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Short Communication

Development of a Multiplex Polymerase Chain Reaction Method for Simultaneous Detection of Genetically Modified Soy and Maize

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ABSTRACT - This study was aimed to develop a novel qualitative multiplex polymerase chain reaction (PCR) for simultaneous detection of genetically modified (GM) soy and maize within a single reaction. The specific primers designed to detect four respective GM events (A2704-12, MON88017, Bt11, and MON863) were included in the tetraplex PCR system. Each of PCR products for four GM events could be distinguished by agarose gel based on their different lengths. The specificity and reproducibility of this multiplex PCR were evaluated. This multiplex PCR consistently amplified only a fragment corresponding to a specific inserted gene in each of the four GM events and also amplified all four of the PCR products in the simulated GM mixture. These results indicate that this multiplex PCR method could be an effective qualitative detection method for screening GM soy and maize in a single reaction.

Key words: *Genetically modified foods, Maize, Multiplex PCR, Simultaneous detection, Soy*

Labeling systems have been introduced for genetically modified (GM) foods in the European Union, Korea, Japan, and other countries, and each of these systems is distinct. For example, the threshold levels for the unintentional presence of GM materials in non-GM crops have been defined as 0.9% in the European Commission[1)], 3% in Korea[2)], and 5% in Japan[3)]. For the monitoring of labeling systems, it has been necessary to develop methods for detecting genetically modified organisms (GMOs) in foods.

For the detection of GMOs in foods, polymerase chain reaction (PCR)-based detection methods that are able to detect even small amounts of transgenes in raw materials and processed foods have been routinely used. However, the cost and duration of analyses could constitute one of the major bottlenecks for providing consumers with cost effective labeling and detection of the non-GM food supply chain. Thus, it is a need to develop time and cost effective detection methods to preserve the freedom of choice of consumers.

Multiplex PCR is a useful method that employs several primer pairs in the same amplification reaction for a sample DNA and can detect multiple target DNA sequences by simultaneous amplifications in one tube. Because the number of GM crops is continuously increasing, multiplex PCR would be one useful method for screening GMO content in raw materials and/or processed foods. Multiplex PCR methods have been developed and reported as simultaneous detection tools for GM crops[4-7)]. In this study, we have developed a novel qualitative multiplex PCR method for simultaneous detection of GM soy (A2704-12) and maize (MON88017, Bt11, and MON863) within a single reaction.

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Materials and Methods

Materials

Dry seeds of four GM events, i.e., one progeny each of A2704-12 and Bt11 developed by Syngenta Seeds AG (Basel, Switzerland), and one progeny each of MON88017 and MON863 developed by Monsanto Co. (St. Louis, MO) were kindly provided by the developer.

Preparation of test samples and DNA extraction

Dry seeds were ground with a P-14 speed motor mill (Fritsch GmbH, Ibar-Oberstein, Germany) and stored at −20°C until use. DNA extraction was performed using the DNeasy Plant Maxi kit (Qiagen GmbH, Hilden, Germany). The DNA concentration of solutions was determined by measuring UV absorbance at 260 nm, and the quality was evaluated by the absorbance ratios at 260/280 and 260/230 nm. These DNA samples were used for the subsequent experiment.

Oligonucleotide primers

The primer pairs suggested by developers for use in the PCR are listed in Table 1. These primers were synthesized by Bioneer Co., Ltd. (Daejeon, Korea). Each oligonucleotide was diluted to the appropriate concentration to conduct PCR with the appropriate volume of sterilized water.

Multiplex PCR conditions. The specific primers synthesized to detect four respective GM events (A2704-12, MON88017, Bt11, and MON863) were included in the tetraplex (4-plex) PCR system. The reaction volume of 25 μL contained 25 ng of genomic DNA, 0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$, 0.2 μmol/L of the 5'- and 3'-primers, and 1.25 units of AmpliTaq Gold DNA polymerase (Applied Biosystems). The reactions were buffered with the PCR buffer II (Applied Biosystems) and amplified in a thermal cycler, the Silver 96 well GeneAmp PCR 9700 (Applied Biosystems) in a max mode, according the following step-cycle program: preincubation at 95°C for 0.5 min, annealing at 63°C for 1 min, and extension at 72°C for 1 min; 27 cycles consisting of denaturation at 95°C for 0.5 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min; followed by a final extension at 72°C for 7 min.

