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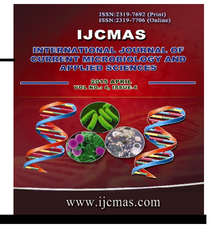

Original Research Article

A novel Multiplex PCR Method for Simultaneous Detection of Genetically Modified Soybean Events

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A B S T R A C T

The increasing number of commercialized GM crops and the growing need for authenticity control of raw materials, feeds and foods set an urgent necessity for the development of sensitive, reliable and cost-effective methods for GMO detection. In the present study, a novel multiplex PCR method for the simultaneous detection of all EU-authorized genetically modified soybean events was developed. The method was based on three gene-specific *(EPSPS*, *PAT* and *Cry1Ac* genes) and one event-specific (DP 356043) DNA sequences. It was characterized with high sensitivity, as the LOD for each sequence was 0.05%. The new method was applied for the screening of 15 soybean products and 36 meat products at the market for the presence of genetically modified soybean events. Results demonstrated that 51% of the tested samples contained *EPSPS* gene, while *PAT* gene was detected in 8% of the DNA extracts. In contrast to that, *Cry1Ac* gene and DP 356043 event-specific sequence were not observed in any of the analyzed products. The data indicated that the proposed method could be used as a reliable routine screening assay of various food products for the presence of EU-authorized genetically modified soybean events.

Keywords

Multiplex PCR, Genetically modified soybean events, EU legislation, Soybean products, Meat products

Introduction

Genetically modified organisms (GMOs) are defined as organisms ″in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination″ (Directive 2001/18/EC). Since the GMOs entered the food chain, a scientific and public debate concerning their safety and the need for labeling information came up especially in Europe (Mafra *et al.*, 2008). Therefore, the EU has dedicated special attention to consumer information by requiring a mandatory labeling for food products containing more than 0.9% of authorized genetically modified (GM) material (Regulation (EC) No 1829/2003). The increasing number of commercialized GM crops and the growing need for authenticity control of raw materials, feeds and foods sets an urgent necessity for the development of sensitive, reliable and cost-effective methods for GMO detection.

The polymerase chain reaction (PCR) remains the most widely used analytical system for detection of GMO due to its high sensitivity and reliability (Peano *et al.*, 2005). Multiplex PCR methods based on simultaneous amplification of multiple sequences save considerable time and effort by decreasing the number of reactions required to assess the possible presence of GMO in a food sample (Nikolić *et al.*, 2009). Several approaches based on multiplex PCR have been developed for detection of genetically modified organisms in different food products (Nikolić *et al.*, 2009; Germini *et al.*, 2004; Jinxia *et al.*, 2011; Guo *et al.*, 2012; Demeke *et al.*, 2002; Choi, 2011). Peano *et al.* developed a multiplex PCR system for simultaneous amplification of seven DNA target sequences – *lec* gene, *zein* gene and five gene-specific sequences (Peano *et al.*, 2005). Guo *et al.* reported for an effective quadruplex PCR assay targeting the commonly used trait genes in GMOs (Guo *et al.*, 2012) and Nikolic *et al.* applied a multiplex PCR method for detection of *lec* gene, 35S promotor and T-nos terminator (Nikolić *et al.*, 2008). The numerous advantages of genetically modified crops are facing the increasing concern of consumers for the potential risk associated with the spread of GMOs into the environment, with the preservation of biodiversity, human health and food safety. With the growing number of GMOs that may be simultaneously present in a food product, the analysis for a particular GMO is time consuming and economically inefficient. Therefore, the initial screening for the presence of taxon-specific and widely spread genetically modified DNA elements can contribute to a rapid and cost-effective discrimination of the samples to such containing transgenic DNA and to GMO-free ones. Currently, the matrix approach is applied in the majority of GMO laboratories throughout the world and is adopted as a model for conducting GMO assays (European Commission. A decade of EU-funded GMO research (2001–2010). Report: EUR 24473 EN). Waiblinger *et al.* used the matrix approach for screening 81 transgenic events by detection of five target DNA sequences (Waiblinger *et al.*, 2010). Leimanis *et al.* also validated a multiplex method for GMO screening based on the matrix approach (Leimanis *et al.*, 2008).

