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Measurement of genetically modified (GM) genes in different corn products

Meltem Asicioglu[1,2], Burhanettin Yalçınkaya[1] and Muslum Akgoz[1*]

[1]*Bioanalysis Laboratory, TUBITAK National Metrology Institute, UME, P.Box:54, 41470, Türkiye*

[2]*Prof. Dr. Aziz Sancar Biological Clock Laboratory, Department of Molecular Biology and Genetics, Gebze Technical University, 41400, Türkiye*

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Abstract: Nowadays, genetically modified (GM) plants are widely used in several commercial products. Corn, cotton, soybean and canola are the most widely consumed foods globally and their derived products have reached the global markets. Specifically, corn has the greatest diversity of approved GM events globally (a total of 130 varieties approved in one or more countries). It is one of the most widely used food and feed ingredient, highly integrated into food and feed supply chains. Since GMOs carry environmental risks and human health related risks, it is required that the food and their ingredients that either contain GMOs or derived from GMOs should be labelled. In this study, we investigated the taxon-specific cauliflower mosaic virus 35S promoter region (CaMV) and *Agrobacterium tumefaciens* nonpalin synthase terminator (tNOS) gene in addition to the control gene of High Mobility Group (HMG). Corn-containing foods were obtained from grocery stores in Turkey. DNA extraction was performed by modified CTAB DNA extraction method for all samples and genomic DNA were extracted. The DNA extracts were screened by qPCR and it was shown that genetically modified DNA sequences of tNOS and CaMV were not present in the samples investigated. For the security of society, randomized screens should be carried out by scientist in random bases.

Keywords: Biometrology; food safety; GMO quantification; GMO screening; corn.

1. Introduction

Recombinant DNA technology has been widely used in modern agriculture since the first commercial genetically modified (GM) plant (the Flavr Savr tomato) was approved for marketing in 1994 [1]. Production of genetically modified organisms (GMOs) has revolutionized the agricultural industry which has been increasing steadily worldwide. Corn, cotton, soybean and canola are the most widely consumed foods in several countries and their derived products have reached the global marketplace to 181.5 million tons by 2014 [2].

Several transgenic crops with beneficial characteristics have been developed by insertion of foreign microbial genes into the plant genomes. The most common GM plants are insect-resistant and herbicide-tolerant corn, soybeans and cotton plants. In contrast to important benefits of GM plants, known and unknown risks to human health and environment may prevail in future. For instance,

* Corresponding author E-Mail: muslum.akgoz@tubitak.gov.tr

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allergens and toxins due to antibiotic resistance markers may reveal health problems in short and long term. Cross pollination may lead to transfer of transgenes and this may cause the loss of flora and fauna biodiversity [3,4,5]. Specially, corn has the greatest diversity of approved GM events globally (a total of 130 varieties approved in one or more countries), and is one of the most widely used food and feed ingredients, highly integrated into food and feed supply chains [6,7].

Highly accurate measurement of GM genes is critical in food and feed product analysis. According to the European Union (EU) regulations, it is mandatory to label food and feed products containing more than 0.9 % authorized GMO and more than 0.5 % unauthorized GMO material for the consumer information [8]. Turkey also regulates GMOs according to EU labelling regulations. The regulation requests accurate monitoring and traceable detection of GMOs in raw materials of food and feed. As a result, several analytical methods have been developed and validated worldwide for GMO detection. The most widely used reference method for GMO detection approach is the Real-Time Polymerase Chain Reaction (qPCR) which is fast, cheap, and accurate [3,9] in detecting the presence of GMOs either accidentally or intentionally [10].

In this study, 11 different corn seeds or corn containing products were screened for the presence of CaMV and tNOS sequences using the taxon specific method. HMG (High mobility group) gene, an endogenous gene of corn was used as a control gene.

2. Experimental

2.1. Sample collection

Eleven corn samples were randomly collected from local markets selling both local and imported foods in Turkey. For sample identification, cornflakes, corn seed-1, corn seed-2, popcorn & rice crisp, corn chips, popcorn, corn flour-1 and corn flour-2, corn starch, corn on the cob and canned corn samples were labelled from 1 to 11, respectively. MON88017 certified reference material was used as positive control material.

