- Short communication

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Detection of Maize Intrinsic and Recombinant *Cry1Ab* Gene Fragment in Genetically Modified Maize

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Polymerase chain reaction (PCR) assay was performed to detect and identify the genetically modified maize (*Bt11*). Primer pair Bt11 1-5′ and Cry1Ab 1-3′ detected the region of the insect resistant *Cry1Ab* gene sequence inserted in GM Bt11 maize and a primer pair *ZE01-ZE02* were used to detect the maize intrinsic *zein* (*Ze1*) gene in maize DNA. The presence of the corresponding DNA segments was specifically detected in GM maize by the designed primers. It was concluded that this method is useful for fast and easy screening of *Bt* gene in the food products and GM *Bt* crops.

The methods of plant genetic transformation are applied for the insertion of genes of interest into specific crop plants for their improvement (Estruch et al. 1997). These transformed plants are commonly known as genetically modified plants or GM plants. A number of GM plants have already been developed for commercial use in different parts of the world (James 2006). Before commercialization, the expression of the introduced trait(s) is needed to be assayed. This detection of the expression of genetically modified trait is essential not only to determine the existence of transgenes in crop plants before commercial exploitation but also to satisfy the required biosafety protocols for the safeguard of human and animal health as well as in protecting the environment. Thus, for the safety assessment of GM products detection of genetically modified trait is very important and it is regarded as the first step of the safety assessment procedure and also for the identification of GM and non-GM plants and their products.

Freeze-dried flour from genetically modified (GM) maize and non-GM (*Bt11*) maize isoline were used as GM and non-GM reference materials for the present study. Genomic DNA was isolated from maize grain powder using phenol-chloroform isoamyl method and also carried out using Wizard[R] Genomic DNA Purification Kit (Promegra Corporation. 2800 Woods Hollow Road. Madison, USA) following manufacturer instructions.

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Two pairs of oligonucleotide primers were used to detect the maize intrinsic genes and the recombinant *cry1Ab* gene. *ZE01* and *ZE02* (1st BASE Pti. Ltd., 41 Science Park Road, Singapore) were used for the detection of maize intrinsic *Ze1* gene. Bt11 1-5′and Cry1Ab 1-3′(same manufacturer) was used to detect *cry1Ab* gene. Sequences of these primers are presented in Table 1.

Table 1. Primers and their sequences used in the study.

Primer	Sequence	Specificity
ZE01	5'-TGC-TTG-CAT-TGT-TCG-CTC-TCC-TAG-3'	*Ze1*/ sense
ZE02	5'-GTC-GCA-GTG-ACA-TTG-TGG-CAT-3'	*Ze1*/ antisense
Bt11 1-5'	5'-CCA-TTT-TTC-AGC-TAG-GAA-GTT-C-3'	*Adh1-1S IVS6*/sense
Cry1Ab 1-3'	5'-TCG-TTG-ATG-TTK-GGG-TTG-TTG-TCC-3'	*Cry1Ab*/antisense

The r-DNA components introduced into the GM-maize lines and an intrinsic gene are shown in Figs 1 and 2. Two pairs of oligonucleotide primers shown in Table 1 were used to amplify r-DNA to detect and distinguish the respective GM maize from non GM maize. In the study the oligonucleotide primer pair Bt111-5′ and cry1Ab1-3′ were synthesized to detect a part of the *cry1Ab* gene cassette of *Bt11* maize (Fig.1). The oligonucleotide primer ZE01-ZE02 was used for detection of the intrinsic *Zein* (*Ze1*) gene to assess the efficiency of all reactions, thereby eliminating any false negative reactions.

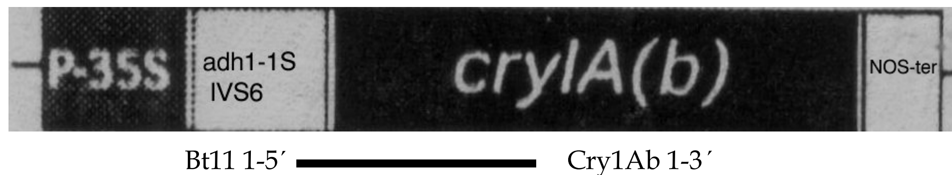

Fig. 1. Shows schematic diagram of the *cry1Ab* gene cassette of Bt11 maize. ▬▬ indicates part of the cassette that is amplified by the Bt11 1- 5′ – cry1Ab 1-3′ primer pair. The unit of an insect-resistant trait consists of [CaMV P-35S]-[DNA fragment containing the No.6 intron sequence (IVS6) from maize alcohol dehydrogenase 1 gene (*adh1-1S*)]-[synthetic *cry1Ab*]-[NOS-ter derived from *Agrobacterium tumefaciens*].