At present, seven genetically modified soybean events are authorized in the European Union: MON 40-3-2, MON 89788, MON 87701 and MON 87701 x MON 89788 developed by Monsanto Company, A 2704-12 and A5547-127 by Bayer CropScience AG and GM soybean event DP 356043 by Pioneer Hi-Bred International, Inc. (GMO Compass, 2014). Our literature search did not find a multiplex PCR method for the simultaneous detection of the EU-authorized genetically modified soybean events. Therefore, the aim of the present study was to develop such method and implement it for the routine screening of soybean and meat products at the market.

Materials and Methods

Samples

The samples used in this study included 15 soybean products and 36 meat products. The soybean products were divided into four groups: raw soybeans (2); soy protein isolates and flours (5); textured soy proteins (5) and vegetarian foods (3). The meat products were divided into four groups: fresh processed meat products (13); raw-cooked meat products (9); cured meat products (6); cooked and smoked salami (5) and dried meat products (3).

Certified Reference Materials ERM-BF 410dk and ERM-BF 425d (IRMM, Geel, Belgium), and CRM AOCS 0707-B3 and CRM 0809-A (AOCS, USA) were used as positive controls, targeting respectively *EPSPS* gene, DP 356043 event, *PAT* gene and *Cry1Ac* gene. Certified Reference Materials ERM-BF 410ak and ERM-BF 425a (IRMM, Geel, Belgium) and CRM AOCS 0707-A3 and CRM 0906-A (AOCS, USA) were used as negative controls.

DNA extraction

All samples were homogenized with a laboratory homogenizer VWR 431 (VWR, USA). DNA extraction from samples and reference materials was conducted by a modified CTAB method, according to Stefanova *et al.* (2013).

The DNA concentration was determined by measuring the absorbance at 260 nm. The purity of DNA extracts was calculated by the ratio of the absorbance at 260 nm and 280 nm (Shimadzu UV-VIS, Shimadzu Corporation, Japan).

Amplification of the lectin gene was performed according to Meyer *et al.* (1996).

Selection of primers for multiplex PCR

Development of the novel multiplex PCR method was carried out according to the matrix approach.

A matrix with four modules was created, and selection of the primer sequences was done in accordance with the available databases and previous publications. Primer sequences and references were listed in Table 1. The oligonucleotide sequences were synthesized by Metabion GmbH (Germany).

Multiplex PCR conditions

Series of experiments were conducted in order to determine the optimal conditions for multiplex PCR, including different amounts of DNA used (50 ng, 100 ng, 150 ng, 200 ng, 500 ng), as well as combinations of different concentrations of PCR buffer (0.5x, 1x and 2x), $MgCl_2$ (1.0 mM, 1.5 mM and 2.0 mM), dNTP (0.1 – 0.6 mM), primers (0.1 – 0.6 μM) and DNA polymerase (1 U, 1.5 U, 2 U, 2.5 U and 3 U). Additionally, the temperature and duration of the particular stages of the PCR reaction were adapted.

Multiplex PCR was carried out with conventional PCR (2720 Thermal Cycler, Applied Biosystems, USA). The PCR reaction was performed in a final volume of 25 μl, containing 5 μl DNA extract (150 ng DNA), 1x PCR buffer (Fermentas, Canada), 0.5 mM of each dNTP (Fermentas, Canada), 1.5 mM $MgCl_2$ (Fermentas, Canada), 2.5 U TrueStart™ HotStart Taq DNA polymerase (Fermentas, Canada), 0.5 μM of each primer targeting *EPSPS* gene, 0.2 μM of primers for *PAT* gene, 0.3 μM of primers for *Cry1Ac* gene and 0.4 μM of each primer targeting DP 356043 genetically modified soybean event.