2.2. Genomic DNA extraction

All samples, except starch and corn flour, were homogenized after grinding and stored at -80°C until analysis. Genomic DNA was extracted from homogenized samples using the modified CTAB (Cetyltrimethylammonium bromide) method [11]. Total DNA was extracted from 100 mg sample, in triplicates. For each sample, 750 µL CTAB extraction buffer and 20 µL RNase A (10 mg/ml) was added. The suspension was mixed and incubated for 30 min at 65 °C. After incubation, 20 µL (10 mg/mL) Proteinase K was added and incubated for additional 30 min at 65°C. The tubes were gently shaken for 2 h at 65 °C (1,200 rpm). After incubation, the samples were centrifuged for 10 min at 12,000 × g. The supernatant was transferred into a new tube, then, 1 volume of chloroform was added and vortexed. The tubes were centrifuged for 15 min at 12,000 × g and upper phase was transferred to a new tube. 2 volumes of the precipitation buffer (5 g/L CTAB, 0.04 M NaCl) was added and incubated for an hour at room temperature. The samples were centrifuged for 15 min at 12,000 × g and discarded the supernatant. Pellet was dissolved with 350 µL, 1.2 M NaCl then 350 µL chloroform added and mixed well. The samples were centrifuged for 10 min at 12,000 × g. The upper phase was transferred into a new tube and 0.6 volume of isopropanol was added and incubated for 20 min at room temperature. The samples were centrifuged for 15 min at 12,000 × g and aqueous phase was discarded. The pellet was washed with 500 µL, 70% ethanol and then centrifuged for 10 min at 12,000 × g. After centrifugation, the supernatant was discarded. The precipitate was dried and dissolved in 50 µL of 1x TE buffer.

The DNA yield and purity was determined using NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Integrity of the extracted genomic DNA samples was checked by loading 10 µL of extracted DNA on DNA gel electrophoresis (0.8 % agarose gels) containing EtBr in 1x TBE (10 mM Tris base, pH: 8.0, 2.75 g Boric acid/L, 1 mM EDTA buffer) [12].

2.3. Real time PCR analysis

PCR amplifications of DNA samples were carried out using Light Cycler® 480 Real Time PCR Instrument (Roche, Germany) with SYBR® Green I Master Mix (Cat. No.04707516001, Roche, Germany). Forward and reverse primers (Table 1) were synthesized and purified by MOPC (Macrogen, South Korea). qPCR was performed in a total volume of 20 µL with 5 µL of DNA as template (8 ng/µL) unless there is enough DNA in the extract. After an initial denaturation at 95°C for 10 min, 40 cycles of PCR was performed by denaturing at 95°C for 15 s, annealing and extension at 60°C for 1 minute (HMG and tNOS) or annealing and extension at 69°C for 1 min (CaMV).

Table 1. Primer pair sequences and their target elements [13,14]

Primers	Primer Sequences (5` -3`)	Target gene	Amplicon size (bp)
HMG-F	TTGGACTAGAAATCTCGTGCT	High Mobility Group	79
HMG-R	GCTACATAGGGACGCTTGTCC		
tNOS -F	GATTAGAGTCCCGCAATTATACATTTAA	Nos Terminator	69
tNOS –R	TTATCCTAGKTTGCGCGCTATATTT		
CaMV-F	AAAGCAAGTGGATTGATGTGATA	35S Promoter	75
CaMV-R	GGGTCTTGCGAAGGATAGTG		

All samples were analyzed for the presence of tNOS and CaMV genes using qPCR method and PCR products were analyzed in 2% DNA agarose gels by visualizing under UV transilluminator using Ethidium Bromide. HMG gene was used as a positive control.

3. Results and discussion

A total of 11 samples of corn-containing foods were collected from markets in Turkey. All of samples were extracted by CTAB DNA extraction method.

The amount and purity of the extracted genomic DNA samples was determined by NanoDrop 1000 Spectrophotometer by measuring at 260 nm, 280 nm, and 230 nm. The concentrations of genomic DNA extracts are given in Table 2. Genomic DNA was found intact and visible for the samples of corn seed-2, popcorn, corn flour-1, corn flour-2, corn cob, although same degradation of genomic DNA is visible (Figure 1). The extraction efficiency of these samples was also higher than that of other samples (Figure 1). Degradation was observed with corn seed-1, corn chips and canned corn samples. Canned corn is heat processed, therefore, DNA degradation is considered normal. For corn flakes, popcorn&rice crisp and corn starch samples did not reveal any genomic DNA bands at all, consistent with absorbance readings except, absorbance reading of popcorn&rice crisp, 37 ng/µL.

The ratio of absorbance 260 nm to 280 nm ($A_{260/280}$) represents the purity in terms of protein content and as the value is around 1.8 it is considered pure enough for PCR applications. Corn seed-2, corn chips popcorn, corn flour and corn cob has higher $A_{260/280}$ values than 1.8 as well as positive control sample. As expected, highly processed cornflakes and Popcorn & Rice Crisp samples exhibits very low levels of $A_{260/280}$. Contamination with carbohydrates and other organic materials also lead to lower ratios of absorbance 260 nm to 280 nm ($A_{260/280}$) than 2.0. Similarly, processed cornflakes and Popcorn & Rice Crisp samples exhibited lowest $A_{260/280}$ values.

