ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 9 (4): 499-505, 2010
ISSN 1682-296X

Detection of Genetically Modified Maize and Soybean Food Products in the Jordanian Market

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Abstract: The objective of this study was to survey for the genetically modified maize and soybean food products in the Jordanian market. The study was designed to extract genomic DNA of maize and soybean products by cetyltrimethylammonium bromide (CTAB) method and to identify specific genes for maize and soybean, expression control specific genes 35S promoter and NOS terminator by polymerase chain reaction analysis. For confirmation test of the genetically modified maize and soybean food products, nested polymerase chain reaction experiments were performed using internal primers for the detection of the E35S promoter and the hsp70 exon1/intron1 region of maize MON810 and Cp4 EPSPS gene of soybean. Three out of 19 maize food products were identified as carrying amplified DNA fragments of 35S promoter region, the nested PCR test confirmed the presence of MON810 event. One of three soybean food products was identified as carrier DNA fragments of 35S promoter region, Cp4 EPSPS event was not detected.

Key words: Genetically modified food, maize, soybean, 35S promoter, nested PCR

INTRODUCTION

The introduction of Genetically Modified Organisms (GMOs), also called Living Modified Organisms (LMOs) and their products have made a great contribution to the world's economic development (Al-Hmoud *et al*., 2008; Borlaug, 2000; Raveendar and Ignacimuthu, 2010; Vain, 2006). Currently transgenic maize and soybean are worldwide major crops with vital agronomical interests, at present there are several GM products of these crops available in markets, e.g., Roundup ReadyTM soy and MON810 Yield Gard Corn (Greiner and Konietzny, 2008; Ujhelyi *et al*., 2008). However, there are rising international concerns about possible potential risks of GM products, this is because of the results of various studies which reported possible harmful effect might be caused from the use of GMO or their products (Konig *et al*., 2004; De Vendomois *et al*., 2009; Seralini *et al*., 2007, 2009). As a result of possible risks which might be implicated by GMOs, increasing number of countries has adopted labeling policies for GMOs and their products. Nowadays, countries with enforced labeling policies include Australia, China, the European Union, New Zealand, Japan, Russia, Saudi Arabia, South Korea, Switzerland and Taiwan and there are more countries planning to introduce labeling policies (Gruere and Rao, 2007). Consequently studies have been carried out in several countries to ascertain the presence and type of GM events of GM products. Type of genetic modification of GM soybean and maize products in Egyptian market were identified by Polymerase Chain Reaction (PCR), the study revealed 20% of 40 investigated soy samples contained Roundup Ready soybean; 15% of 40 maize samples tested positive for Bt176 and 12.5% positive for Bt11 maize (El-Sanhoty *et al*., 2002). Polymerase-chain-reaction-based methods were used to detect and survey genetically modified soy (Roundup-ReadyTM soy) and maize (Bt176 Maximizer maize; Bt11 maize, MON810 Yield Gard corn, T25 LibertyR Link maize) sold commercially in Brazil, the investigation showed the number of food products containing genetically modified soy in a proportion above 1.0% on the ingredient level, the threshold for labeling according to Brazilian legislation, increased from 11% in 2000 to 36% in 2005 (Greiner and Konietzny, 2008; Greiner *et al*., 2005).

The objective of this study was to determine qualitatively by validated PCR methods the occurrence of products derived from genetically modified maize and soybean in foods sold commercially in Jordan. Maize and soybean were chosen because these are the major transgenic crops grown world-wide.

MATERIALS AND METHODS

Maize and soybean food products: Twenty two commercial food products, 19 of maize origin and three of soybean

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Table 1: Country of origin of analyzed maize and soybean food products

Food product category	Country of origin	No. of tested products	No. of detected GM products
Maize	USA	2	0
	India	2	0
	Thailand	1	0
	Hungary	1	0
	Lebanon	1	0
	Unknown	12	3
Soybean	China	1	0
	USA	1	1
	Netherland	1	0

GM indicates genetically modified products detected in this study

origin (Table 1), were obtained during the period from January 2010 and June 2010 from Food and Drug Testing Administration, the Royal Scientific Society Testing Laboratories, local bakeries and supermarkets in Amman, Jordan. Standard maize genetically non-modified (ERM-BF413a), genetically modified MON 810 maize (BF413f), standard soybean genetically non-modified (BF410a) and genetically modified Roundup ReadyTM soya (ERM-BF410e) were obtained from Dr. Eric Kubler. These samples were originally purchased from European Commission, DG JRC and IRMM, Belgium.

