Biotec.Res.J.2016; Vol 2(1):1-5
eISSN 2395-6763

ORIGINAL RESEARCH

Isolation and detection of CAMv5S promoter and *cry* gene from the genetically modified cotton varieties

Vinoth KANNA A[1, 2]*, Loyola PRINCY E[1]
[1]Department of Biotechnology, Thanthai Hans Roever College, Elambalur, Perambalur – 621 212, Tamil Nadu, India
[2] Department of Molecular Biology, School of Life Sciences, Madurai Kamaraj University, Madurai – 625 017, Tamil Nadu, India
*Corresponding Author email: avinothkanna@live.in

• Received: 12 July 2015 • Revised: 15 September 2015 • Accepted: 05 October 2015 • Published: 04 January 2016 •

ABSTRACT
Cotton (*Gossypium arboretum*) plays an important role in the day and daily life of humans. The production of cotton has been affected by various number of insect pest causes a serious infestation and infection which leads to vast damage to the cotton and its producers. In this study, the pre hybrid varieties such as MECH-184 and RCH 2 have been taken which was compared with a non Bt cotton variety (as a control) were studied for the presence of CaMV 35S promoter and *cry* genes. This has been studied using the PCR and lateral flow methods. From the results, these hybrid varieties have contained both the CaMV 35S promoter and *cry* genes.

KEY WORDS: *CaMV 35s, cry, PCR, lateral flow assay*

Introduction

Today biotechnology is having a role in enabling techniques with wide and diverse application in agriculture, health care industries and environment. Genetically modified organisms (GMOs) have been developed and applied successfully since early 1970s under contained conditions and since mid-1980 for commercial applications in the field and opened environmental trails (Prabhu, 2009).

A genetically modified organisms is an organism whose genetic structure is altered by incorporating a gene, which will express their desirable character, GMO is a typical example that has broad application in agriculture and food industries, the use of genetically modified plants can lead to increased yields in agriculture by generating species resistance to diseases, insect s and products (Phillips, 2008). For the production of transgenic plant, recombinant DNA technology can be used for insertion of genes in plants for higher yield, stress resistance and herbicide resistant (Devlina *et al.,* 2006)

Several commercially important crops such as cotton, maize, soy bean, tomato, potato, rice etc. have been genetically modified. In India, extensive efforts have been initiated for promoting researches and development of transgenic crop (Walden, R. and Schell, J. 1990). A transgenic plant contains a genes of different species which have been artificially inserted instead of the plant acquiring them through pollination and these plants pass these genes to their offspring. Today, plants can be genetically engineered to produce their Bt toxin which would protect them from pest infestations. Genetic (r-DNA) engineering is the modification of DNA molecules to produce changes in plants, animals or

other organisms (Li *et al.,* 1991).

The promoter was named CaMV 35S promoter (**"**35S promoter") because the coefficient of sedimentation of the viral transcript whose expression is naturally driven by this promoter is 35S. It is one of the most widely used, general-purpose constitutive promoters. The 35S promoter is a very strong constitutive promoter, causing high levels of gene expression in dicot plants. However, it is less effective in monocots, especially in cereals. The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors (Ho *et al.,* 1999).

Cotton has historical importance since it provides a livelihood to millions of people's worldwide by way of support in agriculture and use of cotton in textiles. Cotton contributes nearly 30% of the Indian agricultural gross domestic product, and nearly nine million hectares of land in India is used to produce cotton. India is the third largest cotton producer in the world (ICTSD, 2013). But because of the pests the production becomes substantially low. The major reason for this low productivity is damage caused by several insect pest such as *Helicoverpa armigera* (Kulakarni *et al.,* 2009). Chemical pesticides were used to overcome the problems caused by the pest. But after regular spray of chemical pesticides it was found that the pest started developing resistance towards these chemicals. These chemicals are further causing environmental pollution. Biopesticides can be used to overcome the problems raised due to chemical pesticides. But most of the biological pesticides display a narrower host range than chemical pesticides. (Barwale *et al.,* 2004). Bt cotton has been genetically modified by the insertion of one or more genes from a common soil bacterium, *Bacillus thuringiensis*. These genes encode for the production of insecticidal proteins, and thus, genetically transformed plants produce one or more toxins as they grow. The value of Bt cotton as a replacement for insecticide applications for specific pests and as a pest management tool for reducing the insecticide costs considerably has been documented (Bambawale *et al.,* 2004). The present study is to identify the presence of modified gene in the GMO crops using PCR and lateral flow method.

