Indian Journal of Biotechnology
Vol 10, January 2011, pp 143-146

Qualitative detection of GM maize (Bt11) in food and feed sold commercially in Turkey by PCR based methods

Güzin Candan Gürakan[1], Gamze Aydin[1] and Remziye Yilmaz[2]*

[1]Department of Food Engineering and [2]Molecular Biology and Biotechnology R&D Center
Middle East Technical University, 06531 Ankara, Turkey

Received 13 August 2009; revised 11 May 2010; accepted 20 July 2010

To regulate the presence of genetically modified organisms (GMOs) in crops, foods and food ingredients, the development of reliable and sensitive methods for their detection is necessary. In the present study, products derived from GM maize in the Turkish market were monitored using methods to detect the presence of CaMV 35S promoter, *nos* terminator and Bt11 maize. The results demonstrated, for the first time, the presence of GM maize in Turkish food and feed products qualitatively.

Keywords: Genetically modified organisms (GMO), Bt11 maize, PCR

Many genetically modified (GM) crop plants are approved for cultivation worldwide since 1994 (http://agbios.com). Later, their detection in food and food products led to the development of regulations on GM foods[1-3]. Several analytical methods, both qualitative and quantitative, have been developed to determine the presence and the amount of GM organisms (GMOs)[4-6]. These detection methods mainly focus on the newly introduced DNA[7]. In European Union (EU), validation report of the GMO specific detection method for Bt11 in sweet maize was published by the Community Reference Laboratory for GM Food and Feed on 5th August 2004 (http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm). For detecting GMOs, polymerase chain reaction (PCR) was found to be the ideal method for its high sensitivity, specificity and rapidity.

It is the policy of Turkish Government to protect its biological diversity as well as human and animal health against the possible adverse effects of products developed by using modern biotechnology. As a result a new Biosafety Law[8] is promulgated, which demands the regulated use of GMOs. Furthermore, there is no detailed information in the country about the presence of GM crops consumed either as food or feed. Present study describes the screening of different food and feed products, collected from various cities of Turkey in 2004, to detect the presence of GM maize (Bt 11 maize).

A total of 31 food and feed samples, including a variety of highly processed products containing maize ingredients, were collected randomly from the different city markets of Turkey for analysis (Table 1). Certified reference materials (CRMs) consisting of dried maize flour with 0, 0.1, 0.5, 1, 2 and 5% (w/w) Bt11 maize (IRMM-412R), developed by the Institute for Reference Materials and Measurements (IRMM), Geel, Belgium and commercialized by Fluka Chemika, were used as reaction controls.

DNA extraction from maize foods was performed by CTAB[9]. The extracted DNA (6 µL) was subjected to electrophoresis on a 0.7% agarose gel at a constant voltage (90 V for 40 min) to evaluate the quality of DNA. All primers used in the study were synthesized and purified by IONTEK Inc. (Turkey) and their sequences are listed in Table 2.

For screening and identification of Bt11 maize, the PCR reaction mixture was set up in a volume of 30 µL containing 2 µL DNA solution, 1 U Taq polymerase, 3 m*M* $MgCl_2$, 0.2 m*M* of each dNTP, buffer for polymerase and 0.5 µ*M* of each primer. The amplification was carried out in a Techne Progene thermocycler with the following programme: (i) For primers Zein-3/Zein 4, initial denaturation for 3 min at 95ºC, 35 cycles of 1 min at 95ºC, 60 sec at 60ºC and 60 sec at 72ºC, and final extension for 7 min at 72ºC; (ii) For primers 35S-1/35S-2 and NOS-01/NOS-02, initial denaturation for 3 min at 94ºC, 35 cycles of 60 sec at 94ºC, 60 sec at 60ºC, 60 sec at 72ºC and a final extension at 72ºC for 3 min; (iii) For primers IVS2-2/PATB-F/ IVS2-2/PATB-R, initial denaturation for 2 min at 94ºC, 30 cycles of 45 sec at 94ºC, 60 sec at 56ºC and 60 sec at 72ºC, and a final extension at 72ºC for 10 min. PCR products (12 µL) were determined on a 1.5% agarose gel (80 V for 30 min and 100 V for 90 min) containing ethidium

*Author for correspondence:
Tel: +90 312 210 64 06; Fax: +90 312 210 64 77
E-mail: remziye@metu.edu.tr

bromide in TBE buffer (10 m*M* Tris, 2.75 g boric acid/L & 1m*M* Na_2EDTA). The visualization was performed in a gel documentation system (Vilber Laurmot, France).

