# Polymerase Chain Reaction for Evaluation/ Detection of Transgenic Planting Material

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Transgenic crops developed using recombinant DNA technology can make a significant contribution to global food, feed and fibre security, and alleviation of poverty, particularly in the Third World (Dunwell, 1999). Global area under cultivation of transgenic crops has increased from 1.7 million hectares in 1996 to 81 million hectares in 2004 (James, 2004). The commonly used regulatory sequences to transform most of GM plants are Cauliflower Mosaic Virus promoter (*CaMV 35S*) and the *Agrobacterium tumefaciens* nopaline synthase terminator. Consequently, methods detecting one of these regulatory sequences are popular for screening purposes.

With the fast introduction of new transgenic crops the demand to detect exogenous genes is increasing. To differentiate between transgenic and non-transgenic crops, a series of analytical tools is a perquisite. The majority of the methods hitherto developed, mainly focused on detection of DNA. Polymerase Chain Reaction (PCR) has high sensitivity and specificity where a primer is designed based on the regulatory sequence or part of exogenous structural gene for evaluation/detection of transgenic crops. In the present study, the primers were designed and tested for screening to study the feasibility of the PCR for evaluation/detection of transgenic planting material.

Most of the new analytical methods used for detection and quantification of exogenous genes in GM crops are very expensive and time consuming. In India till 2003, 28 imports of GM planting material of eight crops for different traits have been made (Mangal *et al.*, 2003). The present study is carried out under the DBT project, "Development of PCR based methods for testing of transgenic planting material", and methods developed to analyse these transgenic crops are being validated by interlaboratory tests and standardization of upgraded techniques is under progress. The relatively inexpensive, qualitative and sensitive method used in the present study, would be suitable for meeting regulatory obligations of country.

Crushed seeds of transgenic soybean, maize, mustard, and cotton were used for the study. Nucleotide sequences of exogenous genes were obtained from NCBI and primers were designed and synthesized. PCR protocols were standardized for amplification of exogenous gene sequences in transgenic crops.

Results showed that the PCR product with 195bp from *CaMV 35S* promoter was amplified in the GM soybean, maize, mustard, and cotton. While no PCR product was detected from the conventional/non-GM soybean, maize, mustard, and cotton. The PCR analysis with these primers could detect 10-20ng quantity of DNA of plant material. The designed primer pairs were used for detection of exogenous genes in respective GM crops. Respective primer pair amplified the PCR product of 320bp from *CP4EPSPS* gene in soybean and maize, 340bp and 290bp from *barnase* and *barstar* in GM mustard and 447bp from *Cry1Ac* gene in cotton.

Hence, the PCR technology has been effectively employed for testing of transgenic planting material.

## References

Dunwell, JM (1999) Transgenic Crops: The next generation, or an example of 2020 vision. *Annals of Botany* **84:** 269-277.

James C (2004) Preview: Global status of commercialized biotech/ GM crops: 2004. *ISAAA* Briefs **32**. ISAAA:Ithaca, NY. (www.isaaa.org).

Mangal M, K Malik and GJ Randhawa (2003) Import of transgenic planting material: National scenario. *Current science* **85(4):** 454-458.

Markus L, P Brodmann, K Pietsch, J Pauwels and E Anklam (1999) IUPAC collaborative trial study of a method to detect genetically modified soybeans and maize in dried powder. *Food composition and additives* **82(4):** 923-928.