J. Agr. Sci. Tech. (2016) Vol. 18: 805-815

PCR-based Detection of Genetically Modified Soybean at a Grain Receiving Port in Iran

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ABSTRACT

The detection of Genetically Modified (GM) organisms is becoming a legal necessity. This study was carried out to detect genetically modified events in soybeans imported into Iran using simplex and multiplex PCR. Therefore, five samples of imported soybean were obtained from Bandar Imam Khomeini customs. Modified CTAB method was used to extract DNA from soybean seeds. The result indicates that the modified method is suitable for DNA extraction from soybean seeds and probably can be used for other oilseeds. Using specific primers for *CaMV 35S* promoter, *NOS* terminator and *epsps* gene PCR reactions were performed. In this study soybean *lectin* gene was used as internal control. The results revealed that soybean samples imported from Canada and Paraguay were genetically modified and they had *CaMV 35S* promoter, *NOS* terminator and *epsps* gene in their genomes. The result of simplex PCR was the same as multiplex PCR, but multiplex PCR detected the GM soybeans very quickly and in a cost-saving and time-consuming way. Based on PCR analysis using GM soybean event-specific primers, it is suggested that the soybean plants may be GTS 40-3-2. No fragment was amplified when the DNA of US or Non-GM soybeans were used as template in the PCR reaction. This is the first report that shows GM soybeans imported to Iran without use of the "GMO" label in the shipment's documentation.

Keywords: *EPSPS* gene, Genetically Modified Organisms (GMOs), Monitoring of GMOs, Roundup ready soybean, Simplex and multiplex PCR.

INTRODUCTION

Genetic modification is a technology that allows changes to be made to the DNA of a plant to give it a useful characteristic using DNA from any biological source. Since the first Genetically Engineered (GE) tomato, Flavr Savr, was commercialized in the United States in 1994, many Genetically Modified (GM) crops have been developed and commercialized globally (James, 2013). According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA), in the sixteenth year of commercialization of these products (from 1996 to 2013), the areas planted to transgenic crops reached 175 million hectares in 2013, by three percent growth compared to 2012 (James, 2013). Recently, GM stacks, which are considered as Genetically Modified Organisms (GMOs) that come from the combination of two or more single transgenic events, are coming to be an important and growing sector of the transgenic crops in the field (Weber *et al*., 2012). It shows that there has been a new emphasis on GM crops as the solution to world hunger which leads to increasing number of GM crops on the market (James, 2013). Despite benefits of these crops and years of investment by both the government and private sectors, commercially grown GE

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crops remain largely restricted to the two main traits i.e. herbicide tolerance and insect resistance, in four main crops: soybean, maize, canola, and cotton (James, 2013).

In 2013, GM soybean continued to be the principal GM crop, with nearly 80% of the total world soybean area planted to GM soybean (James, 2013). To decrease the herbicide dosage in soybean plant, GM soybean event (GTS 40-3-2) with the glyphosate-tolerant trait has been developed and commercialized worldwide (Liu *et al*., 2009). The importation of genetically modified soybean has been recently increased due to high consumption, which means monitoring genetically modified soybean in imported plant materials is more necessary (Costa *et al*., 2010).

To monitor GM plants, a number of analytical techniques such as herbicide bioassays, immunoassay, ELISA assay as a protein-based method (Enzyme-Linked Immunosorbent Assay and lateral flow strip) and nucleic acid-based detection methods like PCR are available for detecting the presence or absence of transgenes (Liu *et al*., 2009). PCR technique is the most widely used because of its high sensitivity, fast analysis time, and its capability for detection of specific transgenic events (Querci *et al*., 2004). To date, several event-specific detection methods of GM soybean have been reported (Zhang *et al*., 2008).

For more than ten years, GTS 40-3-2 (Roundup Ready soybean) has been the only commercially available GMO derived from soybean (Sisea *et al*., 2010). Roundup Ready plants such as soybean carry a gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (epsps) that confers resistance to glyphosate.

Some regulatory sequences such as promoter and terminator introduced into plant cells along with transgene can be used to detect genetically modified plants (Lipp *et al*., 2001). Usually *cis* acting elements, which are in use for plant transformation, contain *35S CaMV* promoter, derived from cauliflower mosaic virus, and *NOS*, Nopalin synthase, terminator from *Agrobacterium* plasmid (Ovesná *et al*., 2002).

