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Investigation of transgenic elements in genetically modified maize germ (*Zea mays*) and maize germ oil distributed in local market by qualitative PCR method

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Abstract

Background and objective: Detection of genetically modified organisms (GMOs) is a controversial issue in food control. Due to the increased production and consumption of transgenic products in the last decade, their monitoring and characterization has come into force by the regulators. At this study, we examined the transgenic elements in maize germs and their oil by PCR.

Materials and methods: Following the DNA extraction from maize germ and maize oil by CTAB and Wizard Magnetic methods, respectively, PCR test was conducted to analyze the genes CaMV35S, NOS, FMV, and *zein*. Then, the PCR products were electrophoresed on agarose gel 2% containing ethidium bromide. To avoid false negative and false positive results, appropriate control actions were implemented during the experiments.

Results and conclusion: CaMV35S, NOS, FMV, and *zein* genes were detected and identified in transgenic maize germ samples, but no specific band of the transgenes was found for maize oil samples, indicating the DNA degradation during the oil production and purification process. It indicated that the current processes of maize germ oil production remove the residual transgenes. Therefore, the concerns about unintended intake of unknown genetic materials through the genetically modified crops such as maize oil is reduced to some extent.

Keywords: DNA extraction, genetically modified organisms, maize oil, polymerase chain reaction

1. Introduction

By population growth, production of safe food has become the greatest challenge in the world. Production of genetically modified (GM) plants is one of achievements of genetic engineering in agriculture, which can provide food for the growing population [1]. In the last two decades, genetic engineering has succeeded in production of

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GM products. In fact, biotechnologists were able to produce GM plants at high quantities as safe food for human and livestock [2, 3]. For instance, genetically modified organisms (GMOs) are the product of one or more genes' transfer from one or more organisms (donor) to another organism (acceptor) including plants, in a specific way that is different from natural or classical transfer [4]. The area under cultivation of GM plants has increased significantly in the world over the past few years, so that it reached from 1.7 million hectares in 1996 to 189.8 million hectares in 2017 [5]. In this regard, oilseeds are the most cultivated GM products [6].

In economic terms, price of oilseed is directly associated with its oil content. Maize or corn (*Zea mays* L.) contains high amount of oil and is cultivated extensively in various countries [7]. Corn oil contains high concentration of essential polyunsaturated fatty acids, which make it a great source of energy as food and feed [8].

Various analytical methods have been used to evaluate the oil content of seeds including nuclear magnetic resonance, near-infrared spectroscopy, and chromatography [9-11]. In recent years, there has been a growing interest in use of DNA-based analysis for food authentication. DNA molecules are high durable compared to the other compounds present in biological cells such as proteins. DNA markers are effective targets, which are not affected by geographical, climatic, or agricultural factors. Most of DNA-based methods rely on specific amplification of one or more DNA fragments by polymerase chain reactions (PCR) [12]. Due to the appropriateness of DNA-based approaches, we evaluated the genetic structure of maize germ oils and maize germs to determine the residual transgenes in the samples.

2. Materials and methods

2.1. Sample

Maize germ oils were purchased from local market (n = 30) and maize germs (MON89034 event maize; n = 5) were prepared from Department of Agriculture and Plant Breeding, Campus of Agriculture and Natural Resources, University of Tehran.

2.2. Extraction of DNA from maize germ oil and maize germ

Extraction and purification of DNA from complex and highly processed food matrices is important for conduction of efficient PCR technique. Polysaccharides, phenolic acids, and some other compounds are not completely eliminated during classical extraction processes and remain as contaminant in final DNA preparations, which can interfere with, reduce, or completely inhibit the DNA polymerase activity. For this purpose, the Wizard Magnetic method is mostly selected due to its effectiveness in extraction of DNA from complex foodstuffs and fatty matrices such as crude olive oil, degummed soybean oil, and refined sunflower and maize oils [13]. The Wizard Magnetic DNA purification system for food (Promega, Madison, WI, USA) was specifically selected because of its application in extraction of DNA from oil matrix. This method was followed according to the manufacture's instruction, with some modifications. For comparative purposes, 200 g of each oil sample was used instead of 160 g recommended by the manufacturer and transferred to five 50-ml sterile falcon, separately. Then, 2 ml lysis buffer A was added to each falcon and mixed vigorously. One ml of lysis buffer B was further added followed by vigorous mixing. After 10 min incubation at 25 °C under occasional mixing, 3 ml of precipitation solution was added and the mixture was stirred vigorously for 1 min. Final mixture was centrifuged at 40,000 ×g for 25 min. The supernatant (organic phase) was discarded carefully and the lower aqueous phase was transferred to another sterile falcon. 50 lL of MagneSil paramagnetic particles was added to the removed aqueous phase, followed by mixing and addition of 0.9 volume parts of isopropanol. The mixture was homogenized by shaking and incubated at 25 °C for 1 h under occasional mixing. Then, the tube was placed in a PolyATtract System 1000