Parameters of amplification were as follow: initial denaturation at 95°C for 5 min, 35 cycles of 30 s at 95°C, 60 s at 58°C and 30 s at 72°C , and a final extension at 72°C for 10 min. Each extract was amplified in duplicate assays. Further the amplified fragments were analyzed by electrophoresis in a 2% agarose gel carried out in 0.5 x TBE buffer (45 mM Tris-borate and 1 mM EDTA) for 60 min at 100 V, stained with Safe View Nucleic Acid Stain (NBS Biologicals, England). The agarose gel was visualized under UV light using MiniBIS Pro transilluminator and gel documentation system (DNR Bio-Imaging Systems, Israel).

The sensitivity of the multiplex PCR method was determined by preparing seven DNA solutions with equal percentage content of the four targeting DNA sequences (2.5%, 2%, 1%, 0.5%, 0.1%, 0.05% and 0.01%, respectively). Each DNA solution was amplified in four independent PCR reactions. The DNA solution with the lowest percentage of the four target DNA sequences, which was amplified successfully in all independent PCR reactions was determined as limit of detection (LOD).

Result and Discussion

DNA extraction, concentration and purity

Quality and yield of the isolated DNA are critical factors in DNA preparation for further PCR analysis. Results from the spectrophotometric assessment of DNA concentration and purity showed that the extraction method produced high quality DNA extracts. Concentration varied between 60.79 – 806.93 ng DNA/µl DNA extract and the purity of the extracts was also high (A_{260}/A_{280} = 1.65 – 1.86 for all extracts) (data not shown).

The DNA extracts from all reference materials, soybean and meat products were further subjected to PCR analysis for detection of the taxon-specific lectin gene, in order to confirm their suitability for amplification. All extracts gave positive signal for the presence of the lectin gene and were therefore considered suitable for further PCR assay (data not shown).

Matrix approach, selection of target sequences and PCR primers

Currently, seven genetically modified soybean events (MON 40-3-2, A2704-12, MON 89788, MON 87701, DP 356043, A5547-127 and MON 87701 x MON 89788) are authorized for food and feed use in the European Union. The development of the novel multiplex PCR method for detection of all GM events listed above was done according to the matrix approach. The first step was to create a matrix (table) providing information for the presence or absence of certain elements in the GMO. Further, the analytical module for each target DNA sequence was elaborated. The matrix in the present study included four analytical modules for screening of the EU-authorized genetically modified soybean events (Table 2). Selection of the DNA sequences was based on the available information regarding target soybean events. The multiplex PCR assay of three gene-specific modules and one event-specific module allowed the simultaneous detection of all EU-authorized genetically modified soybean events. The primers used in the present study were selected based on their similar annealing temperatures.

Multiplex PCR method and determination of LOD

Development of a multiplex PCR method for simultaneous detection of *EPSPS*, *PAT* and *Cry1Ac* genes and DP 356 043 event-specific sequence was based on the simplex PCR reactions. The novel multiplex PCR method produced four amplicons with distinct sizes (Figure 1) – 145 bp (for *EPSPS* gene), 262 bp (for *PAT* gene), 300 bp (for *Cry1Ac* gene) and 99 bp (for DP 356 043 event). The sensitivity of the multiplex PCR method was evaluated by equivalent DNA mixtures of four GM soybean events (MON 40-3-2 with *EPSPS* gene, A2704-12 with *PAT* gene, MON 87701 with *Cry1Ac* gene and DP 356043, containing the event-specific sequence). Seven DNA solutions with equal content of the four targeting DNA sequences (2.5%, 2%, 1%, 0.5%, 0.1%, 0.05% and 0.01%, respectively) were used for determination of LOD of the method (Figure 1).

The data in Figure 1 demonstrated successful amplification of all four target DNA sequences in DNA extracts from the positive control and the DNA solutions in lanes 1 to 6. This showed the possibility for simultaneous detection of each of these sequences in concentrations up to 0.05%. In lane 7 (DNA solution with 0.01% of each DNA target sequence), only PCR amplicons with size 262 bp and 300 bp were observed, which demonstrated the higher sensitivity of the corresponding simplex methods when they were combined in a multiplex PCR assay. PCR fragments of 99 bp, 145 bp, 262 bp or 300 bp were not detected in the DNA extracts from the negative controls (N and C), which showed the absence of non-specific amplification and confirmed the specificity of the method.