In different studies, various genes or regulatory elements were used to detect GM crops (Park *et al*., 2015) or their products (Costa *et al*., 2010), but *35S CaMV* promoter (Lin *et al*., 2000), *NOS* terminator (Lin *et al*., 2000) and *epsps* gene (Lin *et al*., 2000) are the most common genetic elements used to detect these crops. The use of multiplex PCR can facilitate screening process and can be regarded as a powerful tool for cost- and time-saving GM crops detection (Huber *et al*., 2013).

There are various methods to use for plant DNA extraction. Some of them are the basic methods such as CTAB (Saghai Maroof *et al*., 1984) that are usually modified to get better results. Modification may be carried out to improve their compatibility with samples (Mahmoudi Nasab *et al*., 2010).

The objective of the present study was to detect genetically modified events in soybeans imported into Iran by simplex and multiplex PCR detection method using specific primers for the gene encoding 5-enolpyruvylshikimate-3-phosphate synthase, *35S CaMV* promoter, and *NOS* terminator.

MATERIALS AND METHODS

Sampling and Sample Preparation

Five samples of imported soybean, namely, MV RENUAR and MV ANASTASIA from Canada, MV CLIO and MV ERATO from the United States, and a sample from Paraguay were collected from Bandar Imam Khomeini customs in southern Iran. Also, two Non-GM soybeans genotypes, namely, *Glycine max* cv. Williams and *G. max cv*. Clark were obtained as control from Seed and Plant Improvement Institute, Karaj, Iran. Sampling was carried out randomly according to ISO standard (ISO/21568, 2005).

DNA extraction and purification

Plant genomic DNA was extracted and purified using the cetyltrimethyl ammonium bromide, CTAB (Sigma Aldrich, USA) method (Saghai Maroof *et al*., 1984) with some modifications. The CTAB extraction buffer contained 143 mL of deionized water (dH_2O), 22 mL of Tris (Merck, Germany) 1M, pH 7.5, 22 mL EDTA (Merck, Germany) 0.5M, pH 8 and 30.8 mL NaCl (Merck, Germany) 5M. The seeds were grinded with a mortar and pestle in liquid nitrogen as finely as possible. Around 400 mg of powdered seeds were transferred to a 50 mL sterile tube followed by addition of 9 mL hot extraction buffer and incubated at 65°C for 60 minutes with occasional inverting of the tubes. The tube was left at room temperature for 10 minutes without shaking. Then, 4.5 mL octanol (Sigma Aldrich, USA): chloroform (Sigma Aldrich, USA) (24:1, v/v) was added to each tube, mixed thoroughly and centrifuged at 1,420×g for 15 minutes. The upper phase was transferred to a new tube and 4.5 mL octanol:chloroform (24:1, v/v) was added to each tube. Tubes were shaken for 10 minutes at room temperature. The tubes were centrifuged at 1,420×g for 15 minutes. The upper phase was transferred to a new tube, 10 µL RNase (Vivantis, Malaysia) was added to each tube and was left at room temperature for one hour. After that, 12 mL cold absolute ethanol was added to each tube and centrifuged at 3,440 rpm for 10 minutes at room temperature. After centrifugation the supernatant discarded and DNA was resuspended in 200 µL of sterile deionized water and stored at -20°C until used.

For purification of nucleic acid, DNA was precipitated with absolute ethanol (Merck, Germany) and washed by solutions I [absolute ethanol, 76 mL; acetate sodium (Merck, Germany) 1M, 8 mL; dH_2O, 16 ml] and II [absolute ethanol, 76 ml; acetate ammonium (Merck, Germany) 1M, 1 ml; dH_2O, 23 mL], respectively. The quantity and quality of DNA was assessed by electrophoresis on 1% agarose gel. The amplificability of extracted DNA was verified using plant-specific primers LecSS-f/LecSS-r, and GMO3/GMO4 targeting at *lectin* gene, specific to soybean (Table 1).