Materials and Methods

Plant samples

Samples such as Bollgard^{TM} Cotton MECH-184 and RCH 2 BT cotton seeds were purchased from Maharashtra Hybrid Seeds Co. Ltd, Mumbai and RASI seeds, Attur, Tamil Nadu. Similarly, non-hybrid varieties were purchased from the local seed shop.

Genomic DNA extraction

Fresh leaf samples were collected from the respective samples and they were kept at -70°C for one overnight. To the above, extraction buffer were added and grinded using the methodology of Sambrook and Rusell (2001). The collected DNA samples were labelled and stored in the separate vials at -20°C for further use.

Re-precipitation of DNA

Re-precipitation of DNA is generally performed to obtain pure DNA preparations. Any detergent which forms a complex with the DNA strands can be completely cleaved by using potassium acetate which replaces the sodium ions of SDS and absolute ethanol precipitates and prevents it from forming complexes with the impurities.

DNA quality check

The concentration and condition of DNA quality was checked by Agarose gel electrophoresis. This method is not only used for the identification of DNA, but also for the separation and purification of DNA fragments. Furthermore, the location of DNA within the gel can be determined directly by staining with low concentration of fluorescent intercalating dyes such as Ethidium Bromide (EtBr); bands containing as little as 20pg of double stranded DNA can be detected by direct examination of the gel in UV.

Quantification of DNA

The purity of DNA was estimated by utilizing the property of the heterocyclic rings of the nucleotides of absorbing light strongly in the UV range. DNA absorbs maximum light energy at about 260nm. An optical density of 1.0 corresponds to approximately 50µg/ml of double stranded DNA and 36µg/ml of single stranded DNA. The protein impurities absorb maximum light energy at about 280nm. The ratio of absorbance viz. A_{260}/A_{280} provides an estimation regarding the purity of DNA (Sambrook *et al.,* 1989).

PCR amplification of CaMV35S promoter gene and CrylAc gene

The PCR ingredients and primers were procured from Eppendorf, Germany and Operon technologies, Germany respectively. The sequence details of the primer for the detection of CaMV35 promoter gene and Cry 1 protein gene is given below.

Table 1: Sequence details of the primer

Primers		Sequence
CaMV 35S	35s FP	5'GCTCCTACAAATGCCATCA 3'
	35s RP	5'GATAGTGGGATTGTGCGTCA 3'
CrylAc	IAC1 FP	5'GCTCTCCGCGAGGAAATGCG 3'
	IAC2 RP	5'CACGTGGCTCAACCTGTGGG 3'

Protein Based Method (Lateral Flow Strip Assay)

Protein based methods use antibodies to detect or measure the amount of 'novel' protein(s) produced by the GMO. Strip tests can be carried out by unskilled personnel in the actual field. Typically, a sample for testing is crushed and mixed with extraction buffer. The strip is dipped into this mixture and the result monitored as the colour of the strip changes indicating whether or not the GMO variety is present. This test is relatively cheaper than other methods of GMO detection and it can be performed quickly within 20 min (Chen and Wu, 2012).

Results and Discussion

Extraction of DNA

The DNA samples were extracted and further analyzed using the agarose gel electrophoresis which exhibited the presence of the DNA samples. Further, these DNA samples were treated with various enzymes in order to find the stability of the DNA such as proteinase K enzyme, high concentration, low concentration and contaminated DNA have studied which was represented in the Figure 1.

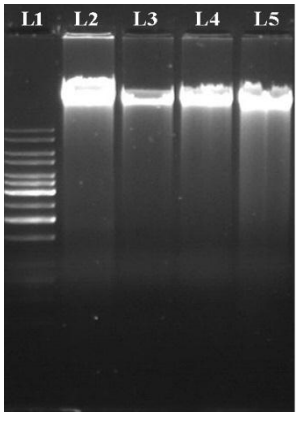

Figure 1: Gel electrophoresis of the hybrid and non-hybrid Cotton plants DNA. Lane 1: Marker, Lane 2: RCH-2 Bt DNA sample, Lane 3: RCH-2 Non-Bt DNA sample, Lane 4: MECH-184 Bt DNA sample, Lane 5: MECH Non Bt DNA sample.

Quantification of the amplified DNA

The DNA which was obtained from both the hybrid and non-hybrid cotton plants were quantified using the UV spectrophotometer was shown in the Table 2. The results showed that the quantity and the absorbance was significantly higher in the hybrid variety than the non-hybrid variety. The concentration of the DNA was insignificantly higher in the hybrid variety (df = 1,4; N > 0.05).

