GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Detection of genetically modified maize and soybean in feed samples

S. Meriç[1], Ö. Çakır[1], N. Turgut-Kara[1] and Ş. Arı[1,2]

[1]Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey
[2]Research and Application Center for Biotechnology and Genetic Engineering, Istanbul, Turkey

Corresponding author: Ö. Çakır
E-mail: ozgurckr@istanbul.edu.tr

Genet. Mol. Res. 13 (1): 1160-1168 (2014)
Received May 29, 2013
Accepted December 2, 2013
Published February 25, 2014
DOI http://dx.doi.org/10.4238/2014.February.25.2

ABSTRACT. Despite the controversy about genetically modified (GM) plants, they are still incrementally cultivated. In recent years, many food and feed products produced by genetic engineering technology have appeared on store shelves. Controlling the production and legal presentation of GM crops are very important for the environment and human health, especially in terms of long-term consumption. In this study, 11 kinds of feed obtained from different regions of Turkey were used for genetic analysis based on foreign gene determination. All samples were screened by conventional polymerase chain reaction (PCR) technique for widely used genetic elements; cauliflower mosaic virus 35S promoter (CaMV35S promoter), and nopaline synthase terminator (T-NOS) sequences for GM plants. After determination of GM plant-containing samples, nested PCR and conventional PCR analysis were performed to find out whether the samples contained Bt176 or GTS-40-3-2 for maize and soy, respectively. As a result of PCR-based GM plant analysis, all samples were found to be transgenic. Both 35S- and NOS-containing feed samples or potentially Bt176-containing samples, in other words, were analyzed with Bt176 insect resistant *cryIAb* gene-

specific primers via nested PCR. Eventually, none of them were found Bt176-positive. On the other hand, when we applied conventional PCR to the same samples with the herbicide resistance CTP4-EPSPS construct-specific primers for transgenic soy variety GTS-40-3-2, we found that all samples were positive for GTS-40-3-2.

Key words: Genetically modified organism; PCR; Feed; Maize; Soybean

INTRODUCTION

In the past two decades, recombinant DNA technology has been widely used in modern agriculture. Advances in molecular biology and recombinant DNA techniques have made it possible to manipulate plant genomes. Nowadays this technology is used in changing and improving plants for different purposes. A genetically modified (GM) plant is a plant whose genetic material has been altered. GM plants are created to possess several desirable traits such as herbicide tolerance (Block et al., 1987), insect resistance (Moellenbeck et al., 2001), virus resistance (Abel et al., 1986; Register and Nelson, 1992; Baulcombe, 1994), disease tolerance, stress tolerance (Cushman and Bohnert, 2000; Zhang et al., 2005), improvement of nutritional content (al-Babili et al., 2001), and delayed ripening (Redenbaugh et al., 1992; Kramer et al., 1992). The total global areas planted with GM crops increased rapidly from 1.7 to 160 million ha during the period of 1996-2011 (James, 2011). Maize, soybean, canola, cotton, potato, and papaya are commercially the most popular transgenic crops for field trials in the world. Especially, maize (24% of total maize crops) and soybean (60% of total soybean crops) are two main GM crops. Maize (*Zea mays* L.) is one of the three major cereal crops in the world. Soybean is one of most important protein sources used in feed and food. Due to their importance, maize and soybean are among the first crops to be genetically engineered and commercially used. There are several registered GM maize and soybean plants available worldwide (Aulrich et al., 2001; Tony et al., 2003; Greiner et al., 2005; Ujhelyi et al., 2008).

GM maize and soybean crops, which have an important place in agriculture, are increasingly used in the production of food and feed (Flachowsky et al., 2005; Sieradzki et al., 2006; Vain, 2006; Yoke-Kqueen et al., 2011). Herbicide-tolerant, insect-resistant, virus-resistant, disease-tolerant plants have taken a place in the world's food and feed market. Particularly, GM feed products have received more interest during the last few years. This increase in GM plant-derived feed causes more concern in biosafety and health. As a result of these international concerns about the possible potential risks of GM products, many countries have set several regulations regarding their production, import and risk assessments. Turkey is one of these countries that declared GM organism regulations under a biosafety law in 2010 (Official Gazette of the Republic of Turkey, 2010). Within the biosafety law, none of the GM events (i.e., varieties or lines) have been approved for use as food, but certain events [Bt11, GA21, NK603, DAS1507, DAS59122, MON89034, MON40-3-2 (GTS-40-3-2), MON89788, A2704-12] of GM plants have been approved for use as feed (Official Gazete of the Republic of Turkey, 2011a,b).

