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# Introgression of Bt genes into the commercial cotton lines

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## ABSTRACT

*Cotton bollworm (Helicoverpa armigera) is one of the most serious insect pests of cotton. As every organism has a pathogen surviving on it, Bacillus thuringenesis, a bacterium has been identified to cause bacterial diseases in insects. This bacterium produces a protein (cry toxins) which paralyzes the digestive system of the insect, making it incapable to digest the ingested food, ultimately leading the insect to starvation. If these proteins are injected into the cotton plants, the problem due to bollworms can be overcome. For this, the gene responsible for producing the cry protein is genetically engineered into the cotton plants. Such plants called the transgenic varieties are capable of expressing resistance to the insects. To check if the plants are resistant against the insects, it is necessary to observe them grow in the field, which consumes a lot of time and labor, hence creating economical problems. To skip this tedious process, molecular markers have been produced, which recognize the presence of the transgene in the plant, in its early stages. This even reduces the time span for producing desired plants when compared to the traditional methods. These transgenic plants are further subjected to breeding techniques to produce stable transgenic cotton plants with various desired characters. Bt cotton has been widely planted around the world, and this has resulted in efficient control of bollworm population With reduced use of synthetic insecticides.*

***Key Words:*** *Bacillus thuringenesis, Molecular markers , Bt cotton , protein, Cotton bollworm, MAS.*

## INTRODUCTION

Cotton is an important cash crop. A long-term challenge facing a cotton breeder is the simultaneous improvement of yield and fiber quality to meet the demands of the cotton producer as well as the textile industry. In recent years, improvement of cotton fiber quality has been extremely important because of changes in spinning technology. However, a negative association between lint yield and fiber quality is still present after many years of exhaustive breeding for improved fiber properties. Conventional breeding procedures are difficult for further improving fiber quality because of high costs, long duration, and low selective efficiency [1]. Cotton, a high valued agricultural commodity for more than 8000 years, has long been recognized as a vital component of the global economy [2].Cotton production provides income for approximately 100 million families and approximately 150 countries are involved in cotton import and export [3]. All parts of the cotton plant are useful and it has hundreds of uses. No other fiber comes close to duplicating all of the desirable characteristics combined in cotton. In addition to the fiber used in textile manufacturing, cotton seed is used to produce oil, seed meal (rich in essential amino acids which is lacking in most seed crops) and seed hulls (used for mulch and cattle feed). It has been estimated that 180 million people depend, either directly or indirectly, on the production of cotton for their lively hood [4]. In India, cotton is a major agricultural commodity and a large part of the Indian economy. According to the World Bank, India is the second largest producer and consumer of cotton [5]. The cotton industry in India has 1,543 spinning units, more than 281 composite mills, 1.72 million registered looms and an installed capacity of 36.37 million spindles [6]. Cotton provides a livelihood to more than 60 million people in India by way of support in agriculture, processing and use of cotton in textiles and also contributes 30% to the Indian agricultural gross domestic product and thus cotton is a very important cash crop for Indian farmers [7]. Albeit India's cotton area representing 25% of the global area of cotton, it produced only 12% of world production. Yields of cotton in India are low, with an average yield of 300 kg/ha compared to the world average of 580 kg/ha [8]. The major limiting factors to both cotton production and quality in India are biotic and abiotic stresses. As with many cotton growing areas of the world, major damage is due to insect pests, especially the bollworm complex, sucking pests and viruses. The productivity is still worsened by abiotic stress such as drought and heat. It is worth to mention here that most of the cotton in India is grown under rain fed conditions and about a third is grown under irrigation [9], which also experiences water stress during certain growth periods. Rising production costs to combat biotic and abiotic stresses and stagnant pricing are the additional factors that threaten cotton production. The low pricing of recent years due to poor quality of fiber resulted to biotic and abiotic stresses and has forced many growers to plant alternative crops, even in the face of farm subsidies. Hence, to cope with the growing demand on cotton fiber and by products, genetic enhancement of cotton is indispensable which will ensure competitiveness in the market of this natural-renewable product with petroleum-derived synthetic fibers, given the projected future decline in petroleum reserves. Moreover, modifications to expand the use of seed derivatives for food and feed could profoundly benefit the diets and livelihoods of millions of people in food-challenged economies [10]. Microbial Bacillus thuringiensis (Bt)-based products have been used commercially for almost 40 years by growers, including organic growers, to control selected insect pests [11]. More recently, the gene(s) encoding the insecticidal proteins in these Bt microbial products have been cloned [12] and introduced and expressed in genetically modified plants [13-15]. The use of commercial, nuclear transgenic crops expressing Bacillus thuringiensis (Bt) toxins has escalated in recent years because of their advantages over traditional chemical insecticides. However, in crops with several target pests with varying degrees of susceptibility to Bt (e.g., cotton), there is concern regarding the suboptimal production of toxin, resulting in reduced efficacy and increased risk of Bt resistance [16, 17]. Additionally, reliance on a single (or similar) Bt protein (s) for insect control increases the likelihood of Bt-resistance development [18]. Plant-specific recommendations to reduce Bt resistance development include increasing Bt expression levels (high-dose strategy), expressing multiple toxins (gene pyramiding), or expressing the protein only in tissues highly sensitive to damage (tissue-specific expression) [19].

