleles in a population (Ellstrand, 2003; Cerdeira and Duke, 2006).

Pollens and seeds are a source for gene flow. Gene flow by pollens is vital process, the export of seeds cause intentional dispersal of seeds at long distances and in this way transgenes spread (Squire, 2005; Pons *et al.* 2011; Scorza *et al.* 2013). Mechanisms for natural dispersel of seeds involve wind, water or animals while humans also disperse seeds by various ways. Generally, natural means of seeds dispersal work on short distances at about 10 meters from source fields but by human actions, seeds can be dispersed at longer distances because seeds are more persistent than pollens (Squire, 2005; Scorza *et al.* 2013).

In cotton mostly self pollination occurs but pollinating insects are a source for cross pollination and it occurs readily. Consequently if *Bt* and non-*Bt* plants grown in close proximity then out-crossing occurs and results in *Bt* gene transfer to seeds of *non-Bt* refuge bolls (Zhang *et al.* 2000). In china, out-crossing rate is reported up to 10.5% of *Bt* genes to non *Bt* plants grown in adjacent rows (Zhang *et al.* 2005). Pakistan is cultivating Bt cotton for the last more than a decade but there are no data available regarding gene flow. So, this project was designed to determine the frequency of gene flow in transgenic cotton in Faisalabad region.

MATERIALS AND METHODS

Experimental Material and Sample Collection: Leaf samples of non-Bt and Bt cotton were collected from fields of four different locations of Faisalabad. Total of 316 (Three hundred and sixteen) samples of Bt and non-Bt cotton were collected including 240 samples of Non-Bt and 76 samples of Bt cotton. The leaf samples were brought to the laboratory and stored at -20 °C till further analysis.

DNA isolation: DNA was isolated form leaf samples collected from different locations by using the procedures developed by Doyle and Doyle (1987).

Polymerase Chain Reaction (PCR): The extracted DNA was used as a template in PCR analysis. DNA extracted from non-transgenic plants was used as negative control while DNA isolated from confirmed transgenic plants was used as positive control. PCR reaction was carried out in 20µl of reaction volume using genomic DNA (as template) 200 ng, gene/event specific forward and reverse primers 20 pmol each (**Table – 1**), dNTPs 200 mM, PCR buffer 1X (50mM KCl, 2.5mM $MgCl_2$ and 10mM Tris-HCl pH 9.0) and Taq polymerase 0.5 unit in a PCR machine under the following conditions: Initial denaturation at 98 °C for 5 minutes; denaturation at 94 °C for 1 minute, Annealing at 55 °C (for cry1Ac and cry2A) and 58 °C (for MON531 event) for 1 minute, extension at 72 °C for 1 minute (30 cycles); final extension for 72 °C for 10 minutes and stop at 4 °C.

Frequency of vertical gene flow of *Non-Bt* genes: Frequency of cross-pollination was calculated by the procedure described by Mahmood-ur-Rahman *et al.* (2007) and Noor *et al.* (2010).

RESULTS AND DISCUSSION

This study was commissioned to monitor gene flow in genetically modified (GM) cotton. GM pollens or seeds cross pollinate with non-GM plants and produce hybrids which then stabilized in populations by introgression of transgenes. Although the frequency of hybrids varied among non transgenic populations (0-29%) grown alongside to transgenic populations. There are several ways of vertical gene flow of *Bt* genes such as pollens and seed dispersal. In *non-Bt* cotton, the average rate of gene flow was 13.3% while in *Bt* cotton samples, it was 15.3% (**Table – 2**).

Total 316 cotton samples were PCR amplified (**Figure – 1**). Out of these, 76 samples were of *Bt* cotton and 240 samples of *Non-Bt* cotton. Out of 76 *Bt* cotton samples 59 were positive and 17 were negative for MON-531 primers and showed 22% gene flow, 64 were positive and 12 were negative for *cry1Ac* genes showing 15% gene flow and PCR amplification for *cry2A* gene showed 69 positive and 7 negative samples, and 9% gene flow was calculated. *Non-Bt* samples were also PCR amplified and the results shows that out of 240 samples 44 were positive and 196 were negative for MON-531primers and revealed 20% gene flow, 24 were positive and 216 were negative for *cry1Ac* showing 10% of gene flow and 24 samples were positive and 216 were negative for *cry2A* and revealed 10% gene flow.

Distance is an important factor which influence cross pollination. In cotton transgene flow rarely observed but pollen-mediated gene flow of the *cry1Ac* transgene observed below 1% at field edges. Shen *et al.* (2005) observed transgene flow from GM cotton in China. Results of this study showed that gene flow occurred at distances of 0-6 m at maximum and it decreased as distance increased. In self pollinating wheat *Triticum aestivum*, the overall outcrossing was 3.4% as a result of cross pollination in close proximity and at the distance of 0.5-2.5m, the cross pollination rate declined from 0.7% to 0.03% (Rieben *et al.* 2011). Henry *et al.* (2003) showed up to 95 % out-crossing rate in maize in GM and conventional crops. Previously <1.0% gene flow was studied in Bt rice in Pakistan (Mahmood-ur-Rahman *et al.* 2014).

Out-crossing between *Bt* and *non-Bt* cotton decreases with distances of 20 m within two crops (Zhang *et al.* 2005). Unharvested buffers of non-transgenic plants are commonly used as a sink to contain transgenic pollen (Beckie *et al.* 2006). Cotton is low out-crossing crop and gene flow can be detected by sampling at the edges more efficiently (Goggi *et al.* 2006; Bannert and Stamp, 2007; Heuberger *et al.* 2010).