Processed products (e.g., foods and feeds) derived from GM crops can be identified by testing for the presence of a transgene or by detecting expressed novel proteins encoded by

the genetic material. Polymerase chain reaction (PCR) (Mullis and Faloona, 1987) techniques, which have become essential for many common procedures such as cloning specific DNA fragments have allowed the investigation of new fields such as the control of the presence of genetically modified DNA. PCR is used in the growing analytical approach for GM organism detection (Holst-Jensen and Berdal, 2004). Among DNA-based methods, PCR technology is preferred by many analytical laboratories interested in the detection of GM organisms because of its high sensitivity, specificity, and wide range of gene constructs (Ahmed, 2002; Anklam et al., 2002; Holst-Jensen et al., 2003). PCR-based detection can be evaluated in at least four categories related to the level of specificity namely screen-specific, gene-specific, construct-specific, and event-specific evaluation. The first category targets the cauliflower mosaic virus (CaMV) 35S promoter (P-35S) regions, T-NOS (nopaline synthase terminator) regions and/or gene encoding resistance to ampicillin (*bla*) and neomycin/kanamycin (*nptII*) antibiotics used in selection. In the second category, detection is achieved by the amplification of specific genes such as *cryIA(b)*. In category 3, the junctions between the adjacent elements of gene construct, for example, promoter and genes and in category 4, the junction between the gene and its integration locus are targeted for PCR amplification (Holst-Jensen et al., 2003).

The objectives of our study were to collect comprehensive qualitative analytical data by identifiying GM plants using gene- and construct-specific screening methods and to survey the status of GM feed in Turkey.

MATERIAL AND METHODS

Feed samples

This study included 11 kinds of feed, which are obtained from different regions in Turkey (Aegean, Anatolian, Black Sea, Central Anatolian, Marmara, Mediterranean, Southeastern, Thracian regions). Feed samples were kindly obtained from Turkish Feed Manufacturers' Association.

Reference samples

Powdered certified reference material (CRMs) ERM-BF411 (Bt176) and ERM-BF410k (GTS-40-3-2) containing 0 and 5% GM maize and 0 and 5% GM soybean, respectively, were purchased. These samples were used for screen-specific, gene- and construct-specific PCR analysis. In this study, 100 ng/µL genomic DNA from Bt176 maize and GTS-40-3-2 soybean lines were prepared as positive control samples for PCR. DNA extracted from blank reference materials was used as a non-GM control. *Hordeum vulgare* L. (barley) DNA (Istanbul University, Molecular Biology Laboratory DNA bank) was used as negative control for species-specific PCR.

Genomic DNA isolation

All feed and reference material DNAs were extracted from 100 mg samples by the cetyltrimethylammonium bromide (CTAB) method (Lipp et al., 1999; Somma, 2006) with minor modification. A homogeneous sample of 100 mg was transferred to a sterile 1.5 mL microcentrifuge tube,

with 300 µL sterile deionized water and mixed with a loop. CTAB buffer, 500 µL, was added and mixed. Proteinase K (20 µL 20 mg/mL) was added, shaken and incubated at 65°C for 60-90 min. 20 µL RNase A (10 mg/mL) was added, shaked and incubated at 65°C for 15-20 min. Following centrifugation for 10 min at 16000 *g*, the supernatant was transferred to a microcentrifuge tube containing 500 µL chloroform and shaken for 30 s. After additional centrifugation for 10 min, 500 µL of upper layer was transferred to a new microcentrifuge tube containing 500 µL chloroform and shaked. After centrifugation for 5 min, the upper layer was transferred to a new microcentrifuge tube. Two volumes of CTAB precipitation solution was added and mixed by pipetting and the tube incubated at room temperature for 60 min. The supernatant was discarded after centrifugation, and 350 µL NaCl (1.2 M) and a 0.6 volume of isopropanol were then added, followed by mixing and centrifugation for 10 min. Upper layer was transferred to a new microcentrifuge and 0.6 volume of isopropanol was added. Samples were centrifuged for 10 min. The supernatant was discarded and pellet was washed with 70% ethanol, air-dried and dissolved again in 50 µL sterile deionized water. DNA concentrations and quantities were measured using a spectrophotometer. Genomic DNA samples and DNA marker were separated on an EtBr-stained 1% agarose gel to estimate their quality.