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## MATERIALS AND METHODS

***Plant materials:***

In the present investigation comprising Pest- resistant crops can be developed by genetic modifications The primers used here are the molecular markers in the primers are primer1, primer2, & primer3.

***DNA extraction and PCR conditions:***

DNA was extracted from fresh leaves by the Cetyl Trimethyl Ammonium Bromide method [20]. Thermal cycler program for PCR comprised $95^0$C for 3 minutes for initial denaturation, followed by 38 cycles of $94^0$C for 15 seconds, $58^0$c for 30 seconds, $72^0$C for 1minute and ending up with 7 minutes at $72^0$C for the final extension. The annealing temperature was adjusted based on the specific requirements of different primer combinations. The PCR products were resolved by electrophoresis in 1.4% agarose gel containing 0.5 µg/ml of Ethidium Bromide prepared in 1X TAE buffer at a constant voltage of 80v for period of 2 hrs. The gel was visualized in UV transilluminator and documented using SYNGENEGENESNAP G-BOX gel documentation system.

## RESULTS AND DISCUSSION

For analyzing the zygosity of the samples to see whether the given sample is homozygous or heterozygous for a particular trait, primers specific to that trait are used in PCR. If that particular trait is present in the plant DNA sample, then it is amplified and a band is obtained. The plants are tested to see if they are hybrids for a trait. As hybrids derive their characters from both the female and male parents, they get a double band. Whereas, the female and male parents get a single band with difference in their band lengths.

The first development of our program was the construction of genetic maps of tetraploid cotton combining RFLP, SSR, and AFLP markers, generated separately for the first two backcross generations (BC1 and BC2). The initial BC1 map, comprising 888 loci grouped in 37 linkage groups, and spanning 4400 cM [21], benefited from the development and integration of new additional microsatellite markers [21]. This updated saturated BC1 map now spans 5500 cM and comprises a total of 1160 loci ordered along 26 chromosomes or linkage groups. On the other hand, the BC2 map (512 loci in total) constructed using AFLP and SSR markers had 393 loci in common with the BC1 map. The two maps agreed for loci order, thus allowing their merging into a combined map. This new consensus BC1/BC2 map then served for 3 separate QTL analyses of fiber quality components and as a support for the MAS program.

The process used is efficient in selecting for chromosomal regions of interest (foreground selection), while letting the rest of the genome return towards that of the recurrent parent. In this particular example, the BC4 plant has retained, at the heterozygous state, genomic regions carrying favorable alleles on chromosomes/linkage groups c6, c25 and c26. The other regions carrying QTLs on c3, c23, c20, A01 and A03, which were heterozygous on the BC1 plant have partly or completely returned to the homozygous *Gh/Gh* state. Most of the non-carrier genome (91% of the genotyped loci) of the BC4 plant has returned to the homozygous state [22].

The 565 bp and 600 bp fragments in case of PCR showed the integration of *Cry1Ac* and *Cry2A* in advance lines and a 68 Kda band of protein, which is a truncated *Cry1Ac* toxin from integrated Bt gene showed the expression of *Cry1Ac* gene. This appearance of 68 Kda band indicates the expression of gene in transgenic cotton lines, as this band of protein is absent in non- transgenic control samples [23, 24]. In a study of heterosis and varietal origins reported the first RFLP evaluation in upland cotton [25] found that 64% of cotton RFLPs is co-dominant. Detailed RFLP maps of cotton with 41, 5, 31, 24 and 17 linkage groups were developed by [24, 26-29] respectively [30]. identified RFLP marker linked to resistance allele for bacterial blight pathogen. Moreover using a detailed RFLP map, genes affecting density of leaf and stem trichomes have been mapped by [31]. An effort for mapping the trait of low gossypol seed and high gossypol plant was made using 49 RFLP probes to trace introgression of parental DNA segment in tri species hybrid and in three back cross generations [32].

