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QUALITATIVE TRIPLEX PCR FOR THE DETECTION OF GENETICALLY MODIFIED SOYBEAN AND MAIZE

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ABSTRACT

A molecular screening method based on multiplex PCR that involves amplification of specific soybean or maize sequences from plant DNA (lectin or zein) and the amplification of 35S promoter and NOS terminator for the detection of genetically modified soybean and maize was developed. The new method is proposed for the simultaneous detection of tree genetic elements in the same run as reliable method for rapid detection of genetically modified plants with sensitivity of 0.1%.

Keywords: GMO detection, triplex PCR, maize, soybean

Introduction

Analytical methods to detect and quantify genetically modified organisms (GMOs) fall into two main categories: protein analysis to detect the specific protein expressed by the transgene in the GMO through the use of ELISA (enzyme-linked immunosorbent analysis) and lateral flow strip tests or DNA analysis to detect the specific transgene in the GMO or specific elements associated with the transgene (14).

Among those, the polymerase chain reaction (PCR) is the most widely applied method, which can be used for both qualitative and quantitative analyses (5). The PCR method for GM soybean and maize detection has been utilized for qualitative analysis (9, 6, 13).

Genetic control elements such as the Cauliflower Mosaic Virus 35S promoter (CaMV P35S) and the *Agrobacterium tumefaciens* NOS terminator are present in around 95% of currently commercialized GMO plants in EU (3). In order to discriminate between negative and positive results obtained due to inhibition in the amplification, amplification of specific sequence from plant DNA is necessary. If the samples are found to be positive they should be subjected to specific transgenic analysis in order to determine the strain of GMO presents (8).

Screening methods are of great importance for routine analysis and are used for control labelling regulations. The development of a practical detection method is required to confirm the validity of labeling system and to monitor the status of circulation for GMO.

In this study simultaneous amplification technique by triplex PCR has been described as reliable tool for qualitative screening of GMO.

Materials and Methods

Reference materials

The Certified Reference Materials (CRM) standards containing Roundup Ready soybean (RR), MON 810, Bt 176, Bt 11 with 0%, 0.1%, 0.5%, 1% GMO produced by the Institute for Research Materials and Measurements (IRMM) were used.

DNA extraction

DNA extraction was carried out using CTAB method (16). 200 mg of flour was used for DNA isolation. The concentration and purity of extracted DNA were analyzed by agarose gel electrophoresis and with a spectrophotometer Ultraspec 2000 (Pharmacia, England).

PCR primers

The sequences of oligonucleotide primers used in this study are reported in **Table 1**. The primers were synthesized by Metabion GmbH (Germany).

TABLE 1

Oligonucleotide primers

Gene	Sequence (5'-3')	Amplicon (bp)	Reference
LECTIN	TCCACCCCCATCCACATTT GGCATAGAAGTTGAAGGA	81	(1)
ZEIN	AGTGCGACCCATATTCCA G GACATTGTGGCATCATCATTT	277	(14)
NOS	GACACCGCGCGCGATAATTTATCC TTATCCTAGTTTGCGCGCTA	118	(10)
35S	GCTCCTACAAATGCCATCAGATAGT GAT AGT GGG ATT GTG CGT CA CCACGTCTTCAAAGCAAGTGG TCCTCTCCAAATGAAATGAACTTCC	195 123	(8) (10)

Triplex PCR

The multiplex PCR was carried out using premix of 2x PCR Master Mix, (Fermentas, Lithuania) containing 4 mM $MgCl_2$, 0.4 mM dNTP, 0.05 units/µl Taq DNA Polymerase (recombinant).

PCR was performed in a final volume of 25 µl of PCR mix containing 0.8 µM primers for 35S and NOS, 0.2 µM primers

for lectin and zein, Taq polymerase was corrected to 2 units/ reaction (Fermentas) and approx. 100 ng DNA were used.

Amplifications were carried out in a Mastercycler ep gradient S termocycler (Eppendorf, Germany) under the following programs: denaturation at 95 °C for 2 min followed by 40 cycles of 95 °C for 25 sec, 60 °C for 30 sec, and 68 °C for 45 sec and final extension at 68 °C for 10 min.