In view of the high risk of accidental contamination of samples and reagents by cross-contamination, individual PCR steps were performed separately in terms of physical space and equipment. A reaction with water instead of DNA extract was carried out with every mix as reagent control and with every set of samples, a series of positive and negative controls were run to ensure the accuracy of the results[10].

Table 1—Maize samples collected from different parts of Turkey

Location/Sample no.	Kind of sample
Eskişehir/1-4; Ankara/5	Commercial feed
Ankara/6; Isparta/7; Ankara/8; Antalya/9; Canakkale/10	Kernel corn[1]
Ankara/11 & 12; Istanbul/13; Ankara/14 & 15	Maize flour
Ankara/16; Eskişehi /17; Ankara/18 & 19	Maize starch
Eskişehir/20; Ankara/21; Istanbul/22	Corn flakes
Ankara/23-25	Maize chips
Istanbul/26; Ankara/27 & 28	Pop corn
Adapazarı/29-31	Conventional non-modified maize

[1]Not treated with heat to prepare it for eating

The DNA extracted from the samples was amplified using plant-specific primers for zein (ze-03 & ze-04) and an amplicon of 277 bp was visualized on the agrose gel. However, soybean and tomato DNAs, which were utilized to control the specificity of primer pairs, showed no positive signals. Earlier, it was reported that the analysis of products containing maize seemed to be difficult probably due to low concentration of maize ingredients or to some processing steps in food production that strongly contributed to the failure of DNA extraction[11]. However, in the present study, amplification of all the samples yielded almost equal concentration of zein specific PCR products (data is not shown here).

The samples with positive signal for maize specific gene were analyzed for detection of GMO by targetting CaMV 35S promoter and *nos* terminator. The 35S promoter positive samples gave a PCR product of 195 bp using the primer pair 35S-1 and 35S-2 (Fig. 1). On the other hand, positive samples for *nos* terminator gave an amplicon of 180

Table 2—Primers utilized for detection and identification

Target sequence	Origin of sequence	Primer name	Length (bp)	Sequence	Product length*
Zein	*Zea mays*	Ze-03(R)	19	AgTgCgACCCATATTCCAg	277 bp[5]
		Ze-04(F)	21	gACATTgTggCATCATCATTT	
CaMV 35S promoter	*Agrobacterium tumefaciens*	35S-1(R)	19	gCTCCTACAAATgCCATCA	195 bp[4]
		35S-2 (F)	20	GATAgTgggATTgTgCgTCA	
nos terminator	*A. tumefaciens*	NOS-01(R)	20	gAATCCTgTTgCCggTCTTG	180 bp[4]
		NOS-02(F)	20	TTATCCTAgTTTgCgCgCTA	
Bt11 specific primer	*Bacillus thuringiensis*	IVS2-/PATB(F)	22	CTgggAggCCAAggTATCTAAT	189 bp[1]
		IVS2-2/PATB (R)	22	gCTgCTgTAgCTggCCTAATCT	