Out of the 45 ISSR and 40 RAPD primers used, only 19 ISSR and 35 RAPD primers were scorable. All the 19 scorable ISSR primers were reproducible, while out of 35 RAPD primers, only 21 were reproducible; 19 ISSR 21 RAPD primers generated 90 and 150 markers, respectively. Out of them 12 ISSR and 15 RAPD primers were polymorphic and produced 49 and 76 markers, respectively. Size of the amplified products for ISSR and RAPD was 100-1444and 100-2000 bp, respectively (**Figs 1 & 2**). The per-centage of polymorphic bands for ISSR and RAPD were 54 and 50, respectively [33].

**Table No. 1: Primers used**

| S. No. | Primer Name | Primer Sequence |
|---|---|---|
| 1 | Primer 1 | 5'AATTACCCATTatatcgcaCAAATTAC 3' |
| 2 | Primer 2 | 5' GGAATGctctactaCCTGAG 3' |
| 3 | Primer 3 | 5' ACActcccAGGtacgtcCAGA 3' |

**Table No. 2: Transgenic Results interpretation**

| S. No. | Genotype | Amplification for Transgene |
|---|---|---|
| 1 | Non Transgenic | 300 bp |
| 2 | Homo | 600 bp |
| 3 | Hetero | 300 and 600 bp |

***Experiment:*** Initially we have carried out the Qualitative assay for 20 homozygous plants and 20 non transgenic plants.

**Table No. 3: Following are the results presented for the transgenic zygosity assay**

| S. No | Sample # | Trait (Transgenic) |
|---|---|---|
| 1 | T-1 | Homozygous |
| 2 | T-2 | Homozygous |
| 3 | T-3 | Homozygous |
| 4 | T-4 | Homozygous |
| 5 | T-5 | Homozygous |
| 6 | T-6 | Homozygous |
| 7 | T-7 | Homozygous |
| 8 | T-8 | Homozygous |
| 9 | T-9 | Homozygous |
| 10 | T-10 | Homozygous |
| 11 | T-11 | Homozygous |
| 12 | T-12 | Homozygous |
| 13 | T-13 | Homozygous |
| 14 | T-14 | Homozygous |
| 15 | T-15 | Homozygous |
| 16 | T-16 | Homozygous |
| 17 | T-17 | Homozygous |
| 18 | T-18 | Homozygous |
| 19 | T-19 | Homozygous |
| 20 | T-20 | Homozygous |

| S. No | Sample # | Trait (Transgenic) |
|---|---|---|
| 21 | NT-21 | Non-transgenic |
| 22 | NT-22 | Non-transgenic |
| 23 | NT-23 | Non-transgenic |
| 24 | NT-24 | Non-transgenic |
| 25 | NT-25 | Non-transgenic |
| 26 | NT-26 | Non-transgenic |
| 27 | NT-27 | Non-transgenic |
| 28 | NT-28 | Non-transgenic |
| 29 | NT-29 | Non-transgenic |
| 30 | NT-30 | Non-transgenic |
| 31 | NT-31 | Non-transgenic |
| 32 | NT-32 | Non-transgenic |
| 33 | NT-33 | Not amplified |
| 34 | NT-34 | Non-transgenic |
| 35 | NT-35 | Non-transgenic |
| 36 | NT-36 | Non-transgenic |
| 37 | NT-37 | Non-transgenic |
| 38 | NT-38 | Non-transgenic |
| 39 | NT-39 | Not amplified |
| 40 | NT-40 | Non-transgenic |

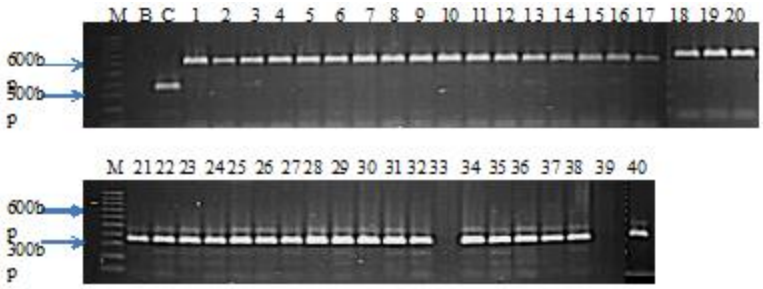


**Fig. 1: Representative gel image for the samples. 1 to 20 is positive and 21 to 40 are negative (Non Transgenic plants) samples.**