Amplified products detection

PCR products were determined using electrophoresis on 2% agarose gel containing ethidium bromide (0.5 g/mL). A Step Ladder 50 bp (Sigma, Germany) consisting of DNA fragments ranging in size from 3000 to 50 bp was used as marker.

The visualization was performed in UV transilluminator and the images were captured with DOC PRINT system (Vilber Lourman).

Results and Discussion

The multiplex PCR system applied to reference materials showed only specific amplicons of the expected size. The results related to Bt 176 and MON 810 standards with 0%-1% GMO content showed zein amplicons in all samples and 35S amplicons from 1% to 0.1% (**Fig. 1**). The results related to Bt 11 maize standards with 0% to 1% GMO content gave the zein, 35S and NOS amplicons of size 277 bp, 195 bp and 118 bp (**Fig. 2**).

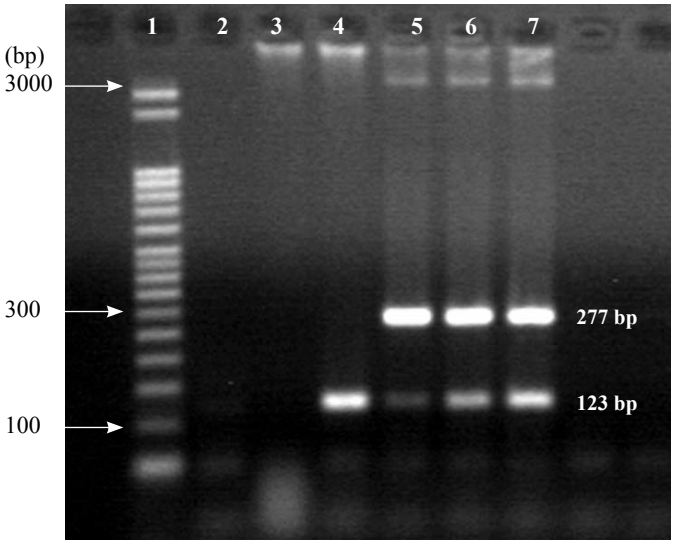

Fig. 1. Electrophoretic profile of multiplex PCR products of zein and 35S promoter: Line 1: DNA ladder; Lane 2: negative (no DNA) control; Line 3: 0% soybean, Line 4: GM soybean; Line 5: 0.1% Bt 176 maize; Line 6: 0.5% Bt 176 maize; Line 7: 1% Bt 176 maize

The multiplex results referred to Roudup Ready soybean standard with 0% to 1% GMO contents showed the lectin amplicons of size 81 bp in all samples exept DNA free negative control and NOS and 35S amplicon from 1% to 0.1% (**Fig. 3**).

The maize and soybean 0% GMO content results indicated the presence of lectin and zein amplicons only. The template free negative control did not show the presence of any amplicon.

Most PCR protocols for GMO detection involve reactions that amplify a single target (10). In triplex PCR tree targets are simultaneously amplified in the same reaction. The combination of several primer pairs in the same reaction tube would result in a higher number of information in a shorter time than single PCR and with less consumption of reagents (4). This detection method saves time and reduced cost (7).