Table 2. Determination of DNA concentration and purity by absorbance spectroscopy

Lane #	Sample Name	Conc. (ng/µL)	$A_{260/280}$	$A_{260/230}$	Lane #	Sample Name	Conc. (ng/µL)	$A_{260/280}$	$A_{260/230}$
P	Positive Control	80	1.96	1.05	**6**	Popcorn	200	2.06	2.26
1	Cornflakes	7.5	1.01	0.35	**7**	Corn Flour-1	123.4	2.05	2.17
2	Corn Seed-1	92	1.68	1.07	**8**	Corn Flour-2	44.8	1.73	1.34
3	Corn Seed-2	138	2.10	2.28	**9**	Corn Starch	-	-	-
4	Popcorn & rice crisp	37	1.34	0.46	**10**	Corn Cob	289	2.10	2.37
5	Corn Chips	79	1.81	1.45	**11**	Canned Corn	509	1.69	1.70

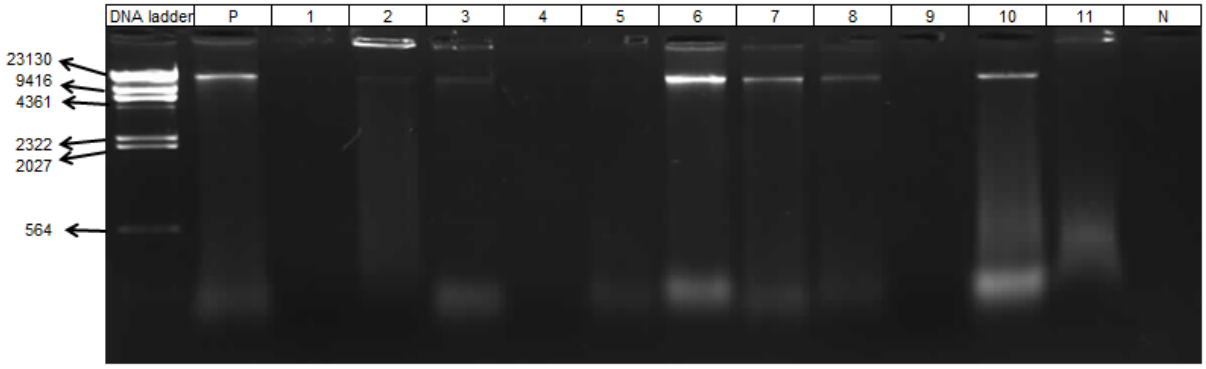

Figure 1. Extracted DNA samples were loaded and analyzed by DNA agarose gel (0.8%) electrophoresis and visualized under UV light. Lanes: DNA ladder, Lambda DNA HindIII digest molecular weight standard marker; P: positive control; lane 1: cornflakes; lanes 2 and 3: corn seeds; lanes 4: Popcorn & Rice Crisp; lane 5: Corn Chips; lanes 6: Popcorn; lanes 7 and 8: corn flour-1 and corn flour-2; lane 9: corn starch; lanes 10: cob and lane 11: canned corn; N: negative control.

Before the investigation of the presence of GMO sequences, to assure the amplification of DNA extracts in PCR, firstly, the extracted DNAs were tested with specific primers for an endogenous HMG gene. All tested samples, even if they are not visible on the DNA gels or even they are not detected with UV absorbance, resulted in 79 bp DNA bands, correct size for HMG gene (Figure 2). According to qPCR analysis, Ct values of samples differed significantly which evidence the presence of PCR inhibitors during the processing of products. Negative control lane did not reveal any signal.

Table 3. HMG gene qPCR result of samples

Lane #	Sample Name	Ct Value	Lane #	Sample Name	Ct Value
P	Positive Control	25,0	**6**	Popcorn	23,2
1	Cornflakes	31,5	**7**	Corn Flour-1	24,8
2	Corn Seed-1	23,7	**8**	Corn Flour-2	24,1
3	Corn Seed-2	24,4	**9**	Corn Starch	32,5
4	Popcorn & Rice Crisp	31,5	**10**	Corn Cob	23,6
5	Corn Chips	23,6	**11**	Canned Corn	27,7
				Negative	-

Secondly, we tested the presence of cauliflower mosaic virus (CaMV) 35S promoter and *Agrobacterium tumefaciens* nonpalin synthase (tNOS) terminator DNA sequences, which are mostly present in several GMOs products worldwide [9]. These methods are available on CRL-GMFF database according to EU legislation [8]. Taxon specific method was applied to screen CaMV and tNOS gene sequences in collected samples. Real Time PCR method (qPCR) was utilized to compare

relative extraction efficiency of the DNA extraction. The amplified DNA was run on 2% DNA agarose gel to evaluate the correct size of DNA bands (Fig 2). The positive control sample (P) exhibited 69 bp and 75 bp PCR products for tNOS and CaMV genes, respectively, which are the correct band size. None of the samples revealed any PCR band specific for tNOS and CaMV genes. This mean that these samples do not contain any genetically modified DNA sequences for P35S or CaMV.