*Super script designates the reference

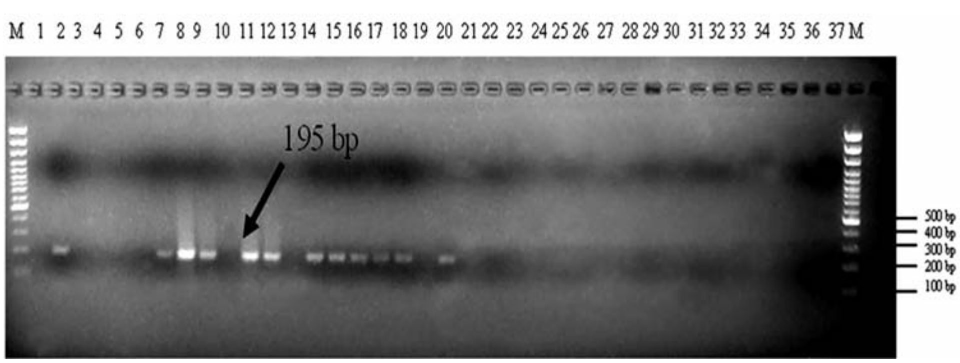

Fig. 1—PCR amplification by 35S-1 and 35S-2 primer sets (M: Molecular markers, DNA ladder (100bp); lane 1: no template; lanes 2-7: 5, 0, 0.1, 0.5, 1 & 2% CRM; lanes 8-17: samples 1-10; lanes 18-22: maize flour; lanes 23-26: maize starch; lanes 27-29: corn flakes; lanes 30-32: maize chips, lanes 33-35: pop corn; & lanes 36-38: non-modified kernel).

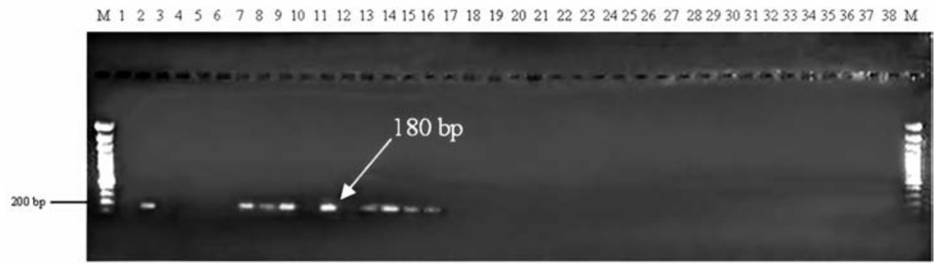

Fig. 2—PCR amplification of NOS terminator sequences (M: Molecular markers, DNA ladder (100bp); lane 1: no template; lanes 2-7: 5, 0, 0.1, 0.5, 1 & 2% CRM; lanes 8-17: samples 1-10; lanes 18-22: maize flour; lanes 23-26: maize starch; lanes 27-29: corn flakes; lanes 30-32: maize chips, lanes 33-35: pop corn; & lanes 36-38: non-modified kernel).

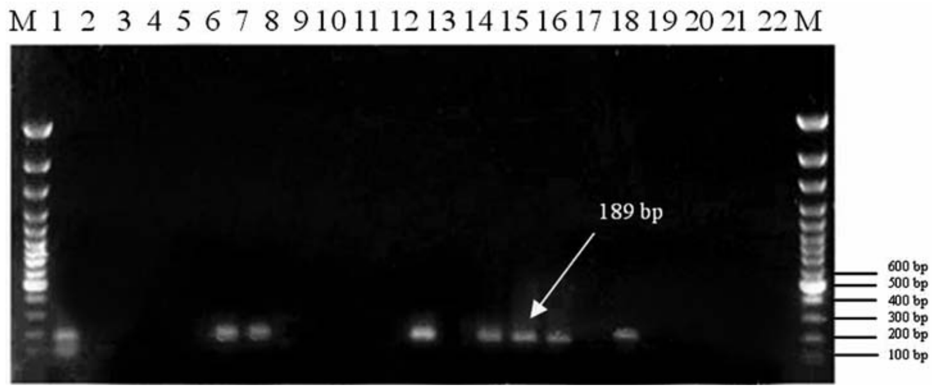

Fig. 3—PCR amplification for Bt11 maize lines (M: Molecular markers, DNA ladder, 100 bp; lanes 1-5: 5, 0, 0.1, 0.5, 1 & 2% CRM; lanes 7-16: samples 1-10; lanes 17-20: maize flour; lane 21: non-modified maize; & lane 22: no template).