Table – 1. List of primers for the detection of *cry1Ac, cry2A* and MON531 event in cotton leaf samples

S. No.	Primer Name	Sequence (5' - 3')	Target Gene/ event	PCR Product Size (bp)
1.	AC-F	AAGTGCCAATGCTTTTACAT	*cry1Ac*	410
2.	AC-R	CTTGGATGGTAATCCCTGTA		
3.	2A-F	CTTCAAGTACCAGTCCCTCA	*cry2A*	296
4.	2A-R	AGTTATGGTTGAGGTTGGTG		
5.	531-F	AAGAGAAACCCCAATCATAAA	MON531 event	346
6.	531-R	GAGAATGCGGTAAAGATACGC		

Table – 2. Percent gene flow in transgenic cotton in Faisalabad region

S. No.	*Bt*/*Non*-Bt	Total Samples	Gene/event	PCR Positive	PCR Negative	% Gene Flow
1.	*Bt*	76	MON531 event	59	17	22
			cry1Ac	64	12	15
			cry2A	69	7	9
2.	Non-*Bt*	240	MON531 event	44	196	20
			cry1Ac	24	216	10
			cry2A	24	216	10

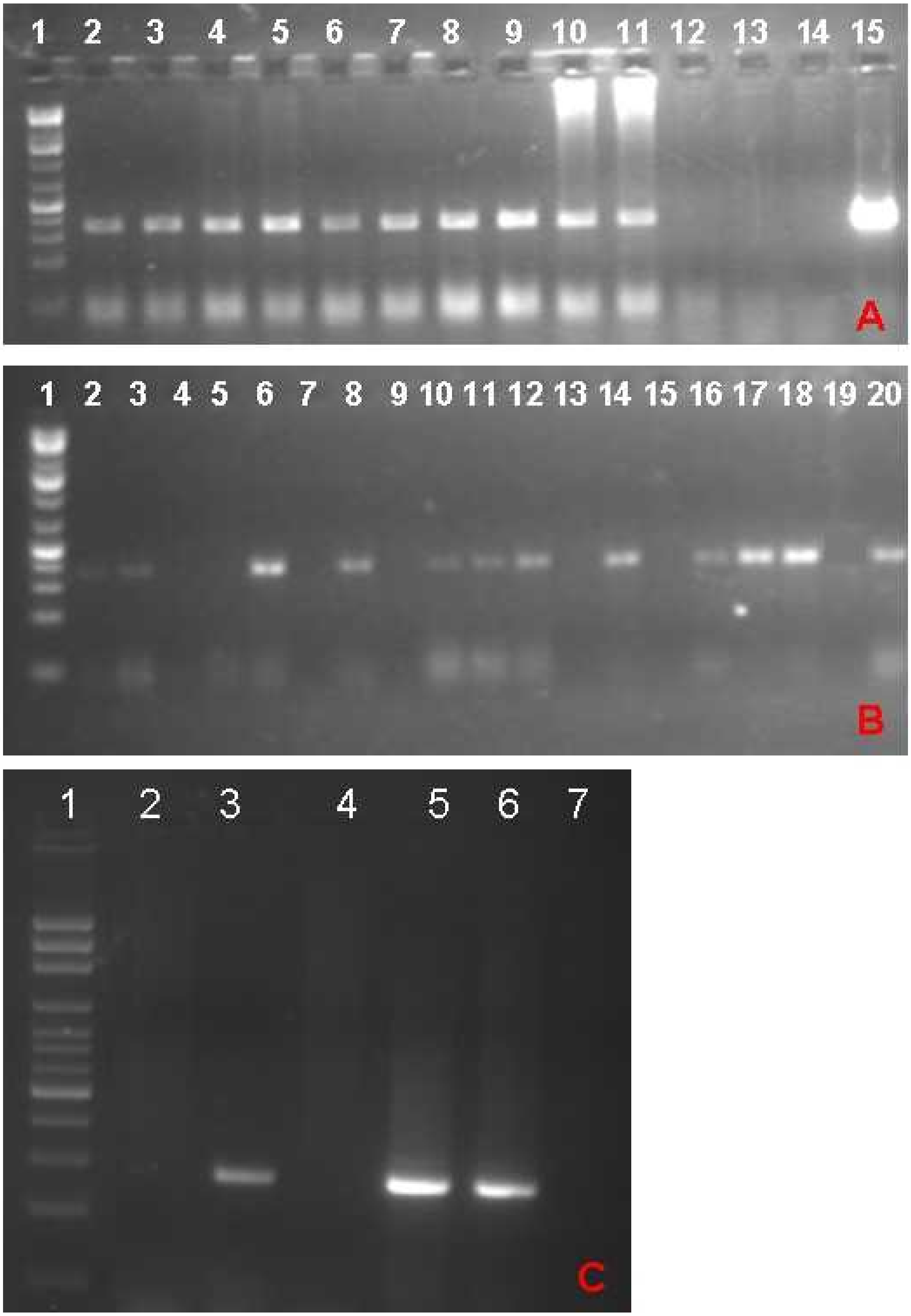

Figure – 1: PCR amplification of Bt genes in cotton leaf samples; (A) PCR amplification to detect MON-531 event. Lane 1 contains marker, lane 2 to 13 contains DNA from cotton leaf samples, lane 14 contains negative control and lane 15 contains positive control, (B) PCR amplification to detect *cry1Ac* gene. Lane 1 contains marker, lane 2 to 18 contains DNA from cotton leaf samples, lane 19 contains negative control and lane 20 contains positive control, (C) PCR amplification to detect *cry2A* gene. Lane 1 contains marker, lane 2 to 5 contains DNA from cotton leaf samples and lane 6 and 7 contains positive and negative control respectively.

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