Analysis of PCR products

The PCR products were analyzed by agarose gel electrophoresis. The agarose gel electrophoresis was performed in 3% (w/v) LO3 agarose (Takara Bio Inc., Japan) gel with 0.5 μg/mL ethidium bromide (Sigma-Aldrich, St. Louis, MO). A 5 μL aliquot of each PCR product was electrophoresed at a constant voltage (100 V) for 30 min in 1 × TAE buffer 40 mmol/L Tris-HCL, 40 mmol/L acetic acid, and 1 mmol/L EDTA (pH 8.0) (Wako Pure Chemical Industries, Ltd., Japan). After the electrophoresis was finished, the gel was scanned by a Molecular Imager FX system (Bio-Rad Laboratories Inc., Hercules, CA).

Results and Discussion

We examined the specificity of the synthesized primer pairs under the multiplex PCR conditions. Tetraplex PCR for the detection of four events of GM soy and maize, i.e., A2704-12, MON88017, Bt11, and MON863, was performed using a reaction mixture including four primer pairs (Table 1) at a concentration of 0.2 μmol/L each. The DNAs extracted from four events of GM soy and maize, non-GM soy, and non-GM maize as template DNAs. As shown in Fig. 1, tetraplex PCR amplified only a fragment that corresponded to the expected DNA length in each of the four target events and clearly amplified all four of the PCR products in the simulated GM mixture. In contrast, no amplification was observed with the DNA extracted from non-GM soy and maize. These results indicated that these four primer sets were compatible in the multiplex PCR system.

In this study, we proposed a novel qualitative multiplex PCR method for the simultaneous detection of four GM events in soy and maize that were currently being grown for commercial purposes. This multiplex PCR method could be a useful qualitative method to screen for the presence of GM soy and maize in non-GM materials.

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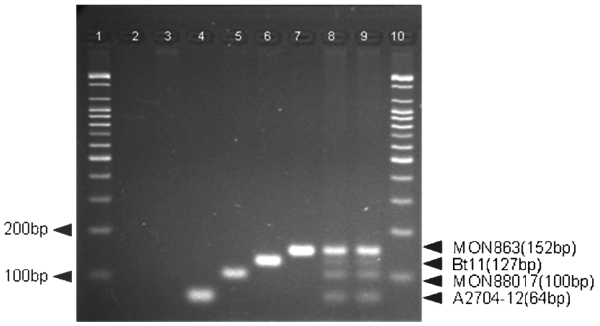

Fig. 1. Specificities of multiplex PCR analyses for the detection of four GM events (A2704-12, MON88017, Bt11, and MON863). The specificities were investigated by multiplex PCR conditions including the four primer pairs of MON88017, Bt11, MON863, and A2704-12 for the template DNA mixture extracted from the seeds of the GM events. Lane1,10: 100bp ladder; Lane2: non-GM soy template; Lane3: non-GM maize template; Lane4: A2704-12 DNA template only; Lane5: MON88017 DNA template only; Lane6: Bt11 DNA template only; Lane7: MON863 DNA template only; Lane8,9: mixtures of four GM DNA templates.

Table 1. List of primers for the multiplex PCR

Target GM	Name	Sequence	Amplicon (bp)
A2704-12	KVM175-5' SMO001-3'	5'-GCA AAA AAG CGG TTA GCT CCT-3' 5'-ATT CAG GCT GCG CAA CTG TT-3'	64
MON88017	MON88017-G-5' MON88017-G-3'	5'-GCT AGC TTG ATG GGG ATC AGA TTG-3' 5'-GAT TGG TTT GTT TTC GGC AGT ATG-3'	100
Bt11	Bt11 3-5' Bt11 3-3'	5'-AAA AGA CCA CAA CAA GCC GC-3' 5'-CAA TGC GTT CTC CAC CAA GTA CT-3'	127
MON863	tahsp17-5' MON 3-3'	5'-GTG TTT TTT GGA TCC CCG G-3' 5'-CCA TCA TGG TTG GTT GGA CTT-3'	152

provision of the GM soy and maize seeds. This work was funded by grants from Korea Food & Drug Administration (09081-095).

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