It is important to note that the sensitivity of the novel multiplex PCR method was the same as the sensitivity of the simplex methods for detection of *EPSPS*, *PAT* and *Cry1Ac* genes. In contrast, the LOD of the PCR method for detection of DP 356 043 GM soybean event changed from 0.01% to 0.05% (data not shown). Similar results were obtained by Leimanis *et al.*, demonstrating the slight alteration in sensitivity of the multiplex method in comparison with the simplex ones (Leimanis *et. al.*, 2008).

Based on the results in Figure 1, the concentration of 0.05% was determined as LOD of the novel multiplex PCR method. The current EU legislation stipulates that any food or feed which consists of or is produced from or contains GMO more than 0.9% must be labeled (Regulation (EC) No 1829/2003). The multiplex PCR method developed in the present study was characterized with a significantly higher sensitivity (0.05%), therefore it could be used as a reliable routine screening assay of various food products for the presence of the EU-authorized genetically modified soybean events. The established LOD of the developed PCR method corresponds to about 66 soybean genomic copies of each target DNA sequence.

Leimanis *et al.* reported that the sensitivity of their multiplex PCR method was 0.045% for each DNA sequence, with lower sensitivity when the *EPSPS* gene was targeted (Leimanis *et. al.*, 2008). Results from the present study also demonstrated higher sensitivity in detection of the other target DNA sequences in comparison with the *EPSPS* gene. Taski-Ajdukovic *et al.* used a duplex PCR method for the detection of *lec* gene and 35S promoter sequence in meat products and estimated LOD of 0.1% of the method (Taski-Ajdukovic *et al.*, 2009). Agodi *et al.* achieved an LOD of approximately 9 soybean genomic copies when analyzing milk for the presence of genetically modified DNA (Agodi *et al.*, 2006). Peano *et al.* developed a multiplex PCR method for GM soybeans and maize with a detection limit of 0.4%, which is significantly lower compared to the LOD estimated in the present study (Peano *et al.*, 2005). Forte *et al.* developed a multiplex PCR method for the detection of 35S promoter, T-nos terminator and *lec* gene (Forte *et al.*, 2005). The sensitivity of the method was 0.5% for the 35S promoter and the T-nos terminator sequences, which is also much lower than the present results.

The LOD of a PCR method depends on a wide variety of experimental conditions such as DNA target sequence, selection of appropriate primers and the analyzed food matrix, and all these factors affect the sensitivity of the method. Results from the present study show that the newly developed multiplex PCR method is characterized by higher sensitivity than any other multiplex methods described so far in the literature.

Application of the multiplex PCR method

The novel multiplex PCR method was applied for screening of 15 soybean products and 36 meat products for the presence of the following genetically modified soybean events: MON 40-3-2, A2704-12, MON 89788, MON 87701, DP 356043, A5547-127 and MON 87701 x MON 89788. PCR amplification of each sample was performed in two replicates.

PCR analysis of soybean products

Figure 2 presented the results from the multiplex PCR assay of soybean products. The data showed that all four PCR amplicons with the sizes of 99 bp, 145 bp, 262 bp or 300 bp were observed in each positive control, corresponding to DP 356 043 event, *EPSPS* gene, *PAT* gene and *Cry1Ac* gene, respectively. Fragments of 145 bp showing the presence of the *EPSPS* gene were detected in the DNA extracts from one of raw soybean samples (Fig. 2A), four of the soybean flours and isolates (Fig. 2B), four soybean granulates (Fig. 2C) and two products from the group of vegetarian foods (Fig. 2D). The amplicon of 262 bp corresponding to *PAT* gene was detected only in one soybean granulate and two vegetarian foods. PCR products with sizes of 99 bp and 300 bp were not observed in any of the analyzed soybean products, which demonstrated the absence of *Cry1Ac* gene and DP 356 043 event in the DNA extracts of these samples. Fragments of 99 bp, 145 bp, 262 bp and 300 bp, corresponding to the target DNA sequences were not observed in the negative controls (N and C). These results confirmed the purity of the PCR components and the absence of non-specific amplification.