Genomic DNA extraction: Genomic DNA was extracted from flour, meal or ground samples of maize and soybean products by Cetyltrimethylammonium bromide (CTAB) method (Querci *et al*., 2006). Five hundred microliter of CTAB was added to 100 mg of homogeneous sample already mixed with 300 μL of sterile deionized water. The mixture was subjected to protinase K (20 mg mL^{-1}) and RNase (10 mg mL^{-1}) treatments at 65°C for 30 and 10 min, respectively and then centrifuged for 10 min. The supernatant mixed was with 500 μL chloroform and centrifuged for 15 min, this step was repeated and the mixture was centrifuged for 5 min. Two volumes of CTAB precipitation solution was added to supernatant, mixed gently by pipetting and incubated at room temperature for 60 min then centrifuged for 5 min. The supernatant was discarded and the precipitate was dissolved in 350 μL NaCl (1.2 M), 350 μL of chloroform was added and mixed for 30 sec followed by centrifugation for 10 min. The upper layer was transferred to new microcentrifuge tube and 0.6 volume of isopropanol was added, mixed gently by inversion then centrifuged for 10 min, the supernatant was discarded. Five hundred microliter of 70% ethanol was added to the precipitate, followed by brief gentle mixing and centrifuged. The supernatant was discarded and the DNA precipitate was allowed to dry, then the DNA pellet was re-dissolved in 100 μL sterile deionized water. The prepared DNA solution was divided into aliquots of 10 μL then stored at -20°C for up to six months. Centrifugation was carried out at 16000 rpm for aforementioned indicated periods.

Table 2: Sequences of primers used in PCR amplification experiments (Querci *et al*., 2006)

Primers	Sequence (5'-3')
CaMV 35S promoter	
p35S-cf3, F	CCACGTCTTCAAAGCAAGTGG
p35S-cr4, R	TCCTCTCCAAATGAAATGAACTTCC
Nopaline synthase (nos) terminator	
HA-nos 118-f, F	GCATGACGTTATTTATGAGATGGG
HA-nos 118-r, R	GACACCGCGCGCGATAATTTATCC
Maize specific	
ZEIN3, F	AGTGCGACCCATATTCCAG
ZEIN4, R	GACATTGTGGCATCATCATTT
Soybean specific	
GM03, F	GCCCTCTACTCCACCCCCATCC
GM04, R	GCCCATCTGCAAGCCTTTTTGTG
MON 810 specific (nested PCR)	
mg 1	TATCTCCACTGACGTAAGGGATGAC
mg 2	TGCCCTATAACACCAACATGTGCTT
MON 810 specific (nested PCR)	
mg 3	ACTATCCTTCGCAAGACCCTTCCTC
mg 4	GCATTCAGAGAAACGTGGCAGTAAC
Soybean RRS (nested PCR)	
GMO5	CCACTGACGTAAGGGATGACG
GMO9	CATGAAGGACCGGTGGGAGAT
Soybean RRS (nested PCR)	
GMO7	ATCCCACTATCCTTCGCAAGA
GMO8	TGGGGTTTATGGAAATTGGAA

Determination of concentration and purity of extracted DNA: The concentration of extracted DNA was determined by measuring at 260 nm against a blank. The ratio A260/A280 was used to estimate the purity of extracted DNA (Querci *et al*., 2006). The measurements were performed using Jenway Spectrophotometer (Genova/UK).

Primers: The forward and reverse primers specific to zein gene (ZEIN3 and ZEIN4), CaMV 35S promoter primers: forward primer (p35S-cf3) and reverse primer (p35S-cr4) and primers for E35S promoter/hsp70 exon-intron cassette of maize MON810 (mg 1/mg 2, mg 3/mg 4); specific primers for soybean (GM03 and GM04) and soybean RRS (GMO5/GMO9, GMO7/GMO8) were used in the amplifications (Table 2). The primers were obtained from Alpha DNA/Canada.