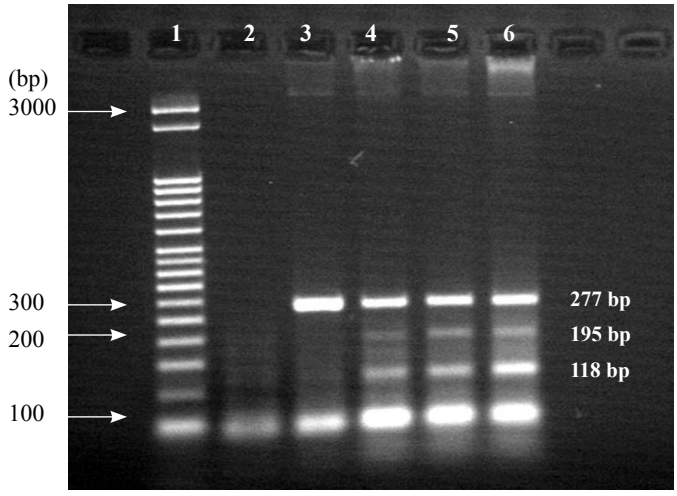

Fig. 2. Electrophoretic profile of multiplex PCR products of zein, 35S promoter and NOS genes: Line 1: DNA ladder; Lane 2: negative (no DNA) control; Line 3: 0% Bt 11 maize, Line 4: 0.1% Bt 11 maize; Line 5: 0.5% Bt 11 maize; Line 6: 1% Bt 11 maize

An inhibition control in the method is a requisite to monitor potential inhibition arising from an individual sample in routine application of the method (12). Host specific internal target (the gene of lectin or zein) has been tested in all assays as control to evaluate DNA quality and PCR efficacy, reducing the risk of false negatives, thereby increasing reliability. The amplifiacability of the DNA extracted from the samples was confirmed using plant specific primers through visualization of amplicons 81 bp for lectin and 277 bp for zein.

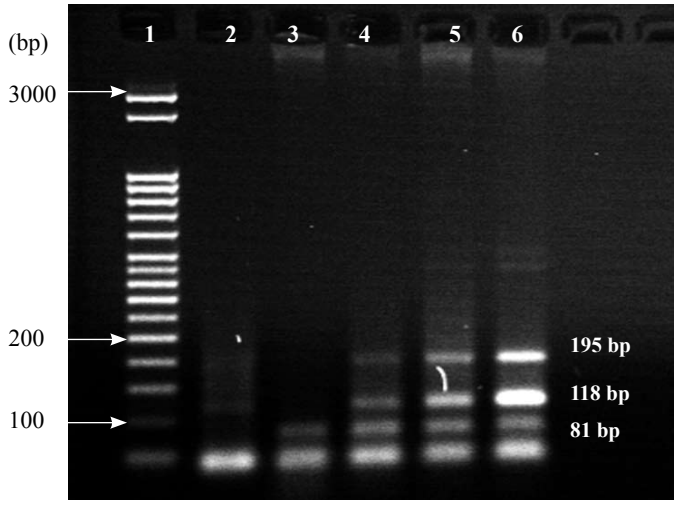

Fig. 3. Electrophoretic profile of multiplex PCR products of lectin, 35S promoter and NOS genes. Line 1: DNA ladder; Lane 2: negative (no DNA) control, Line 3: 0% soybean, Line 4: 0.1% soybean; Line 5: 0.5% soybean, Line 6: 1% GMO soybean

35S promoter regulates the gene expression of Roundup Ready soybean, Bt 11, Bt 176 and MON 810 maize, and his presence is shown in triplex PCR reaction.

The sensitivity and robustness of the procedure were demonstrated by the detection of certified reference material containing GM soybean and GM maize ranging from 0% to 1%. Sensitivity in triplex PCR was 0.1% what is higher than sensitivity obtained by Forte et al. (3) 0.5% for GM soybean and Germini et al. (4) 0.25% for maize.

The method has been validated according to International Seed Testing Association (ISTA) procedure for evaluation of performance data in context of laboratory accreditation for determination of specific traits (GMO).

The triplex PCR has a series of advantages over common PCR including sensitivity, cost saving, efficiency and reproducibility and could be suitable for statutory control activities with respect to labelling and establishing quality assurance systems for non-transgenic organisms producing.

Most countries that have adopted mandatory labelling rules for food or feed have set tolerances for the adventitious presence of GM material in grain products or the final foods based on a percent GM content. In order to reduce detection time and cost, and also promote the accuracy of detection, multiplex PCR technique has been developed. The methods assessed in the present study would become the fundamentals for the establishment of routine tests of GMO and to be referenced by the food labeling requirements in Serbia.

Conclusions

We developed the PCR screening method which is from practical point of view very suitable, saves time and reduced cost of analysis. This study shows that method could be employed to distinguish GM from GM products, because the legal requirement for labelling of foods containing GMO.

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