PCR analysis of meat products

The data from the novel multiplex PCR assay of meat products was presented in Figure 3. Experimental data showed specific fragments with size of 145 bp in 7 of the fresh processed meat products (Fig. 3A), 2 of the raw-cooked meat products (Fig. 3B), 4 cured meat products (Fig. 3C) and one product from the dried meat group (Fig. 3E). An amplicon corresponding to *PAT* gene was detected only in one sample of raw-cooked meat products (Figure 3B), showing the presence of any of the GM soybean lines containing this gene. The other two fragments with sizes of 99 bp and 300 bp (DP 356043 event and *Cry1Ac* gene, respectively) were not found in any of the meat samples, therefore the analyzed products did not contain the corresponding GM soybean events. The DNA extracts from all tested cooked and smoked salami (Figure 3D) did not contain fragments of 99 bp, 145 bp, 262 bp and 300 bp, which indicated that any of the EU-authorized genetically modified soybean events were not presented in the extracts from these products. None of the target amplicons was detected in the negative controls (N and C), which demonstrated the absence of non-specific amplification as well as the purity of the components in the PCR analysis.

Data analysis

The next stage of the matrix approach was to compare the results obtained from the screening with the matrix data used as a standard. Table 3 summarized the content of each target DNA sequence in the DNA extracts from the tested soybean products. Results demonstrated that the *EPSPS* gene was present in 12 of the 15 tested soybean products, therefore these samples could contain any of the GM soybean events (MON 40-3-2, MON 89788 or MON 87701 x MON 89788) or all of them. The multiplex PCR assay showed the presence of *PAT* gene only in three soybean products, indicating that the GM soybean events A2704-12 and A5547-127 could be present in the tested products. None of the analyzed soybean products contained *Cry1Ac* gene and DP 356043 event-specific sequence, which showed that the products did not contain MON 87701, DP 356043 and MON 87701 x MON 89788. This result excluded the possibility for the 12 soybean products mentioned above (with positive signal for *EPSPS* gene) to contain GM soybean MON 87701 x MON 89788. One of the explanations for that could be the later authorization of GM events MON 87701, DP 356043 and MON 87701 x MON 89788 in comparison with the others.

The data presented in Table 4 showed that 14 of the tested meat products contained *EPSPS* gene, indicating the possible presence of GM soybean events MON 40-3-2, MON 89788 or MON 87701 x MON 89788. *PAT* gene was observed in only one product from the group of raw-cooked meat products, therefore this product could contain soybean events A2704-12 and A5547-127.

Results obtained for the meat samples showed absence of *Cry1Ac* gene and DP 356043 event-specific sequence, clearly indicating that GM soybean events MON 87701, DP 356043 and MON 87701 x MON 89788 were not present in the tested meat products.

Results from the application of the novel multiplex PCR method show that 51% (26 samples) of the tested products contained *EPSPS* gene. *PAT* gene was detected in 8% of the samples, while *Cry1Ac* gene and DP 356043 event-specific sequence were not observed in any of the analyzed products. In summary, the total number of GM-containing products was 27, which represented 53% of all tested samples.

Greiner *et al.* analyzed 100 soy-containing products during the period 2000-2005 and reported that in 2000 13% of the samples contained DNA material from the GM soybean event MON 40-3-2 (Roundup Ready®), while in 2005 this percentage increased to 78% (Greiner *et al.*, 2008). The survey demonstrated the fast increase of the use of GM soya in Brazil. Ujhelyi *et al.* carried out a screening of 208 soy products at the Hungarian market for the presence of 35S promoter and T-nos terminator sequence (Ujhelyi *et al.*, 2008), showing that 39% of the analyzed samples contained transgenic DNA. Taski-Ajdukovic *et al.* tested meat products for the presence of GM soybean with a duplex PCR method targeting the taxon-specific *lec* gene and 35S promoter sequence. They reported that 24% of the meat products contained genetically modified material (Taski-Ajdukovic *et al.*, 2009). Nikolic *et al.* reported the presence of genetically modified DNA in 11% of the examined variously processed soy products (Nikolić *et al.*, 2009), while a screening survey of soy products in Jordan established the presence of GM soybean DNA in 33% of them (Al-Hmoud, *et al.*, 2010).