Polymerase chain reaction (PCR)

The total volume of the PCR mixture was 25 µL and contained: 50 ng DNA extracted from feed samples (2 µL), 2.5 µL 10 X buffer, 2.5 µL 25 mM $MgCl_2$, dNTPs, primers, 0.1 µL 5 U/µL Taq DNA polymerase and nuclease-free water. The primers and their sequences used in the PCR amplification experiments are given in Table 1. Variable contents of primers, dNTPs and the amount of water are shown in Table 2. The reaction was carried out using the PCR instrument.

Table 1. Primers used in study and their sequences.

Target sequence	Primer name	Sequence (5'-3')	Products length (bp)
Zein region	Zein_1-L	5'-GCCATTGGGTACCATGAACC-3'	104
	Zein_1-R	5'-AGGCCAACAGTTGCTGCAG-3'	
35S promotor region	P35S-cf3	5'-CCACGTCTTCAAAGCAAGTGG-3'	123
	P35S-cr4	5'-TCCTCTCCAAATGAAATGAACTTCC-3'	
NOS terminator region	tNOS 2-5'	5'-GTCTTGCGATGATTATCATATAATTTCTG-3'	151
	tNOS 2-3'	5'-CGCTATATTTTGTTTTCTATCGCGT-3'	
Bt176 nested 1	CRYIA1	5'-CGG CCC CGA GTT CAC CTT-3'	430
	CRYIA2	5'-CTG CTG GGG ATG ATG TTG TTG-3'	
CryIAb nested 2	CRYIA3	5'-CCG CAC CCT GAG CAG CAC-3'	189
	CRYIA4	5'-GGT GGC ACG TTG TTG TTC TGA-3'	
Lectin region	GMO3	5'-GCCCTCTACTCCACCCCCATCC-3'	118
	GMO4	5'-GCCCATCTGCAAGCCTTTTTGTG-3'	
CTP4/EPSPS	RRS01-5	5'-CCTTTAGGATTTCAGCATCAGTG-3'	121
	RRS01-3	5'-GACTTGTCGCCGGGAATG-3'	

Table 2. Different contents for primers and dNTPs.

	CaMV 35S PCR	NOS PCR	Zein PCR	Lectin PCR	GTS4/EPSPS PCR
10 µM forward primers	0.5 µL	0.4 µL	0.5 µL	0.6 µL	0.6 µL
10 µM reverse primers	0.5 µL	0.4 µL	0.5 µL	0.6 µL	0.6 µL
2 mM dNTP	1 µL	0.4 µL	0.5 µL	0.5 µL	1 µL
Nuclease free water	15.9 µL	16.7 µL	16.4 µL	16.2 µL	15.7 µL

The conditions for PCR amplification of CaMV 35S promoter, NOS terminator, maize-specific gene (*zein*) and soy-specific gene (*lectin*) are summarized in Table 3, and PCR products were analyzed by 2% agarose gel electrophoresis.

Table 3. PCR programs.

	CaMV 35S PCR	NOS PCR	Zein PCR	Lectin PCR	GTS4/EPSPS PCR		
	Temperature					Time	No. of cycle
Initial denaturation	95°C	95°C	95°C	95°C	95°C	5 min	1
Denaturation	95°C	95°C	95°C	95°C	95°C	30 s (40 s)*	35 (30)**
Annealing	60°C	61°C	60°C	63°C	58°C	30 s (40 s)*	
Extention	72°C	72°C	72°C	72°C	72°C	30 s (40 s)*	
Final extention	72°C	72°C	72°C	72°C	72°C	5 min	1
Waiting	4°C	4°C	4°C	4°C	4°C	∞	

*GTS4/EPSPS PCR, **Zein PCR.

Nested PCR

For testing maize content of the feed samples in terms of Bt176 event CRYIA1/CRYIA2 (nested 1) and CRYIA3/CRYIA4 (nested 2) primer pairs were chosen for the detection of synthetic *cryIA*(*b*) gene (Studer et al., 1997). CRYIA1/CRYIA2 external primers and CRYIA3/CRYIA4 internal primers are complementary to the *cryIA*(*b*) gene sequence. All DNAs extracted from feed samples were analyzed using these primer pairs via nested PCR.