Magnetic Separation Stand support for 2 min, until the all particles were attached to the support. The clear solution was discharged carefully, and the particles were washed with 1.5 ml of ethanol 70% (v/v) at -20 °C. The particles were transferred to a 2-ml sterile reaction tube and washed three times with 1.5 ml of ethanol 70% (v/v) at -20 °C. After ethanol removal, the particles were dried at 50 °C for 20 min. DNA was released from the magnetic particles by addition of 50 lL TE 0.19 solution (10 mmol/l Tris, 1 mmol/l EDTA) during 5 min at 65 °C. The final clear DNA extract was collected by pipetting, after placing the tube on a magnetic separation support for 1 min. The extracts were kept at 20 °C until analysis. Extraction was done in duplicate for each sample.

For extraction of DNA from maize germ, 0.5 ml of hexane (Hex) was mixed with 1 ml of sample in microtubes and the mixture was mixed with 0.5 ml of CTAB lubricating buffer (2% CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 50 lM DTT). The microtubes were incubated at 60 °C for 30 min. Then, chloroform: isoamyl alcohol (24:1) was added and the mixture was centrifuged at 20,000 ×g for 30 min at 4 °C. The supernatant was removed and mixed with isopropanol to help DNA deposition. It was mixed slowly with serum and kept at -20 for 24 h. The upper phase was discarded after centrifugation (20000 ×g, 4 °C, 30 min). The tubes were washed with 0.5 ml of ethanol 70% and dried [14].

2.3. Purity evaluation of the extracts

Concentration of the extracted DNA was measured with UV spectrophotometer (UV-1700, Shimadzu, Japan). Final concentration was adjusted to 50 ng/µl for PCR. Purity of the extracted DNA was evaluated by measuring the ratio of UV absorption at 260 to 280 and 260 to 230 nm. For maize germs and their oil, ranges of 1.6-1.9 and 0-0.03 were observed for 260/280 nm ratio, respectively.

2.4. Oligonucleotide primers

Purified and desalted primers were purchased from Eurofins (France). The primers were diluted by sterile double-distilled water to a final concentration of 10 µM and stored at -20 °C until use. Sequences of the oligonucleotide primers are given in Table 1 [15-17].

Table 1- The oligonucleotide primers used to detect the transgenic DNA sequences in the samples by PCR

Sequence	Primer	Target	Amplicon length (bp)	Ref.
5'- GCT CCT ACA AAT GCC ATC A -3'	35s-1	CaMV 35S promoter	195	[16]
5'- GAT AGT GGGATT GTG CGT CA -3'	35s-2	(Cauliflower Mosaic Virus (CaMV) promoter)		
5'- gCA TgA CgT TAT TTA TgA gAT ggg-3'	Forward primer	Agrobacterium tumefaciens NOS-terminator	177	[16]
5'-gAC ACC gCg CgC gAT AAT TTA TCC-3'	Reverse primer			
5'-CGC CAG AAA TCG TTT TTC AT-3'	MZ for	Maize zein	139	[15]
5'-GGT GGT GTC CTT GCT TCC TA-3'	MZ rev			
5'-AGG CGC ACC TAC CAA AAG C-3'	FMV-1	Figworth Mosaic Virus 35S promoter	196	[17]
5'-CGT TAT TTT GTT CCC CAC TTG TAC T-3'	FMV-2			

2.5. Polymerase chain reaction (PCR)

PCR was done in total volume of 25 µl containing 1 x (2.5 µl) buffer, 10-50 ng (l µ1) DNA extracted from the samples, 0.2 µmol/l (2 µl) of each primers, 0.8 mmol/l (2 µl) dNTPs, 1.5 mmol/l (2.1 µl) $MgCl_2$, 1.5 units (0.1 µl) Taq polymerase

(Invitrogen, Barcelona, Spain), and 15.9 µl water. The reactions were conducted in Master cycler gradients (Eppenddorf AG, Hamburg, Germany) according to the following program: initial denaturation at 95 °C for 3 min, conduction of 40 replication cycles at 95 °C for 30 sec, 60 °C for 30 sec, 72 °C for 60 sec, and final extension at 72 °C for 10 min.