The available information on the presence of genetically modified soybean DNA in food products showed high variability of the GM content. On one hand, GM content in foods increased over time due to the constant influx of the number of new GM soybean events as well as the expansion of transgenic soybeans compared to conventional plants.

Table.1 Target DNA sequences and primers

Target	Primers	Sequence	Amplicon (bp)	Reference
EPSPS gene	Sttmf3a Sttmr2a	GCAAATCCTCTGGCCTTTCC TTGCCCGTATTGATGACGTC	145 bp	James et al. (2003)
PAT gene	PAT 2F PAT 2R	GAAGGCTAGGAACGCTTACG GCCAAAAACCAACATCATGC	262 bp	Permingeat et.al (2002)
Cry1Ac gene	Cry1Ac-f Cry1Ac-r	GAGAACGGATTGAGACTGGTT GGCAGGATTGGTCGGGTCTGC	300 bp	Choi (2011)
DP 356043 event	DP356-f1 DP356-r1	GTCGAATAGGCTAGGTTTACGAAAAA TTTGATATTCTTGGAGTAGACGAGAGTG T	99 bp	Shrestha et al. (2008)

Table.2 Matrix with four analytical modules for screening of the EU-authorized genetically modified soybean events

GM soybean event	*EPSPS* gene	*PAT* gene	*Cry1Ac* gene	DP 356043 event
MON 40-3-2	+ [a]	-	-	-
A2704-12	- [b]	+	-	-
MON 89788	+	-	-	-
MON 87701	-	-	+	-
DP 356043	-	-	-	+
A5547-127	-	+	-	-
MON 87701 x MON 89788	+	-	+	-

a Presence of the target DNA sequence in the genome of each GM soybean event;
b Absence of the target DNA sequence in the genome of each GM soybean event.

Table.3 Presence of EPSPS gene, PAT gene, Cry1Ac gene and DP 356043 event-specific sequence in the analyzed soybean products

Products	No. of samples	*EPSPS* gene	*PAT* gene	*Cry1Ac* gene	DP 356043 event
Raw soybeans	2	1	0	0	0
Soy protein isolates and flours	5	4	0	0	0
Textured soy proteins	5	5	1	0	0
Vegetarian foods	3	2	2	0	0
Total	15	12	3	0	0

Table.4 Presence of EPSPS gene, PAT gene, Cry1Ac gene and DP 356043 event-specific sequence in the analyzed meat products

Products	No. of samples	*EPSPS* gene	*PAT* gene	*cry1Ac* gene	DP 356043 event
Fresh processed meat products	13	7	0	0	0
Raw-cooked meat products	9	2	1	0	0
Cured meat products	6	4	0	0	0
Cooked and smoked salami	5	0	0	0	0
Dried meat products	3	1	0	0	0
Total	36	14	1	0	0

Figure 1. Agarose gel electrophoresis profile of PCR products of DNA solutions with equal content of the four DNA sequences, targeting EPSPS gene, PAT gene, Cry1Ac gene and DP 356043 event. M: 100 bp ladder; P: positive control; 1: DNA solution with 2.5% of each DNA target sequence; 2: DNA solution with 2% of each DNA target sequence; 3: DNA solution with 1% of each DNA target sequence; 4: DNA solution with 0.5% of each DNA target sequence; 5: DNA solution with 0.1% of each DNA target sequence; 6: DNA solution with 0.05% of each DNA target sequence; 7: DNA solution with 0.01% of each DNA target sequence; N: negative control (0% of each DNA target sequence); C: negative PCR control (containing deionized water)