DNA amplifications: Amplification reactions were performed according to the reported methods (Querci *et al*., 2006). PCR reactions were carried out in a total volume of 50 μL. Each reaction mixture contained 5 μL of 10x PCR buffer, 5 μL of 25 mM $MgCl_2$, 0.25 μL of Taq DNA polymerase which were obtained as TopTaq TM PCR kit (Qiagen/Germany), 2.5 μL 4 mM dNTPs (Promega/Germany), 1.25 μL of 20 μM of each primers (Table 2), 32.75 μL nuclease-free water and 2 μL of extracted DNA. The parameters of amplification were carried out according to the reported protocols (Table 3). Each run included standard genetically non modified maize and soybean, GM maize or soybean products and

Table 3: Parameters for PCR amplifications experiments for various primers used for detection of GM maize and soybean food products

Primers	ID (1X)	D	A	E	NC (X)	FE (1X)
CaMV 35S promoter:	95°C	95°C	62°C	72°C		72°C
p35S-cf3, F	3 min	25 sec	30 sec	45 sec	50	7 min
p35S-cr4, R						
Nopaline synthase (nos) terminator:	95°C	95°C	62°C	72°C		72°C
HA-nos 118-f, F	3 min	25 sec	30sec	45 sec	50	7 min
HA-nos 118-r, R						
Maize specific:	95°C	96°C	60°C	60°C		72°C
ZEIN3, F	3 min	1 min	1 min	1 min	40	3 min
ZEIN4, R						
Soybean specific:	95°C	95°C	63°C	72°C		72°C
GM03, F	3 min	30 sec	30 sec	30 sec	40	3 min
GM04, R						
MON 810 specific (nested PCR):	95°C	95°C	60°C	72°C		72°C
mg 1	3 min	45 sec	50 sec	50 sec	35	3 min
mg 2						
MON 810 specific (nested PCR):	95°C	95°C	60°C	72°C		72°C
mg 3	3 min	45 sec	50 sec	50 sec	40	3 min
mg 4						
Soybean RRS (nested PCR):	95°C	95°C	60°C	72°C		72°C
GMO5	3 min	30 sec	30 sec	40 sec	25	3 min
GMO9						
Soybean RRS (nested PCR):	95°C	95°C	60°C	72°C		72°C
GMO7	3 min	30 sec	30 sec	40 sec	35	3 min
GMO8						

ID: Initial denaturation, D: Denaturation, A: Annealing, E: Extension, FE: Final extension, NC: No. of cycles, F: Forward primer, R: Reverse primer, X indicates number of cycles during PCR amplification. The amplification conditions were as reported by Querci *et al.* (2006)

no-template control containing all PCR mix component except DNA. The amplifications were performed in the Applied Biosystem Thermocycler 9902 with heating lid.

Gel electrophoresis: The amplification products in parallel with DNA marker ladder of 100 bp (Qiagen) were separated on 1.5% agarose gel, run with 3 volt cm^{-1} and visualized under UV light after staining with ethidium bromide for molecular size determinations in base pair (bp) of DNA fragments (Sambrook and Russell, 2001).

RESULTS

Qualitative PCR-based methods were used to survey and detect the event of genetically modified maize and soya products sold commercially in Amman, Jordan. The majority of collected food products were maize products (Table 1). Maize food products were mainly corn seeds, corn meal or flour, whereas soya products were substitute for meat (soya meat), soy sauce and bread crumbs. Genomic DNA was extracted by CTAB method from various maize and soybean food products, the results showed noticeable variations in the concentrations and purities of extracted genomic DNA. The study revealed four categories which were recognized according to the yield of extracted DNA. The lowest yield's range of extracted DNA was 1 ng μL^{-1}, whereas the highest yield's range was 46 ng μL^{-1} (Table 4). The results of purity of extracted DNA also showed variations and it varied between 1.49 and 1.93. These results might suggest the suitability of CTAB method for DNA extraction from maize and soybean products. Agarose gel analysis of extracted