PCR Conditions for GMO Screening

PCR reactions were performed in a thermocycler (Model iCycler, BIOER Co., China). One microliter (100 ng) of the genomic DNA was used as template for PCR in a final volume of 20 µL, containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl (Merck, Germany), 1.5 mM $MgCl_2$ (Merck, Germany), 200 µM dNTPs (Takara, Japan), 0.3 µM each primer, 0.5 unites of Taq DNA polymerase (Sinagene, Tehran, Iran). Amplification was carried out under the following conditions: 4 minutes at 94°C, 35 cycles (30 seconds at 94°C; 30 seconds at 52°C for LecSS-f/LecSS-r, 63°C for GMO3/GMO4, 51.7°C for 35S-1/35S-2, 57.7°C for P35S-cf3/P35S-cr4, 61°C for HA-nos 118-f/HA-nos 118-r, 53.8°C for GMO7/GMO8; 1 minute at 72°C), with a final extension for 7 minutes at 72°C. Seven microliters of PCR product was analyzed by electrophoresis in 1% agarose (Kawsar Bioteh, Iran) (w/v) gel.

Multiplex PCR

The multiplex PCR was performed with the same conditions as simplex PCR using two primer sets LecSS-f/LecSS-r and 35S-1/35S-2 within a single PCR mixture. Amplification was carried out under the following conditions: 4 min at 94°C, 35 cycles (30 seconds at 94°C; 30 seconds at 52°C, 1 minutes at 72°C), with a final extension for 7 minutes at 72°C.

Cloning and Sequencing of PCR Product

The amplicons were amplified with specific oligonucleotide primers (HA-*nos*

Table 1. List of primers used in this study.

Test	Set of primers	Sequence (5'- 3')	Target DNA sequence	Amplicon size (bp)	Reference
Screening GMO	35S-1 35S-2	*GCTCCTACAAATGCCATCA* *GATAGTGGGATTGTGCGTCA*	p-*35S*	195	Cardarelli *et al.* (2005)
	P35S-cf3 P35S-cr4	*CCACGTCTTCAAAGCAAGTGG* *TCCTCTCCAAATGAAATGAACTTCC*	P-*35S*	123	Cardarelli *et al.* (2005)
	HA-*nos* 118-f HA-*nos* 118-r	*GCATGACGTTATTTATGAGATGGG* *GACACCGCGCGCGATAATTTATCC*	T-*NOS*	118	Cardarelli *et al.* (2005)
Detection of DNA soya	LecSS-f LecSS-r	*AGCTGGAACAAGTTCGTGC* *CGACTTGATCACCAGACTCG*	*Lectin* gene	343	This study
	GMO3 GMO4	*GCCCTCTACTCCACCCCCATCC* *GCCCATCTGCAAGCCTTTTTGTG*	*Lectin* gene	118	Cardarelli *et al.* (2005)
Detection of roundup ready soybean	GMO7 GMO8	*ATCCCACTATCCTTCGCAAGA* *TGGGGTTTATGGAAATTGGAA*	p-*35S*/CTP EPSPS (*EPSPS* gene)	169	Cardarelli *et al.* (2005)
	EPSPS-B1 EPSPS-B2	*TGATGTGATATCTCCACTGACG* *TGTATCCCTTGAGCCATGTTGT*	p-*35S*/CTP EPSPS (*EPSPS* gene)	172	Lin *et al.* (2000)

118f/ HA-*nos* 118r and EPSPS-B1/EPSPS-B2) inserted into pTZ57R/T vector (Fermentase, Germany) and introduced into *E. coli* DH5α cells using an InsTAclone™ PCR Cloning Kit (Fermentase, Germany). The plasmids were extracted from the selected clones using a GeneJET™ Plasmid Miniprep Kit (Fermentase, Germany) and digested with *Eco*RI (Fermentase, Germany) for single digestion and *Eco*RI and *Hind*III (Fermentase, Germany) for double digestion. The recombinant plasmids were sent to BIONEER CO, South Korea, to sequence.