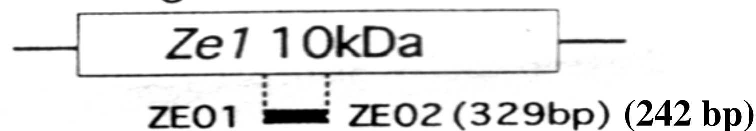

Fig. 2. Schematic diagrams of *zein* (Ze1) gene showing part of the gene that was amplified by *ZE01* - *ZE02* primer pair.

PCR reactions were performed on each DNA sample in a 10 µl reaction mix containing the following reagents: Ampli *Taq* polymerase buffer (10 ×) = 1 µl, primers = .25 µl of each forward and reverse (10 µM), dNTPs (250 µM) =2 µl, Ampli *Taq* DNA polymerase = 1 unit (0.6 µl) (Genei, Bangalore, India), genomic DNA (25 ng/µl) and a suitable amount of sterile deionized water to prepare 10 µl reaction mix. The reaction mix was preheated at 94°C for 10 minutes followed by 45 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C and extension at 72°C for 1 minute. Final extension of 7 minutes at 72ºC was added to allow complete extension of all amplified fragments. Each experiment was repeated at least three times.

The amplified products were separated electrophoretically on 1% agarose gel containing ethidium bromide. Agarose gel electrophoresis was conducted in 1 TE buffer at 100 V for 30 min. One molecular weight marker 100 bp DNA ladder size was electrophoresed alongside the PCR products. DNA bands were observed under UV light on a Transilluminator and photographed by Image Documentation System (Labortechnik, Germany).

The yield of DNA was 600 ng/µl to 1900 ng/µl using both the DNA extraction methods. Both of the protocols were found suitable for the extraction of DNA from the crushed maize samples. Phenol-chloroform-Isoamyl method is cost effective but time consuming. The extraction of DNA using kit was simple but expensive. Although the DNA thus obtained was suitable for the present PCR procedures, the yield of DNA in some of the samples were lower probably due to the fact that maize seeds contain substantial concentrations of polysaccharides. Agarose gel electrophoresis indicated presence of both degraded and good quality of DNA. Degradation was probably due to grinding of the maize samples. It was found that the steeping and wet milling process degrades maize genomic and plastid DNA (Margaret et al. 1999).

The exogenous genes, such as, the *cry1Ab* gene expressed in *Bt* maize are under the control of exogenous promoters, introns and terminators as shown in Fig. 1. The present detection system was based on a PCR system to amplify parts of the promoter or intron, and transgene. The used primer pairs Bt111-5′ and *cry1Ab1-3′* were able to detect a 110 bp product from the above-mentioned recombinant cassette of genetically modified *Bt11* maize (Fig. 2). The size of the expected product is designed to be relatively small. The use of primer with a shorter expected length increases the chance of detection of maize and corn DNA from chickens and cows (Einspanier et al. 2000). Processing of maize for food preparation may degrade or fragments DNA, the primer used in this study was designed to amplify expected length which will work even if fragmented DNA present in the processed foods and in the gastrointestinal tract during safety assessment procedure.

Appropriate design of oligonucleotide primers is important for specific detection of transgene specifically for GM maize present among many types of transgenic varieties and for raw materials of foods.

A detection method to exclude any false judgment owing to any contamination can be performed by using a gene specific primer pair for transgene of GM maize. We were able to obtain reliable and specific detection data by using the primer pair. The primer set to detect *cry1Ab* was designed to amplify DNA from sequence from three different exogenous organisms (part of promoter from CaMV35S, intron from adh1 - 1S gene of maize and part of synthetic *cry1Ab* gene) to prevent false positive reaction in the present study as suggested by Matsuoka *et al.* (2000).

Transgenic maize grain is usually mixed with conventional varieties. Therefore, the present method can be applied to detect GM maize in such mixture of GM maize grains. The PCR products amplified by using *Bt11 1-5'* and Cry1Ab 1-3' primer showed a band of expected size within the genetically modified *Bt11* maize, whereas, no such band was detected in case of non GM maize.