Table 4: Appearance of extracted DNA fragments on agarose gel following exposure to UV light of 365 nm, the obtained concentration and purity of extracted DNA by CTAB method from maize and soybean food products

No. of tested food products	Appearance of extracted DNA	Range of DNA concentration (ng μL^{-1})	Range of DNA purity
1	Weak	1	1.50
8	Moderate	6-12	1.49-1.83
12	Good	17-28	1.64-1.93
1	Very good	46	1.80

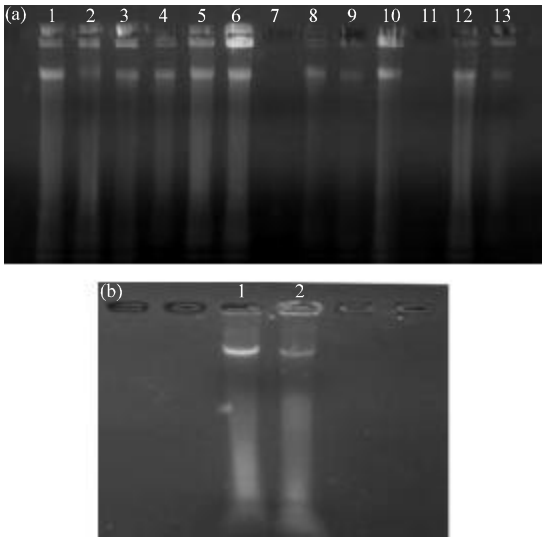

Fig. 1: Agarose gel electrophoresis of extracted DNA from (a) maize and (b) soybean food products by CTAB method. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt cm^{-1}. Lane's numbers represent maize and soybean food products used in this study

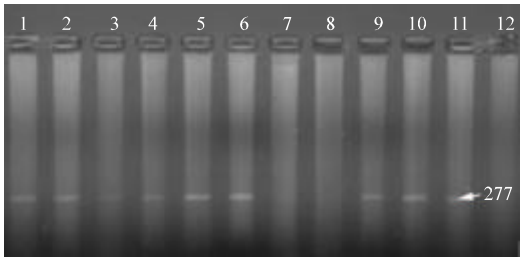

Fig. 2: PCR amplification of zein sequence (277 bp) of maize food products. Electrophoresis was performed on 1.5% agarose gel and run with 3 volt cm^{-1}. Lanes from 1 to 12 represent the maize food products

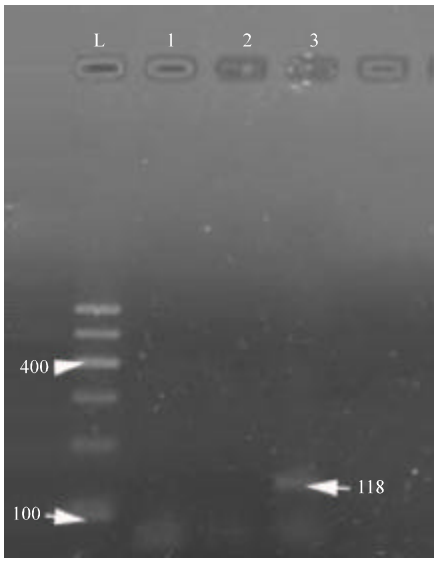

Fig. 3: PCR amplification of lectin (118 bp) sequence in soybean food products. Lane L indicates the 100 base pair ladder; Lanes 1-3 indicate the soybean food products

DNA from various maize and soy products showed that the isolated DNA fragments by CTAB method composed of high molecular weight and a smear of lower molecular weight degraded DNA (Fig. 1a, b).