RESULTS AND DISCUSSION

Detection of Genetically Modified Soybean

DNA samples were used as templates in PCR reactions to detect regulatory sequences, *CaMV 35S* promoter and *NOS* terminator. In all reactions, non-GM soybean sample was used as negative control. To detect *CaMV 35S* promoter, two

set primers were used. A 195 bp fragment with 35S-1/35S-2 primer set and a 123 bp fragment with p35S-cf3/p35S-cr4 primer set should be amplified from the genomic DNA of GM soybean in PCR reaction. The results showed that 2 samples i.e. M.V. ANASTASIA from Canada and the other one from Paraguay, were genetically modified and carrying *CaMV 35S* promoter in their genome, but MV RENUAR, the other sample from Canada, and samples from USA (MV CLIO and MV ERATO) were not genetically modified with these *cis* elements (Figure 1).

It has been reported in some references that these primers should be used with some caution for screening of GMO, since the virus CaMV can infect a wide range of *Cruciferae* (Zhou *et al*., 2007). Hence, samples were used as template in another PCR reaction to amplify a fragment of *NOS* terminator with specific primers, HA-*nos* 118f/HA-*nos* 118r, for more confidence. With this primer set a 118 bp fragment should be amplified from the genomic DNA of GM soybean in PCR reaction. PCR results revealed that only two samples, one from Canada and another one from Paraguay, contained *NOS* terminator in their genome, while the other imported samples were not genetically engineered with these elements (Figure 2). The result being consistent with those obtained from using specific primer sets for *CaMV 35S* region. On the basis of these results, it cannot be said that other imported samples were not genetically engineered because it is possible that these plants were transformed by other regulatory elements.

Specific Detection of GMO

In order to test whether DNA samples preliminarily amplified with specific primers for *CaMV 35S* promoter and *NOS* terminator could be amplified with specific primers that flanked position of the coding region of the *epsps* gene, the PCR reactions with these primers were performed. It was expected that a 169 bp fragment with GMO7/GM primer set and a 172 bp fragment with EPSPS-B1/EPSPS-B2 primer set were amplified by PCR from the coding region of the *epsps* gene.

The results showed that only the positive samples for *CaMV 35S* promoter and *NOS* terminator, M.V. ANASTASIA from Canada, and the imported sample from

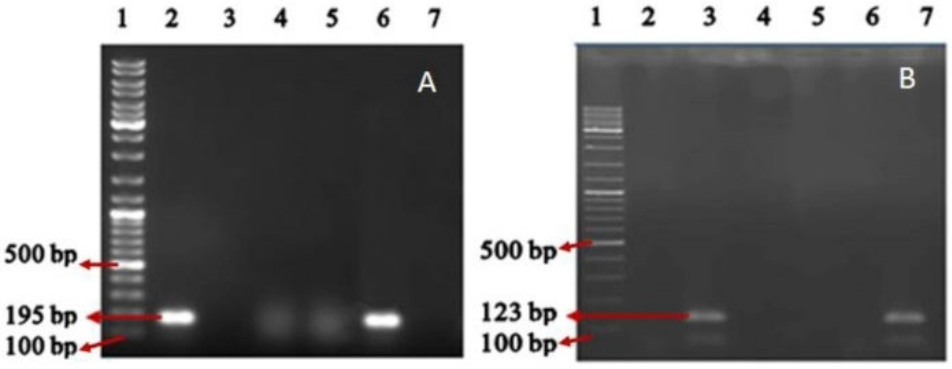

Figure 1. PCR reaction for detection of *CaMV 35S* promoter sequence in DNA extracted from soybean samples. (A) PCR was carried out using 6 different DNA samples as templates with 35S-1/35S-2 primers. (Lane 1) 100 bp DNA size marker; (Lane 2) MV ANASTASIA (Canada); (Lane 3) MV RENUAR (Canada); (Lane 4) MV CLIO (USA); (Lane 5) MV ERATO (USA); (Lane 6) Imported sample (Paraguay), and (Lane 7) Non GM-soybean as negative control. (B) PCR was carried out using 6 different DNA samples as templates with p35S-cf3/p35S-cr4 primers. (Lane 1) 100 bp DNA size marker; (Lane 2) Non GM-soybean as negative control; (Lane 3) MV ANASTASIA (Canada); (Lane 4) MV RENUAR (Canada); (Lane 5) MV CLIO (USA); (Lane 6) MV ERATO (USA), and (Lane 7) Imported sample (Paraguay).