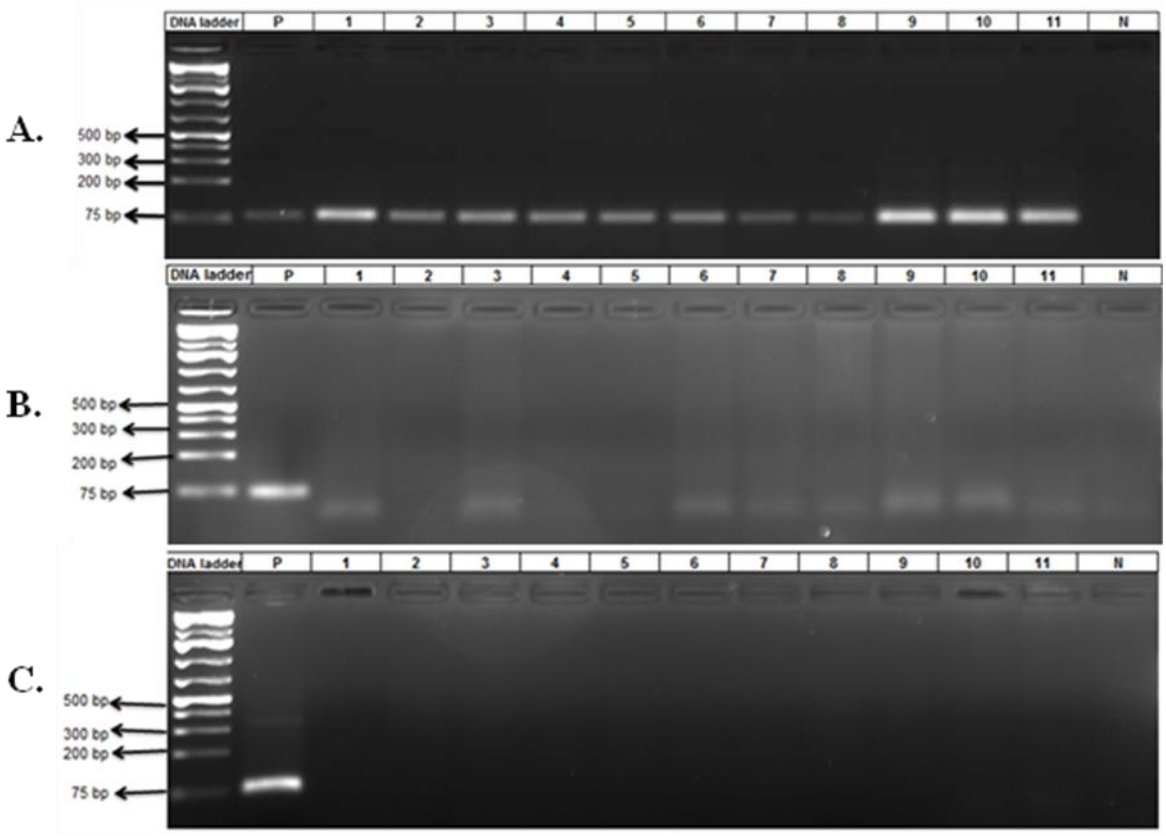

Figure 2. GMO analysis of corn samples. In addition to the control HMG gene (A), tNOS (B) and CaMV genes (C) were screened with qPCR. The PCR products were analyzed in 2% DNA agarose gel using gel electrophoreses. Gene Ruler 1 kb Plus DNA Ladder SM1333 was used as a DNA ladder. (P: positive control, N: negative control, lines 1–11: tested samples).

According to the European Commission GMO legislation, food and feed products containing more than 0.9 % authorized GMO should be labelled for the consumer information. In this study, 11 corn containing foods, collected from Turkish market, were screened for the presence of genetically modified tNOS and CaMV genes. This study has shown that none of these samples carry the DNA sequences for CaMV and tNOS vectors. This kind of analysis should be carried out periodically to test the presence of GMO food and feed in each country on randomized timing.

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ORCID
Meltem Asicioglu: 0000-0002-2718-794X
Burhanettin Yalçınkaya: 0000-0002-3744-6634
Muslum Akgoz: 0000-0001-5065-8087

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