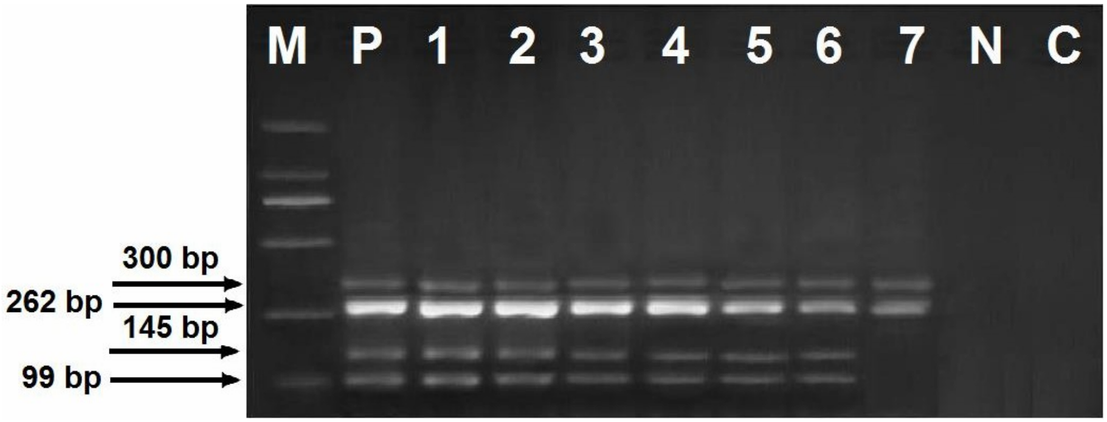


Figure.2 Agarose gel electrophoresis of PCR products of DNA extracts from raw soybeans (A), soy protein isolates and flours (B), textured soy proteins (C) and vegetarian foods (D) for detection of EPSPS gene, PAT gene, Cry1Ac gene and DP 356043 event. M: 100 bp ladder; P: positive control; 1–2 (A), 1–5 (B), 1–5 (C) and 1–3 (D): soybean products; N: negative control (0% of each DNA target sequence); C: negative PCR control (containing deionized water)

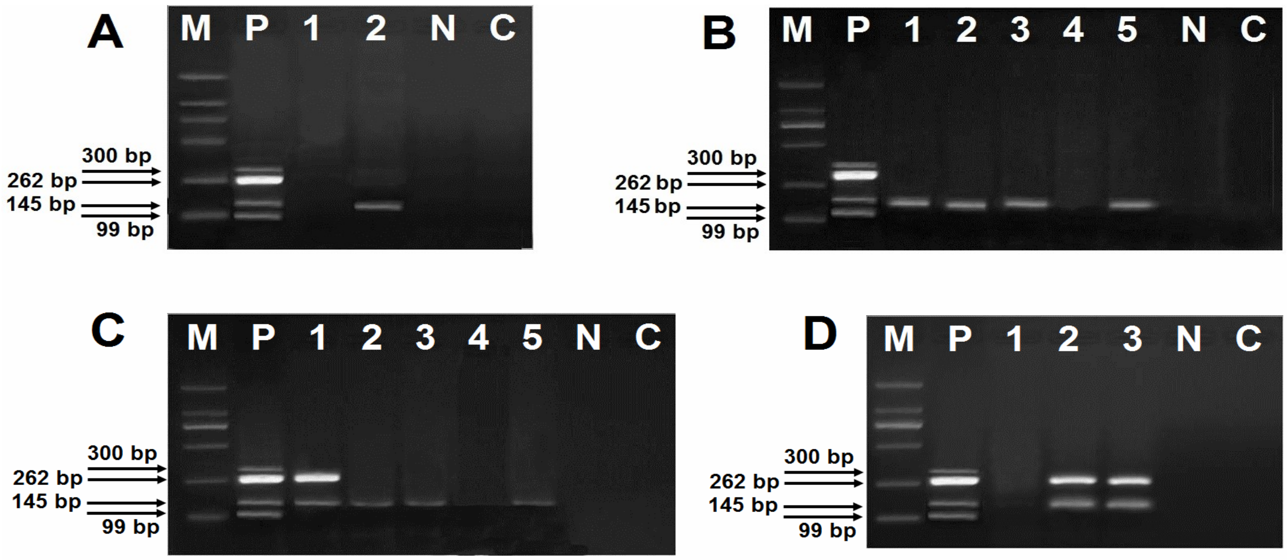
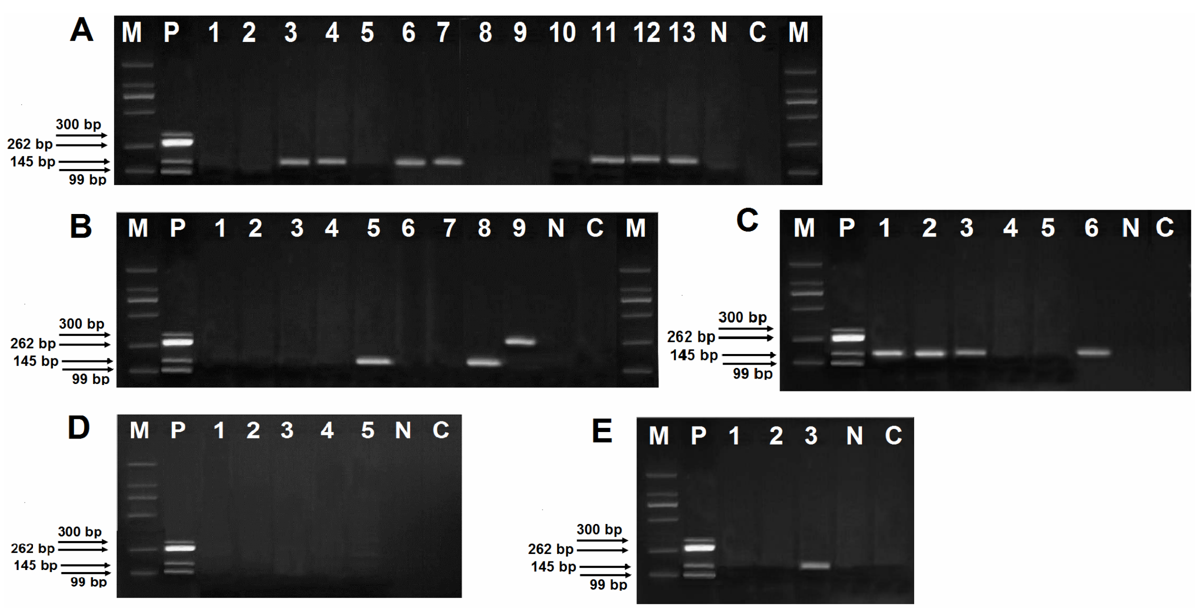