Polymerase chain reaction experiments for the amplification of DNA sequences by the specific primers (Table 2) showed that 4 out of 22 maize and soy products are genetically modified. The plant origin of the food products was confirmed by using two genes specific for maize or soybean. Zein gene was identified in maize food products (Fig. 2), the size of amplified DNA fragment is 277 bp which is specific for zein gene. Whereas lectin gene was identified in soybean food product (Fig. 3), the

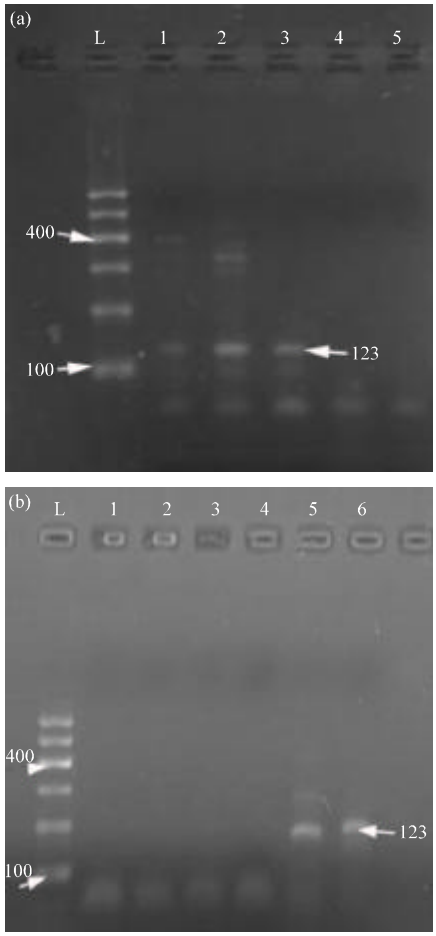

Fig. 4: Detection of PCR amplified 35 S sequence (123 bp) in (a) maize and (b) soybean food products. Electrophoresis was performed on 1.5% agarose gel and run with 3 volt cm^{-1}. Lane L, indicates the 100 bp ladder, Lane's numbers represent maize and soybean products

size of amplified DNA fragment was 118 bp which is specific for lectin gene. DNA fragment for the 35S promoter (123 bp) was identified in three of maize food products (Fig. 4a, b) and one of soy products.

The results of nested PCR experiments showed that the three maize food products contained the hsp70 exon1/intron1 region of maize MON810 (Fig. 5a). The molecular size of the amplified DNA fragment by primers mg1and mg2 was equivalent to 401 bp, while the size of amplified DNA fragment by primers mg 3 and mg 4 was 149 bp (Fig. 5 b). Figure 6 shows the molecular schematic details of 35S promoter/hsp70 exon-intron cassette of maize MON810. The Roundup Ready soybean gene cassette (Cp4 EPSPS) was not detected in the tested soy food samples.

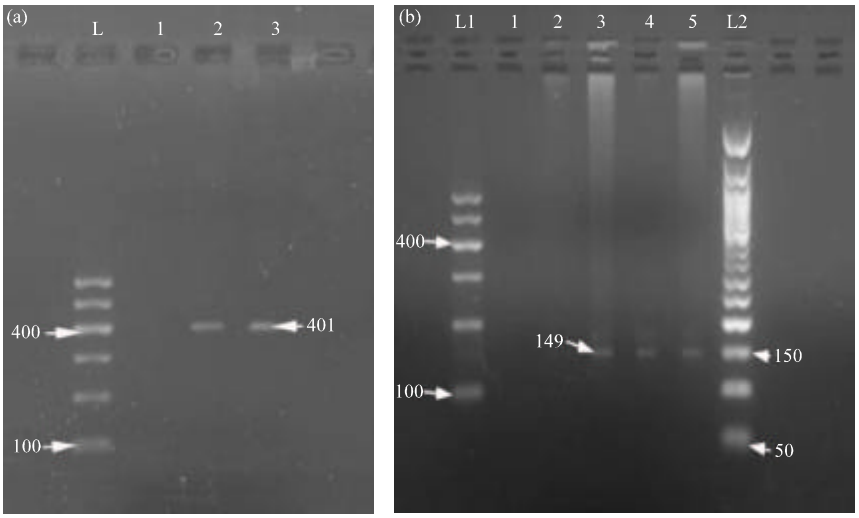

Fig. 5: Detection of amplified nested products in maize food products, (a) 401 bp sequence identified by mg 1/mg 2 primers; (b) 149 bp sequence identified by mg 3/mg 4 primers. Electrophoresis was performed on 1.5% agarose gel and run with 3 volt cm^{-1}. Lanes L and L1 indicate the 100 bp ladder, Lane L2 indicates 50 bp ladder