2.6. Agarose gel electrophoresis

PCR products were electrophoresed on agarose gel 2% containing ethidium bromide. DNA Ladder (50 bp, Fermentas, USA) was used as reference. The genes were studied under 120 v by a UV Trans illuminator. At the end of analysis, the gels were photographed by Dolphin 1D gel analysis software (Wealtec, Nevada, USA).

3. Results and discussion

Today, millions of people consume the foods made from GM plants such as maize, soybean, and rapeseed around the world, but there is not sufficient information and knowledge about possible unfavorable effects of the GM products on human and livestock [18]. In view of safety, it is important to find out whether the new gene or trait has made a difference in the product. One approach is comparative evaluation of the GM products with non-transgenic parent plant in terms of their composition and concentration of the nutrients [19].

Appropriateness of the DNA markers for authentication purposes is important issue specially in complex food matrices such as vegetable oils [12, 20]. In addition, the advances in DNA extraction protocols help to achieve desirable results in analysis of the oils derived from GM crops such as maize. Indeed, following the increased concerns about the GM foods and residual transgenes in the products, advances in DNA analysis have been introduced by the scientists [21, 22]. In this regard, Eugster et al. evaluated the genes of CaMV35s and NOS and FMV promoter by real-time PCR in maize, soy, rapeseed, and tomato. In their study, real-time PCR was sensitive and specific enough for detection of CaMV35S promoter, NOS terminator, and FMV promoter in food and feed. Monitor of these four genetic segments could be considered as a fast method for detection of GM events [23]. In agreement, Rabiei et al. monitored GM maize in processed foods marketed in Iran by qualitative PCR. They found recombinant DNA containing the genes of CaMV35s, Bt-11, MON810, and Bt-176, separately, by high specific primers in the products. The authors detected the events of Bt-11 and MON810 in some maize samples, while the Bt-176 modified gene was not detected in the samples. In their study, presence of GM maize in Iranian food products was approved which needed to be reported on the labels [24]. In addition, Mendoza et al. identified GM maize and soybean products and reported that PCR is efficient in differentiation of GM from non-GM samples, so that it could detect genetic modifications in the marketed GM foods which were introduced as non-GM products [25].

Transgenes in the products that are highly processed or purified (such as starch, sugar, and vegetable oils) are exposed to degradation. Therefore, DNA-based analysis should rely on quality and purity of DNA [26]. Moreover, appropriate strategies should be applied to recover nucleic acids in the matrix and inactivate the PCR inhibitors. For example, polysaccharides and phenolic acids are not removed completely during classical extraction and may remain as contaminant in the environment. Therefore, they could interfere with, reduce, or inhibit activity of DNA polymerase [27-29]. Although, extraction of nucleic acids from vegetable oils are more complicated than the other products because of the small amount of DNA in lipid matrix, which may also become untraceable by electrophoresis after thermal or chemical processing of vegetable oils [23,27,30,31]. In fact, addition of phosphoric acid and sodium hydroxide during the oil purification process destructs the structure of nucleic acids and proteins [32]. However, low

amount of DNA can be amplified through PCR. In accordance, we conducted PCR test to study gene profile of our samples. Otherwise, our control of *zein* could not has been detected in maize germs. The electrophoresis pictures are presented in Figures 1 and 2.