The total volume of nested 1 and nested 2 PCR mixtures was 25 µL and contained: 50 ng DNA (2 µL), 2.5 µL 10 X buffer, 2.5 µL 25 mM $MgCl_2$, 1 µL 2 mM dNTP, 0.6 µL 10 µM of each primer, 0.1 µL 1 U/mL Taq DNA polymerase and 15.7 µL nuclease free water.

The amplification conditions of nested PCR experiments were modified by Querci and Mazzara (2006). Parameters for PCR amplification experiments for specific primers CRYIA1/CRYIA2 and CRYIA3/CRYIA4 were used for the detection of the specific genetic event. Reaction conditions for CRYIA1/CRYIA2 primers were as follows: 5 min initial denaturation at 95°C followed by 35 cycles of 40 s denaturation at 95°C, 35 s annealing at 63°C and 30 s extension at 72°C, and a final 5 min extension at 72°C. In order to amplify CRYIA3/CRYIA4, 35 cycles of 35 s denaturation at 95°C, 30 s annealing at 63°C, and 35 s extension at 72°C, and a final 5 min extension at 72°C was applied.

RESULTS

By using the CTAB method, good quality DNA was extracted from all feed samples. The lowest yield of extracted DNA was 106.7 ng/µL, whereas the highest yield was 657.2 ng/µL. The purity of extracted DNA showed variations between 1.64 (A_{260}/A_{280}) and 1.91 (A_{260}/A_{280}).

Specific primers were used to detect species-specific genes of maize and soybean; these tests were performed for confirming the plant origin of the feed products. Species-specific gene (*zein*) for maize was identified in all feed samples. The size of the detected DNA fragment by primers ZEIN_1-L/ZEIN_1-R was 104 bp (Figure 1), whereas species-specific gene (*lectin*) for soybean was identified in all feed samples by primers GM03F/GM04R, with the size of the amplified DNA fragment of 118 bp (Figure 2).

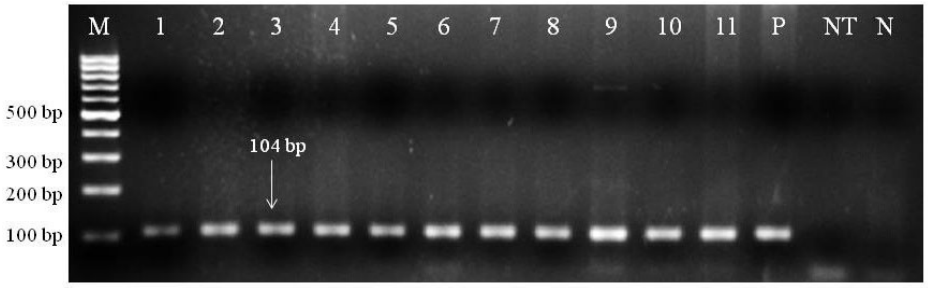

Figure 1. PCR amplification of *zein* sequences by Zein_1-L/Zein_1-R primers. *Lane 1* = Feed 1; *lane 2* = Feed 2; *lane 3* = Feed 3; *lane 4* = Feed 4; *lane 5* = Feed 5; *lane 6* = Feed 6; *lane 7* = Feed 7; *lane 8* = Feed 8; *lane 9* = Feed 9; *lane 10* = Feed 10; *lane 11* = Feed 11; *lane P* = 0.1% CRM (positive control); NT = no template; N = negative control (*Hordeum vulgare* L.); *lane M* = marker (100-bp DNA ladder, 100-1000 bp).