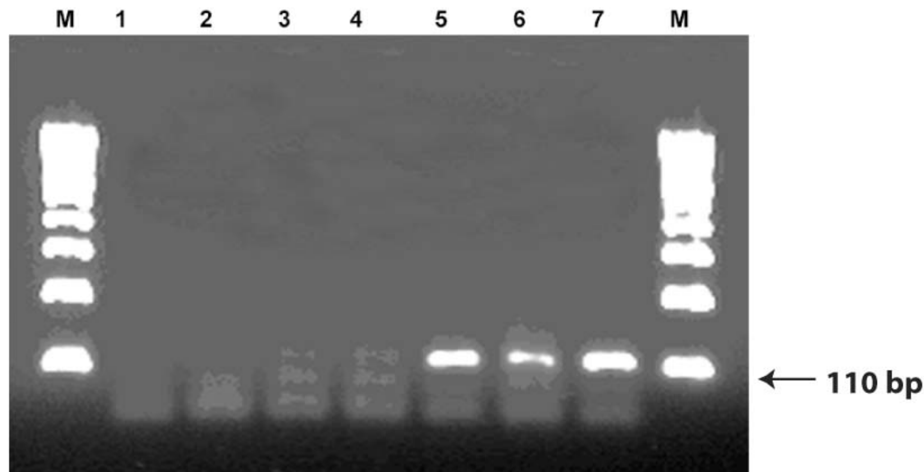

Fig. 3. Agarose gel electrophoresis of PCR products amplified from non GM-maize and GM-maize using primer pair *Bt11 1-5'* and *Cry1Ab 1-3'* which detected the *cry1Ab* gene indicates the expected PCR amplification products.
Lane 1. Control condition (no DNA sample); Lanes 2-4: non GM maize (negative for *cry1Ab* gene); Lanes 5-7: GM maize. M: DNA marker.

In all samples, the primer pair *ZE01-ZE02* detected the intrinsic Ze1 gene (Fig. 4). Thus, the present method has sufficient sensitivity to test the mixing level of GM maize during transportation.

However, we have developed a PCR method capable of identifying genetically modified *Bt* maize. The future work in this laboratory should focus on evaluation of DNA extraction method more closely, designing of different primers and establishment of PCR protocols to detect different transgenes in GM seeds and processed foods, and also develop other detection methods in addition to PCR.

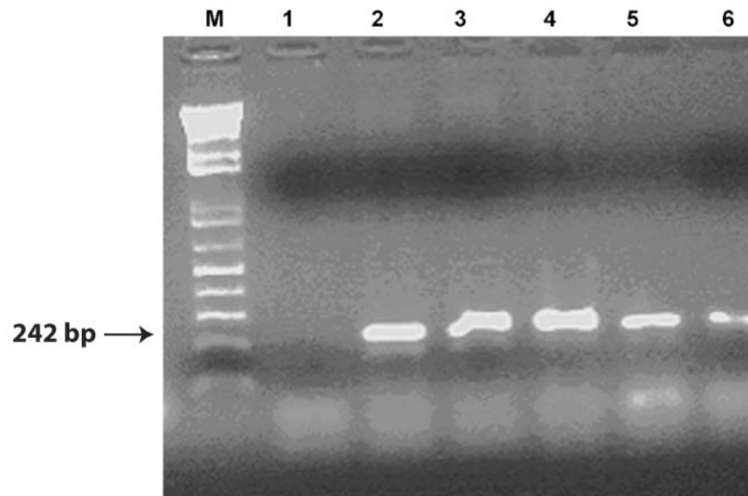

Fig. 4. Agarose gel electrophoresis of PCR products amplified from non GM-maize and GM-maize using primer pair *ZE01-ZE02* that detected the maize intrinsic gene. Lane 1 : Control condition (no DNA sample); Lanes 2-4: Non GM-maize, Lanes 5-6: GM maize. All maize DNA was found positive for *Ze 1* gene indicating efficient PCR amplification.

Acknowledgements

Freeze-dried powder of genetically modified *Bt11* (N58- D1 Lot No. 2608611, Novartis Seed, Inc., Greensboro, NC) and non-GM (*Bt11*) maize isoline were provided by National Institute of Animal Health, Tsukuba, Japan as GMO and non-GMO reference material as a gift which is gratefully acknowledged.

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