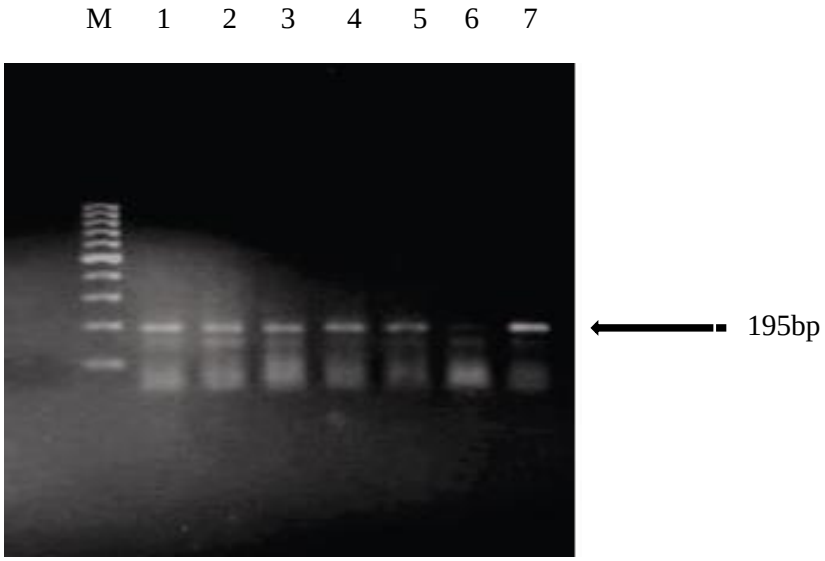

Figure 1. Agarose gel electrophoresis for detection of the genes CaMV35s, NOS, FMV, and *zein*; Lines 1 to 5: DNA extracted from the five maize germ samples, Line 6: DNA without pattern control, Line 7: positive control (35s primer), Line M: DNA Ladder (100 bp).

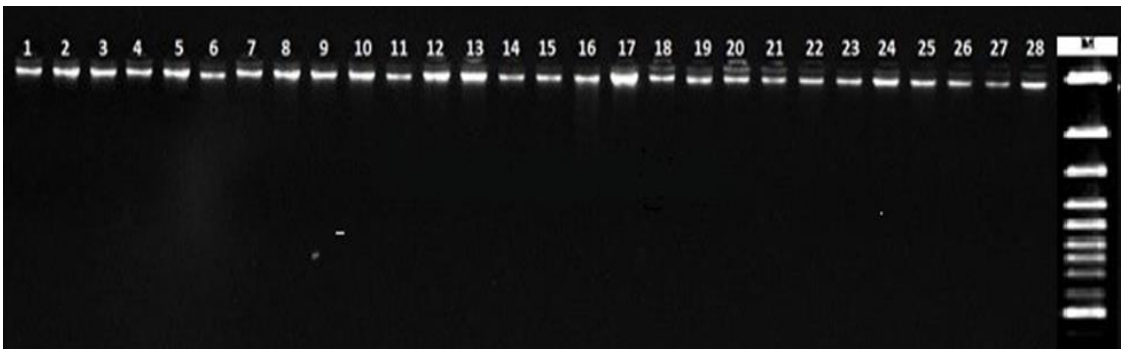

Figure 2. Agarose gel electrophoresis for detection of the genes CaMV35s, NOS, FMV, and *zein*; Lines 1 to 28: DNA extracted from the 28 oil samples, Line M: DNA Ladder (300 bp). Because of the limited number of wells, 28 samples were examined by gel electrophoresis.

In the process, the CaMV, NOS, and FMV genes were studied to find the genetic modification and the *zein* gene, as plant-specific structure, was used to follow the presence of maize DNA in the samples. Compared to the markers of FMV with 196 bp, CaMV35s with 195 bp, NOS with 177 bp, and *zein* with 139 bp, transgenic and plastid genes were detected and identified in maize germ samples. However, none of the transgenes (CaMV, NOS, and FMV) and plastid of *zein* were detected in maize germ oils. It shows that the genes were destructed in the process of oil production from the GM maize germ.

4. Conclusion

Results of our study confirmed the presence of CaMV35S, NOS, FMV, and *zein* transgenes in

the GM maize germs, but no band of the genes was observed after analysis of the maize oil samples. This observation indicated the DNA destruction during oil purification and production. Therefore, use of appropriate methods of oil production to remove residual DNA may decrease the concerns about the presence of genetic compounds in the products derived from GM crops such as maize. At this study, no transgene was detected in the maize germ oils by the Wizard Magnetic extraction and PCR method. However, examination of the other extraction methods is suggested for further studies.

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6. Conflict of interest

The authors have declared no conflict of interest.

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