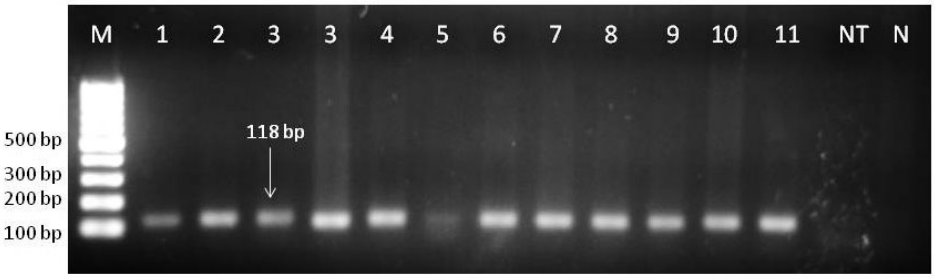

Figure 2. PCR amplification of *lectin* sequences by GMO3/GMO4 primers. *Lane 1* = Feed 1; *lane 2* = Feed 2; *lane 3* = Feed 3; *lane 4* = Feed 4; *lane 5* = Feed 5; *lane 6* = Feed 6; *lane 7* = Feed 7; *lane 8* = Feed 8; *lane 9* = Feed 9; *lane 10* = Feed 10; *lane 11* = Feed 11; NT = no template; N = negative control (CRM Blank); *lane M* = marker (100-bp DNA ladder, 100-1000 bp).

All feed samples were found to be GM according to the transgene abundance (Table 4). The DNA fragment for the NOS terminator (151 bp) and 35S promoter (123 bp) were identified in all feed samples (Figure 3 and 4). The results of nested PCR assays showed that GM feed samples did not contain the *cry1A*(*b*) region of maize event Bt176. On the other hand the results of PCR also showed that GM feed samples were contained Cp4-EPSPS region of the soybean event GTS-40-3-2. This region was identified in all feed samples tested, and the size of the amplified DNA fragment by the primers RRS01-3/RRS01-5 was 121 bp (Figure 5).

Table 4. Screening of various feed samples for transgene content.

Samples	Zein	Lectin	35S	NOS	Bt176 Cry1A(b)	CP4-EPSPS	Presence of transgene
Feed 1	+	+	+	+	-	+	+
Feed 2	+	+	+	+	-	+	+
Feed 3	+	+	+	+	-	+	+
Feed 4	+	+	+	+	-	+	+
Feed 5	+	+	+	+	-	+	+
Feed 6	+	+	+	+	-	+	+
Feed 7	+	+	+	+	-	+	+
Feed 8	+	+	+	+	-	+	+
Feed 9	+	+	+	+	-	+	+
Feed 10	+	+	+	+	-	+	+
Feed 11	+	+	+	+	-	+	+

(+) = possitive; (-) = negative.

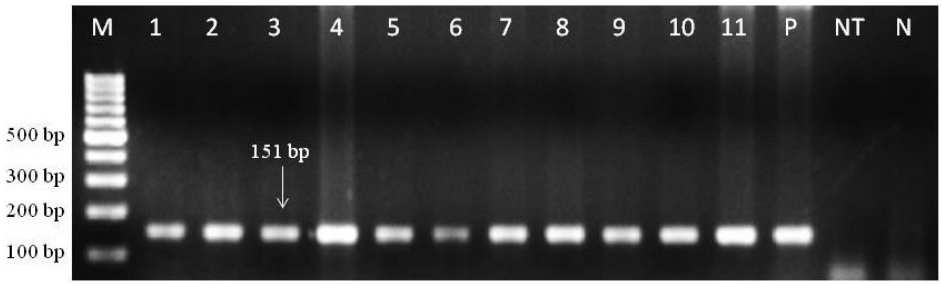

Figure 3. PCR amplification of NOS terminator sequences by tNOS 2-5′/tNOS 2-3 primers. *Lane 1* = Feed 1; *lane 2* = Feed 2; *lane 3* = Feed 3; *lane 4* = Feed 4; *lane 5* = Feed 5; *lane 6* = Feed 6; *lane 7* = Feed 7; *lane 8* = Feed 8; *lane 9* = Feed 9; *lane 10* = Feed 10; *lane 11* = Feed 11; *lane P* = 1% CRM (positive control); NT = no template; N = negative control (CRM Blank); *lane M* = marker (100-bp DNA ladder, 100-1000 bp).