Table 2: Quantification of DNA

Absorbance	Bt cotton	Non-Bt cotton
A260/280	1.81	1.64
A260	0.459	0.547
A280	0.254	0.333
A320	0.036	0.112
Concentration of ds DNA (ng/µl)	23	27

Detection of the CaMV 35S promoter gene

From the Figure 2 which contains Lanes from 1 – 7 and from this figure the presence of the CaMV 35S promoter gene has been amplified using the PCR and analyzed using the DNA marker which gives a detailed information. The lane 1 – 6 which showed up the amplified CaMV 35S promoter gene (band 195kb) that have been present in the Bt cotton varieties whereas in the non Bt variety the particular 195kb gene (Lane 1-6) was found to be absent. The band size was determined by using the 100bp DNA ladder. Lane 7 contained PCR control which is used to neglect the non-specific bands during amplification cycles. This figure which clearly give information regarding the difference between a Bt and non Bt cotton varieties. The screening methods are of a great importance for routine analyses. CaMV35S promoter is used as a universal molecular marker for the analysis of 95% of currently commercialized GMO plants in EU (Holden *et al.,* 2010).

For decades, produced Bt preparations consisting of spores and toxins are registered as Bio-pesticides. Advantages of the Bt preparations are high specificity, short persistence and thus a relatively high environmental compatibility.

Cohen *et al.,* (2000) reported that there is always a risk that insects could become resistant to Bt toxin after prolonged and repeated field exposures. "The most practical approach to prolong the effectiveness of Bt crops has been refugia

strategy and pyramiding of two or more genes in the same cultivar (Cohen *et al.*, 2000).

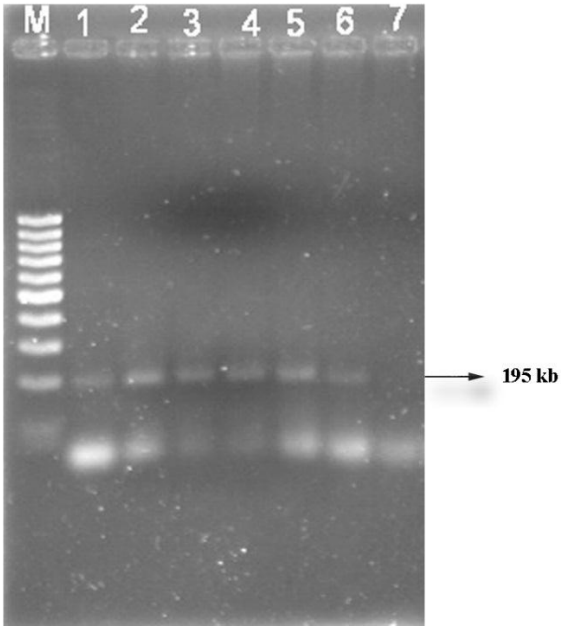

Figure 2: The electrophoretic analysis showed the presence of the molecular weight band 195 Kb has indicated the presence of the BT gene.

The GM crop currently available to EU farmers for cultivation is the GM maize resistant to insects, commonly known as Bt maize (James 2011). On the teritory of Serbia, the number of positive maize samples varied from 12% in 2006 year, to 14.5% in 2008, with the amount of GM mostly below 0.9%, except for two samples with the content above 0.9%, in 2006 (Nikolić & Vujaković 2011).

GMO detection conducted in the Czech republic in 1164 samples over a period of five years indicated that most of the positive samples were mainly RR soya, 3 varieties of GM maize, and one of GM rice (Kyrova *et al.,* 2008). On the ground of the PCR measurements, 38% of the analysed foods, coming from the Hungarian food market, proved to be GMO positive and 6% of these samples contained RR soya above 0.9% (Ujhelyi *et al.,* 2008).

In Ireland, it was found that 12 out of 75 samples tested were positive for GM ingredients and several soya products contained between 0.1% and 0.7% of GM soya (Partridge & Murphy 2004).

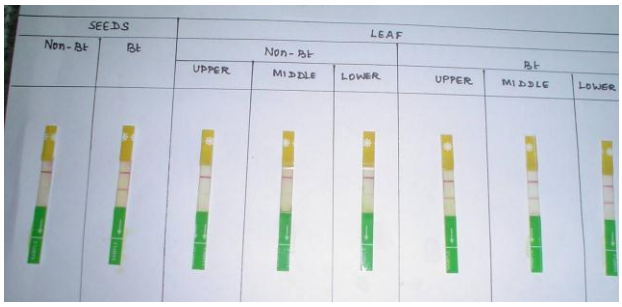

Figure 3: Lateral flow assay method showed the presence of *cry* gene in the strips.