**Table No. 4: Later, the results of the off-springs generated due to crossing the transgene with the R lines was obtained as**

| S.NO | SAMPLE # | TRAIT (Transgenic) |
|---|---|---|
| 1 | P-1 | Homozygous |
| 2 | P-2 | Heterozygous |
| 3 | P-3 | Homozygous |
| 4 | P-4 | Homozygous |
| 5 | P-5 | Heterozygous |
| 6 | P-6 | Heterozygous |
| 7 | P-7 | Heterozygous |
| 8 | P-8 | Heterozygous |
| 9 | P-9 | Heterozygous |
| 10 | P-10 | Homozygous |
| 11 | P-11 | Non-transgenic |
| 12 | P-12 | Non-transgenic |
| 13 | P-13 | Non-transgenic |
| 14 | P-14 | Non-transgenic |
| 15 | P-15 | Homozygous |
| 16 | P-16 | Non-transgenic |
| 17 | P-17 | Non-transgenic |
| 18 | P-18 | Heterozygous |
| 19 | P-19 | Heterozygous |
| 20 | P-20 | Homozygous |
| 21 | P-21 | Heterozygous |
| 22 | P-22 | Heterozygous |
| 23 | P-23 | Non-transgenic |
| 24 | P-24 | Heterozygous |
| 25 | P-25 | Non-transgenic |

| S.NO | SAMPLE # | TRAIT (Transgenic) |
|---|---|---|
| 26 | P-26 | Non-transgenic |
| 27 | P-27 | Non-transgenic |
| 28 | P-28 | Non-transgenic |
| 29 | P-29 | Homozygous |
| 30 | P-30 | Non-transgenic |
| 31 | P-31 | Homozygous |
| 32 | P-32 | Non-transgenic |
| 33 | P-33 | Heterozygous |
| 34 | P-34 | Homozygous |
| 35 | P-35 | Not amplified |
| 36 | P-36 | Heterozygous |
| 37 | P-37 | Non-transgenic |
| 38 | P-38 | Non-transgenic |
| 39 | P-39 | Heterozygous |
| 40 | P-40 | Heterozygous |
| 41 | P-41 | Non-transgenic |
| 42 | P-42 | Non-transgenic |
| 43 | P-43 | Non-transgenic |
| 44 | P-44 | Non-transgenic |
| 45 | P-45 | Non-transgenic |
| 46 | P-46 | Heterozygous |
| 47 | P-47 | Non-transgenic |
| 48 | P-48 | Non-transgenic |
| 49 | P-49 | Non-transgenic |
| 50 | P-50 | Non-transgenic |
| 51 | P-51 | Non-transgenic |

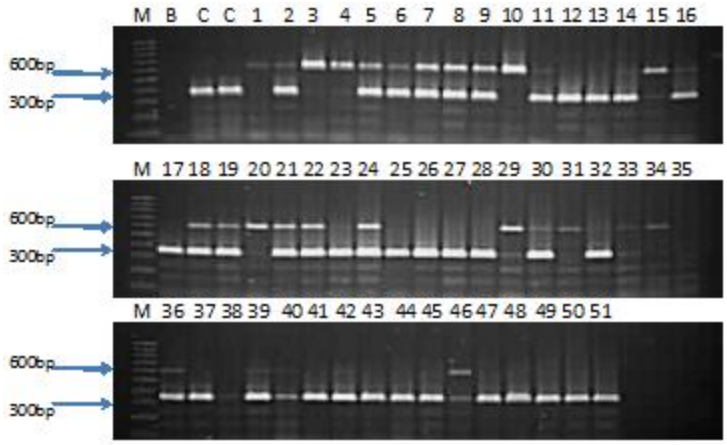


**Fig. 2: Following are the representative gel images of off-spring samples**

**CONCLUSION**

Genes responsible for producing the cry protein is genetically engineered into the cotton plants and transgenic varieties are capable of expressing resistance to the insects. Research work concluded that transgenic plants are resistant against the insects, it is necessary to observe them grow in the field, which consumes a lot of time and labor and also process of molecular markers have been produced, which recognize the presence of the transgene in the plant, in its early stages. Bt cotton has been widely planted around the world, and this has resulted in efficient control of bollworm population With reduced use of synthetic insecticides.

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