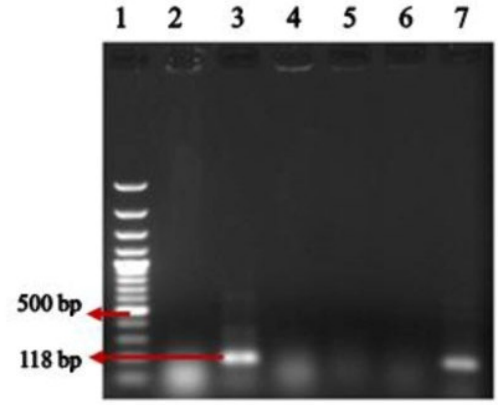

Figure 2. PCR reaction for detection of *NOS* terminator sequence in DNA extracted from soybean samples. PCR was carried out using 6 different DNA samples as templates with HA-*nos* 118f/HA-*nos* primers. (Lane 1) 100 bp DNA size marker; (Lane 2) Non GM-soybean as negative control; (Lane 3) MV ANASTASIA (Canada); (Lane 4) MV RENUAR (Canada), (Lane 5) MV CLIO (USA); (Lane 6) MV ERATO (USA), and (Lane 7) Imported sample (Paraguay).

Paraguay were amplified with specific primer for the coding region of the *epsps* gene (Figure 3). These results indicated that all GM samples used in this study had been genetically transformed with the *epsps* gene that was cloned between *CaMV 35S* promoter and *NOS* terminator. Based on the results of PCR and sequencing, it is suggested that the samples were from GTS 40-3-2 (Roundup Ready) with *epsps* gene, which confers resistance to glyphosate herbicide.

Multiplex PCR for Detection of GM Soybean

A multiplex PCR was performed that consisted of two primer sets, namely, LecSS-f/LecSS-r primer pairs which produce an amplicon of 343 bp and serve as an internal positive control for detecting *lectin* gene, and 35S-1/35S-2 primer pairs

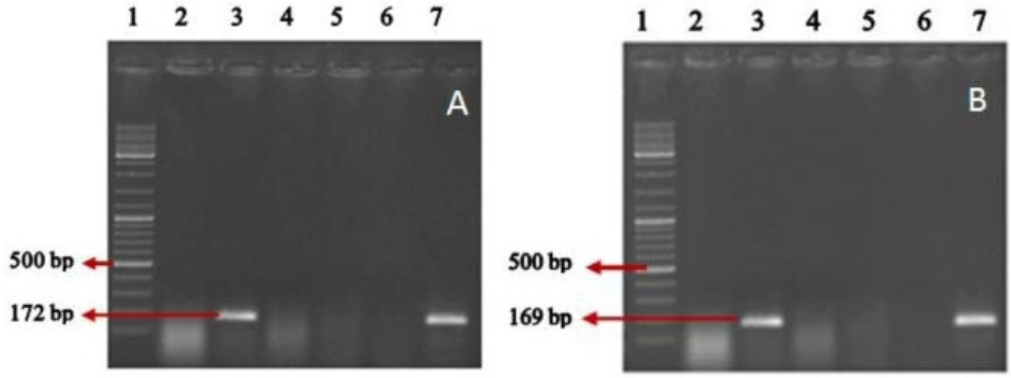

Figure 3. PCR reaction for detection of *epsps* gene sequence in DNA extracted from soybean samples. (A) PCR was carried out using 6 different DNA samples as templates with EPSPS-B1/EPSPS-B2 primers. (B) PCR was carried out using 6 different DNA samples as templates with GMO7/GMO8 primers. (Lane 1) 100 bp DNA size marker; (Lane 2) Non GM-soybean as negative control; (Lane 3) MV ANASTASIA (Canada); (Lane 4) MV RENUAR (Canada), (Lane 5) MV CLIO (USA); (Lane 6) MV ERATO (USA), and (Lane 7) Imported sample (Paraguay).

which produce an amplicon of 195 bp and serve in detecting GMO soybeans. The expected band of 343 bp associated with *lectin* gene was present in all soybean samples that were tested, including non-GM soybean and imported samples, but the 195 bp band associated with *CaMV 35S promoter* was observed only in two GM samples (Figure 4). The obtained results were the same as simplex PCR, thus, the results of PCR using single primer pairs were confirmed by multiplex PCR.