Figure.3 Agarose gel electrophoresis of PCR products of DNA extracts from fresh processed meat products (A), raw-cooked meat products (B), cured meat products (C), cooked and smoked salami (D) and dried meat products (E) for detection of EPSPS gene, PAT gene, Cry1Ac gene and DP 356043 event. M: 100 bp ladder; P: positive control; 1–13 (A), 1–9 (B), 1–6 (C), 1–5 (D) and 1–3 (E): meat products; N: negative control (0% of each DNA target sequence); C: negative PCR control (containing deionized water)



On the other hand, the content of GM soybean depended on the composition and the degree of processing of the tested food products, and was also connected with the geographical region where the studies were carried out. It is important to note that all above cited studies were focused on the detection of only one GM soybean event – MON 40-3-2 (Roundup Ready®) in different food products, whereas the current study investigated the presence of all EU-authorized GM soybean events. This could also be a reason for the estimated high percentage of products containing transgenic DNA (53%).

A novel multiplex PCR method was developed for the simultaneous screening of the EU-authorized genetically modified soybean events. The proposed method targeted three gene-specific and one event-specific DNA sequences and showed high sensitivity (LOD = 0.05%). The new method is rapid, reliable and easy to perform, and was successfully applied for the analysis of various food products. Furthermore, analytical results demonstrated that 51% of the tested samples contained *EPSPS* gene, while *PAT* gene was detected in 8% of the DNA extracts. In contrast, *Cry1Ac* gene and DP 356043 event-specific sequence were not found in any of the analyzed products. In conclusion, 53% of all tested soybean and meat products contained GM material, which demonstrated the rapid increase of the number of food products containing transgenic DNA and therefore implied the urgent necessity of the implementation of multiplex PCR analysis for the efficient screening of foods and feeds at the market.

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