Detection of the genetic modification in heat-treated products of Bt-maize by PCR was described by Hupfer *et al.*, (1998). Conclusion of this work confirms that the higher the processing level (heat, pressure, low pH, etc.), the more degraded the DNA. As a result the length of the PCR amplification to the detection of genetic modification in processed products is very important.

Acknowledgement

The authors greatly acknowledge the MD, Ideal Analytical Research Institute, Pudhucherry for providing necessary facilities to do the work. Also we would express our great attitude to the management of Thanthai Hans Roever College, Perambalur for their timely support to the work.

References

Barwale, R. B., Gadwal, V. R., Zehr, U. and Zehr, B. (2004) Prospects for Bt cotton technology in India. AgBioForum, 7(1&2): 23–26.

Chen, C. and Wu, J. (2012) A Fast and Sensitive Quantitative Lateral Flow Immunoassay for Cry1Ab Based on a Novel Signal Amplification Conjugate. Sensors, 12: 11684-11696.

Cohen, B. M., Gould, F. and Bentur, J. C. (2000) *Bt* rice: practical steps to sustainable use. International Rice Research Notes, 25(2): 4-10.

Devlina, R. H., Sundströma, L. F. and Murib, W. M. (2006) Interface of biotechnology and ecology for environmental risk assessments of transgenic fish. Trends in Biotechnology, 24: 89-97.

Ho, M. W., Ryan, A. and Cummins, J. (1999) Cauliflower mosaic virus promoter – a recipe for disaster. Microbiology Ecology and Health Diseases, 10: 33–59.

Holden, M. J., Levine, M., Scholdberg, T., Haynes, R. J. and Jenkins, G. R. (2010) The use of 35S and Tnos expression elements in the measurement of genetically engineered plant materials. Analytical and Bioanalitycal Chemistry, 396: 2175–2187.

Hupfer, C., Hotze, H., Sachse, K. and Engel. K. H. (1998) Detection of the genetic modification in heat-treated products of Bt mwe by polymerase chain reaction. *Z.* Lebensm Unters Forsch, 206: 203-207.

ICTSD 2013. Cotton: Trends in Global Production, Trade and Policy; Information Note; International Centre for Trade and Sustainable Development, Geneva, Switzerland.

James, C 2011. Global Status of Commercialized Biotech/ GM Crops: 2011. ISAAA Brief No. 43. ISAAA, Ithaca, USA.

Kulkarni, J., Kapse, N. and Kulkarni, D. K. (2009) Plant-based pesticide control for *Helicoverpa armigera* on *Cucumis sativus.* Asian Agricultural History, 13(4): 327-332.

Kyrova, V., Ostry, V., Laichmannova, L. and Ruprich, J. (2008) GENOMON - Specialized monitoring of GM foodstuffs in the Czech Republic during 2002–2007. In: 1st Global Conference on GMO Analysis, June 24.–27, Como, Italy. Abstracts. European Commission, Italy: 100.

Li, B., Xu, X., Shi, H. and Ke, X. (1991) Introduction of foreign genes into the seed embryo cells of rice by electro-injection and the regeneration of transgenic rice plants. Science in China, 34 (8): 923-931.

Nikolić, Z. and Vujaković, M. (2011) Monitoring the occurrence of genetically modified maize in Serbia from 2006 to 2008. Cereal Research Communication, 39: 267–272.

Partridge, M. and Murphy, D. J. (2004) Detection of genetically modified soya in a range of organic and health food products. British Food Journal, 106: 166–180.

Phillips, T. (2008) Genetically modified organisms (GMOs): Transgenic crops and recombinant DNA technology. Nature Education, 1(1):213

Prabhu, K. V. (2009) Use of GMOs under containment, confined and limited field trials and Post-release monitoring of GMOs. In: Biosafety of genetically modified organisms: Basic concepts, methods and issues (Chowdhury, M. K. A., Hoque, M. I. and Sonnino, A. Eds.). pp. 157-220.

Sambrook, J and Russell, D. W. (2001) Polyacrylamide Gel Electrophoresis. In: Molecular Cloning. A Laboratory Manual. Third edition. Cold Spring Harbor Laboratory Press.

Ujhelyi, G., Vajda, B., Beki, E., Neszlenyi, K and Jakab, J. (2008) Surveying the RR soy content of commercially available food products in Hungary. Food Control, 19: 967–973.

Walden, R. and Schell, J. (1990) Techniques in plant molecular biology - progress and problems. European Journal of Biochemistry, 192: 563-576.