Several reports describing PCR techniques used to detect GM soybean have been published (Lin *et al*., 2000). The multiplex PCR assay discriminates the GMO very quickly, reproducibly, and in a cost-saving and less time-consuming way. It is also a flexible assay because it is carried out on the same mixture in the same run (Forte *et al*., 2005). The multiplex PCR method was developed for detection of GM plants in which only one reaction is necessary to detect a plant transformed with two or more separate transgenes. Despite all benefits of multiplex PCR, there are some limitations for this technique that should be considered. Primers designed to amplify the regions in a multiplex reaction should have similar TM, lack significant secondary structure, and have a sensitivity level of at least 10 ng initial DNA concentration (Wilson *et al*., 2002). Another problem inherent to multiplex PCR is that PCR efficiency may be decreased in the presence of more than one primer pair in a single reaction (Exner and Lewinski, 2002). This can lead to increase to obtain spurious amplicons and the formation of primer dimmers.

Sequencing of PCR Products

To verify that the fragments amplified in PCR were associated with GM plants, the fragments amplified with EPSPS-B1/EPSPS-B2 from genomic DNA of two GM samples were cloned and sequenced. The specificity of the amplification was confirmed by sequencing of the PCR products which were identical to the sequence of *NOS* terminator.

GMO Detection in the World

In this study, GM soybeans could be detected by both simplex and multiplex PCR very well, consistent with previous reports (Lee *et al*., 2009), and sensitivity of the two methods was the same.

Based on the Cartagena Protocol on Biosafety to the Convention on Biological Diversity that governs the movements of

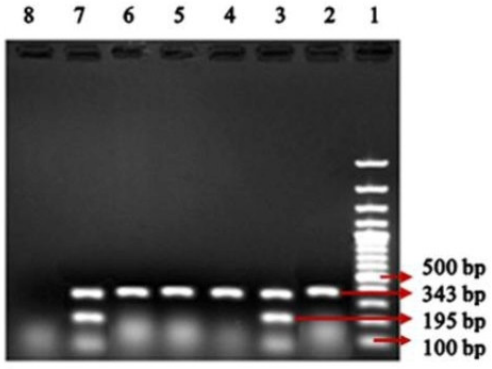

Figure 4. Multiplex PCR for detection of GM soybean. A 343 bp fragment was amplified from all DNA samples with LecSS-f/LecSS-r primer primers, and a 195bp fragment was amplified only from the genome of GM samples with 35S-1/35S-2 primers. (Lane 1) 100 bp DNA size marker; (Lane 2) Non GM-soybean as negative control; (Lane 3) MV ANASTASIA (Canada); (Lane 4) MV RENUAR (Canada), (Lane 5) MV CLIO (USA); (Lane 6) MV ERATO (USA); (Lane 7) Imported sample (Paraguay), respectively, and (Lane 8) No DNA sample.

genetically modified organisms from one country to another, it is ensured that countries are provided with the information necessary to make informed decisions before agreeing to the import of such organisms into their territory. Iran accepted this international treaty on 20 November 2003, and from that date the protocol entered into force in Iran. Hence, as Iran joined the protocol, it is expected that the imported plant materials should be labeled on whether or not they are genetically modified.

On the basis of our results, there were at least two imported soybeans from Canada and Paraguay that were genetically modified, with no statement in this context on the specifications sheets accompanying these transgenic soybeans. This is the first report on genetically modified soybeans imported to Iran lacking "GMO" label in the shipment's documentation. These results revealed that a reliable monitoring system for the detection of GMOs is needed and the Iranian government should prepare a comprehensive official protocol document about the possible consequences if the documents accompanying the imported plant materials are incomplete and/or incorrect.