bp using NOS-01 and NOS-02 primers (Fig. 2). In PCR screening, the sensitivity was 2% (in 5 ng of maize DNA) for 35S promoter and *nos* terminator assays as detected by utilizing positive controls, such as, 0.1-5% CRMs of Bt11 maize lines. No amplification was observed with the negative controls (0% Bt11 and conventional, non-modified maize lines originating from Turkey, provided by Biogenetic Unit, Ankara Province Control Lab, Ministry of Agricultural and Rural Affairs, Government of Turkey), nor with the PCR contamination control (no template DNA). Out of 31 samples tested, 11 were 35S promoter positive. Of these 11 samples, 7 samples were both 35S and *nos* positive. However, the remaining highly processed products containing maize ingredients (maize starch, corn flakes, maize chips & pop corn) were neither 35S nor *nos* positive. The results for all samples are shown in Table 3.

All 31 samples were analyzed for Bt11 maize using ivs/pat region specific primers and 8 of them showed an amplicon of 189 bp, confirming the presence of Bt11 maize (Fig. 3). For these primer combinations, specific Bt11 maize DNA detection had a sensitivity of 2.0% in 5 ng of total maize DNA. The samples (8 in total) those gave a positive amplification signal for Bt11 maize were sample no. 1 (animal feed), 6, 8, 9, 10 (kernel maize) and 13 (maize flour) (Table 3). The other 35S and/or nos positive samples were sample numbers 2, 4, 5 (feed), 7 (kernel corn) and 11 (maize flour). This study did not carry out analysis to detect the presence of other GM maize events.

The maximum threshold for GM contamination of final food and feed products has been defined as 0.9% in European Union, 3% in Korea, and 5% in Japan, so Zhu *et al*[3] suggested a threshold of 0.1% would be suitable for practical detection of Bt11 maize samples. However, in the present study, samples having < 2% level of contamination were not detectable (Table 3). For samples 2, 4 and 7, test results were positive for 35S and *nos* genetic elements, but no positive signals were obtained for Bt11 maize line. These could be due to the presence of a GM maize line other than Bt11. In samples 14 and 15, negative results were obtained for each transgene. For conventional non-modified maize kernels as negative control, always negative results were obtained as expected. In most of the processed samples as maize chips, pop corn, corn flakes, no positive detection was made, whereas

Table 3—Screening of various food and feed samples for presence of Bt11 maize

Sample I D	Sample	Zein	CaMV	NOS	Bt11
0 %		+[1]	-[2]	-	-
0,1 %		+	-	-	-
0,5 %		+	-	-	-
1 %	CRMs	+	-	-	-
2 %		+	+	+	+
5 %		+	+	+	+
1		+	+	+	+
2		+	+	+	-
3	Feed	+	-	-	-
4		+	+	+	-
5		+	+	-	-
6		+	+	+	+
7		+	+	+	-
8	Kernel corn	+	+	+	+
9		+	+	+	+
10		+	+	-	+
11		+	+	-	-
12		+	-	-	-
13	Maize Flour	+	+	-	+
14		+	-	-	-
15		+	-	-	-
16-28	Maize Starch (16-19) , Corn Flakes (20-22), Maize Chips (23-25), Pop Corn (26-28)	+	-	-	-
29-30	Conventional non-modified maize	+	-	-	-

[1]Positive result; [2]Negative result

transgenic elements were detected in maize flour. These results clearly demonstrate the presence of Bt11 maize in Turkish market as food and feed reinforcing the need for development of quantitative methods in all analyses for enforcement of the low level detection sensitivity.

Acknowledgment

Authors thank the Biogenetic Unit, Ankara Province Control Laboratory, Ministry of Agricultural and Rural Affairs, Government of Turkey to provide all conventional non-modified maize lines for this study.

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