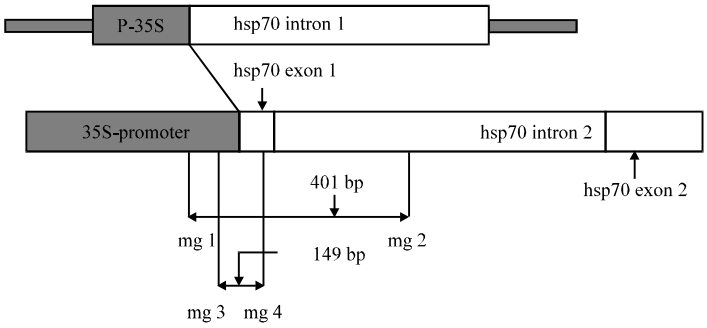

Fig. 6: Schematic details of 35S promoter/hsp70 exon-intron cassette of maize MON810. 401 bp 149 bp represent the amplified DNA fragments by primers mg 1/mg 2 and mg 3/mg 4, respectively

DISCUSSION

Present results showed that 18.2% of maize and soy food products sold in Jordanian markets are genetically modified. These were identified by specific primers and included both common GM detection primers for 35S and event specific. The amplicons of the specific sequences detected are used to build the different GMOs and regulate expression of transgenes, such as promoter 35S and the terminator NOS (Forte *et al*., 2005; Vijayakumar *et al*., 2009). Furthermore, nested PCR system was found useful in detecting the transgenic event, this system of detection was developed for specific detection of transgenic plants (Cankar *et al*., 2008; Zhang *et al*., 2007; Zimmermann *et al*., 1998).

From the time when first generation of GMOs seed were released for plantation in 1995 and their products entered the food and feed markets of various countries in 1996 (Paarlberg, 2006), there have been reports questioning the safety of these organisms and their products when used as food or feed. This brought about an active debate and controversy on possible risks that might be caused by such crops and their products to health (Al-jebreen, 2010; Patel *et al*., 2005; Seralini *et al*., 2007) and environment (Kawata *et al*., 2009; Knispel and McLachlan, 2010; Sanvido *et al*., 2007). As a result two main methods for the identification of GM food and feed have been reported in the literatures; PCR and Enzyme-Linked Immunosorbent Assay (ELISA). PCR is the most accepted technique used worldwide, it showed consistent results when using specific primers for the detection of the regulatory sequence or structural gene in the inserted gene fragment (James *et al*., 2003; Marmiroli *et al*., 2008; Matsuoka *et al*., 2000). The

designed primers must possess some specific characteristics and can be used for GM product screening and product-specificity detection. Thus, it might be convenient to consider these methods for the detection and identification of GMOs. This would represent a new field of diagnostics in which a great deal of development has already been accomplished.

In this study, it was possible to detect genetically modified maize and soybean unlabeled food products sold in the Jordanian market and the genetic event MON810 of GM maize was identified. These results are believed being the first survey for GM products in Jordanian market and might draw the attention for implementation of adequate labeling measures of food products containing materials derived from GM crops. The obtained results suggest the necessity for well-organized efficient monitoring process of GMOs and the need for labeling GM food products in Jordan. The developing world in general and Jordan in particular often lack coherent policies in regard to GM products, hence there is a need to strengthen the indigenous capability in molecular biotechnologies in order to survey and assess the bio-safety of GM food. This will be achieved with the support and assistance of well established international GM laboratories (Al-Hmoud *et al*., 2008).

CONCLUSION

Unlabeled genetically modified maize and soya food products were detected in the Jordanian market. Amplified 35S promoter DNA fragment was found in both GM maize and soya products. Genetic event MON 810 was detected in the GM maize food product, while Cp4 EPSPS event was not detected in GM soy food product. The study showed the need for further concerted efforts in the detection and handling of GM products in Jordan.

ACKNOWLEDGMENTS

The Royal Scientific Society of Jordan appreciates highly the fellowship granted to M. Ibrahim by International Institute of Education. The research team is also thankful to Dr. Eric Kubler from University of Applied Sciences Northwestern Switzerland for his collaboration in supplying the GMO and standard samples of maize and soy.

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