An interesting finding in our study was that the samples imported from the USA were recognized as non-GMO. This detection was carried out using 3 specific primer pairs for screening of GM soybean and 2 specific primer pairs for detection of specific GMO (Roundup Ready soybean) using simplex PCR and multiplex PCR. Considering the fact that over 70 million hectares are covered by GM crops in the USA and also more than 90 percent of soybeans are genetically modified (James, 2013), our suspicion was that the samples from the USA would be genetically modified. There are three possibilities regarding the imported soybean from the USA: (1) The soybean was not directly imported from the US, but it was originally imported from another country; (2) The soybean was genetically transformed by another gene and other genetic elements rather than *CaMV 35S* promoter and *NOS* terminator. Different types of GM soybean currently available for commercial planting in the USA are listed in Table 2. The imported soybean from the USA was truly non-GMO. Therefore, more investigation is required to evaluate these possibilities.

CONCLUSIONS

In this study, it was shown that both simplex and multiplex PCR can detect GM soybeans, but multiplex PCR detected the GM soybeans very quickly and in a cost-saving and time-consuming way. All positive samples detected by simplex PCR were then confirmed by multiplex PCR. The DNA extraction method used in this survey allowed the extraction of amplifiable DNA from soybean seeds and probably can be used for other oilseeds. At least, two samples were genetically transformed with *epsps* gene and have *CaMV 35S* promoter and *NOS* terminator in their genomes, thus, it is suggested to check more samples by specific primers for other *cis* elements.

There were at least two imported soybeans from Canada and Paraguay that had no statement regarding this issue on the specification sheets accompanying the GM soybeans. This is the first report on genetically modified soybeans imported to Iran without the "GMO" label in the shipment's documentation.

ACKNOWLEDGEMENTS

This study was financially supported by Shiraz University.

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Table 2. Types of genetically modified soybeans recorded from 1994 to 2010 in the USA.[a] (http://bch.cbd.int/)

Record ID	Event	Organism	Country and year of cultivation	Description
14852	W98	*Glycine max*–Soybean, soya bean, soya	USA-1996 Canada-1999	Soybean tolerant to glufosinate ammonium herbicide produced by inserting a modified Phosphinothricin AcetylTransferase (PAT) encoding gene (*pat*). (P: 35S; T: SSU-soybean)
14853	W62	*Glycine max*–Soybean, soya bean, soya	USA-1996	Soybean tolerant to glufosinate ammonium herbicide produced by inserting a modified Phosphinothricin AcetylTransferase (PAT) encoding gene (*pat*). (P: 35S; T: SSU-soybean)
14854	GU262	*Glycine max*–Soybean, soya bean, soya	USA-1998	Soybean tolerant to glufosinate ammonium herbicide produced by inserting a modified Phosphinothricin AcetylTransferase (PAT) encoding gene (*pat*). (P: 35S; T: 35S)
14855	A2704-21	*Glycine max*–Soybean, soya bean, soya	USA-1996 Canada-1999	Soybean tolerant to glufosinate ammonium herbicide produced by inserting a modified Phosphinothricin AcetylTransferase (PAT) encoding gene (*pat*). (P: 35S; T: 35S)
14764	A2704-12	*Glycine max*–Soybean, soya bean, soya	USA-1996 Canada-1999	Soybean tolerant to glufosinate ammonium herbicide produced by inserting a modified phosphinothricin acetyltransferase (PAT) encoding gene (*pat*). (P: 35S; T: 35S)
14857	A5547-127	*Glycine max* – Soybean, Soya bean, Soya	USA-1998 Canada- 2000	Soybean tolerant to glufosinate ammonium herbicide produced by inserting a modified Phosphinothricin AcetylTransferase (PAT) encoding gene (*pat*). (P: 35S; T: 35S)
15418	A5547-35	*Glycine max*–Soybean, soya bean, soya	USA-1996 Canada-1999	Soybean tolerant to glufosinate ammonium herbicide produced by inserting a modified Phosphinothricin AcetylTransferase (PAT) encoding gene (pat). (P: 35S; T: 35S)
14769	260-05 (G94-1, G94-19, G168)	*Glycine max*–Soybean, soya bean, soya	USA-1997 Canada-2000	High oleic acid soybean produced by inserting a second copy of the fatty acid desaturase (GmFad2-1) gene. (P: 7Sa-soybean; T: phas)
49073	305423	*Glycine max*–Soybean, soya bean, soya	USA-2010 Canada-2009	Modified fatty acid soybean by inserting *FAD2-1* gene. (P: KTi3; T: KTi3)
101262	DP-305423-1 * MON-04032-6	*Glycine max*–Soybean, soya bean, soya	USA-2010 Canada-2009	Modified fatty acid, herbicide-tolerant soybean and resistance to herbicides glyphosate and sulfonylurea by inserting *GmFad2-1* genes and *gm-hra*. (P: KTi3; T: KTi3 and P: 35S; T: NOS)
48967	DP356043	*Glycine max*–Soybean, soya bean, soya	USA-2008 Canada-2009	Optimum™ GAT™ soybean resistance to herbicides glyphosate, sulfonylurea and imidazolinone herbicides by inserting *gat4601* and *gm-hra* genes. (P: SCP1; T: pinII and P: SAMS; T: gm-als)
14796	GTS 40-3-2	*Glycine max*–Soybean, soya bean, soya	USA-1994 Canada-1995 Paraguay-2004	Roundup Ready™ soybean resistance to herbicide glyphosate by inserting *EPSPS* gene. (P: 7Sa-soybean; T: phas)
40284	MON89788	*Glycine max*–Soybean, soya bean, soya	USA-2007 Canada-2007	Soybean tolerant to herbicide glyphosate by inserting *CP4 EPSPS* gene. (P: TSF1; T: rbcS-E9)
100994	CV127	*Glycine max*–Soybean, soya bean, soya	Brazil–2009 Philippines-2010	Herbicide-tolerant soybean resistance to herbicides imidazolinone and sulfonylurea by inserting *csr1-2* gene. (P: csr1-2-Arabidopsis; T: csr1-2-Arabidopsis)

[a] P: Promoter, T: Terminator.

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شناسایی سویاهای تراریخته وارداتی به ایران با استفاده از تکنیک پی‌سی‌آر

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چکیده

با تولید و کشت روزافزون گیاهان تراریخته، شناسایی این گیاهان و محصولات آنها در محموله‌های وارداتی لازم و ضروری است. به همین منظور این تحقیق با هدف شناسایی دانه‌های سویای تراریخته وارداتی به ایران با استفاده از تکنیک پی‌سی‌آر یگانه و چندگانه صورت گرفت. برای انجام این تحقیق پنج نمونه دانه سویای وارداتی از گمرک بندر امام خمینی دریافت شد. علاوه بر نمونه‌های وارداتی، دو نمونه سویای غیر تراریخته داخلی نیز به عنوان کنترل از موسسه اصلاح نهال و بذر تهیه شد. با استفاده از آغازگرهای اختصاصی برای تکثیر نواحی از پیشبر *CaMV 35S*، پایانبر *NOS* و ژن *epsps* واکنش پی‌سی‌آر انجام شد. در این تحقیق از ژن رمز کننده لکتین به عنوان کنترل داخلی استفاده شد. نتایج نشان دادند که سویاهای وارد شده از کشورهای کانادا و پاراگوئه از نظر ژنتیکی دستورزی شده بودند و در ژنوم خود دارای توالی پیشبر *CaMV 35S*، پایانبر *NOS* و ژن *epsps* بودند. نتایج حاصل از پی‌سی‌آر یگانه با نتایج پی‌سی‌آر چندگانه کاملا هم خوانی داشت. اگرچه نتایج هر دو پی‌سی‌آر شبیه به هم بود اما استفاده از پی‌سی‌آر چندگانه باعث صرفه‌جویی در هزینه و زمان شد و سریعتر دانه‌های تراریخته شناسایی شدند. بر اساس نتایج بدست آمده همچنین مشخص شد که دانه‌های سویای تراریخته مر بوط به سویای تراریخته GTS 40-3-2 هستند. هنگامی که از دی‌ان‌ای سویاهای کنترل یا سویاهای وارد شده از کشور ایالت متحده در واکنش پی‌سی‌آر استفاده شد، هیچ گونه قطعه‌ای طی واکنش پی‌سی‌آر تکثیر نشد که نشان دهنده غیر تراریخته بودن آنها است. نتایج این تحقیق اولین گزارشی است که نشان می‌دهد دانه‌های سویای تراریخته وارد ایران می‌شوند بدون اینکه در مدارک همراه آنها اشاره‌ای به تراریخته بودن آنها شده باشد.