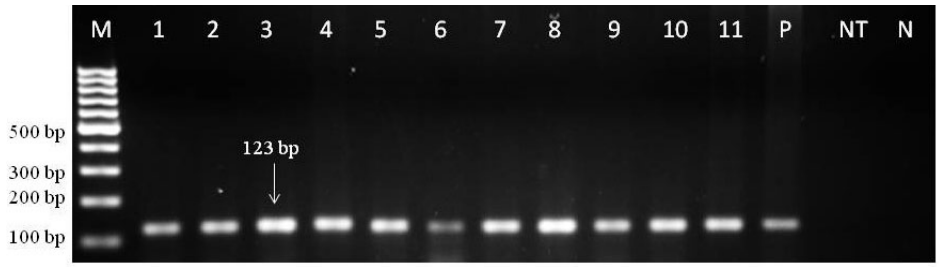

Figure 4. PCR amplification of 35S promotor sequences by P35S-cf3/P35Scr4 primers. *Lane 1* = Feed 1; *lane 2* = Feed 2; *lane 3* = Feed 3; *lane 4* = Feed 4; *lane 5* = Feed 5; *lane 6* = Feed 6; *lane 7* = Feed 7; *lane 8* = Feed 8; *lane 9* = Feed 9; *lane 10* = Feed 10; *lane 11* = Feed 11; *lane P* = 1% CRM (positive control); NT = no template; N = negative control (CRM Blank); *lane M* = marker (100-bp DNA ladder, 100-1000 bp).

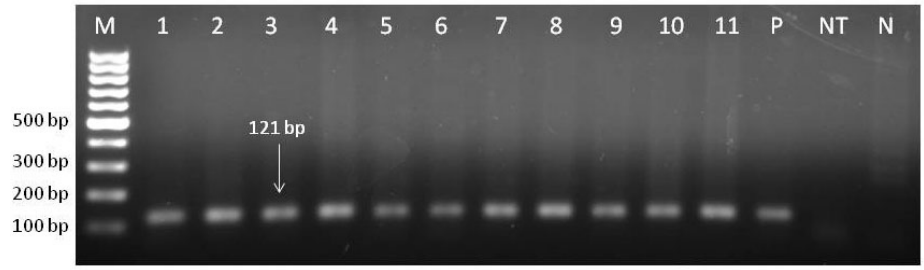

Figure 5. PCR amplification for Round up Ready soy lines by RRS01-5/RRS01-3 primers. *Lane 1* = Feed 1; *lane 2* = Feed 2; *lane 3* = Feed 3; *lane 4* = Feed 4; *lane 5* = Feed 5; *lane 6* = Feed 6; *lane 7* = Feed 7; *lane 8* = Feed 8; *lane 9* = Feed 9; *lane 10* = Feed 10; *lane 11* = Feed 11; *lane P* = 1% CRM (positive control); NT = no template; N = negative control (CRM Blank); *lane M* = marker (100-bp DNA ladder, 100-1000 bp).

DISCUSSION

PCR methods are considered to be the most commonly used techniques for detection of GM organisms. The specific DNA sequences in GM products are targeted and amplified using this technique. This procedure can be carried out by selecting the target DNA sequences and

the appropriate PCR conditions; hence either general or specific detection of GM organisms can be achieved (Holst-Jensen and Berdal, 2004).

In this study, we performed an operative routine analysis for detection of GM plants in feed samples. We used qualitative PCR based on a three-step analysis technique to determine the GM plant content and to estimate the GM plant type. The first step involved the amplification of specific maize and soy gene sequence from feed DNA. The second step entailed amplification of GM plant-specific sequences, represented by the 35S promoter and NOS terminator, to screen for the presence of transgenics in the samples. All GM plant containing samples were subjected to analysis of the specific transgenic event, Bt176 for maize and GTS-40-3-2 for soy in the third step.

Our results showed that all 11 samples contained *lectin* and *zein* genes. This confirmed that the samples tested contained both maize and soybean. The feed samples analyzed were found to be transgenic. All of them were positive for 35S and NOS regulatory elements. To test the specificity of the transgenic event, we applied nested PCR for Bt176 by using specific primers for nested 1 (CRYIA1/CRYIA2) and for nested 2 (CRYIA3/CRYIA4) (Querci and Mazzara, 2006), and they did not contain Bt176. However, we found that 100% of feed samples included GTS-40-3-2 soy. Regarding the evaluation of the risk assessment of GTS-40-3-2 soy by the Turkish Biosafety Committee, the use of this soybean event is allowed in feed and related products with the particular requirements (Official Gazette of the Republic of Turkey, 2011a).

In other countries, e.g., Malaysia, although GM plants are not produced, a related study found that all 24 feed samples analyzed were positive for 35S and NOS regulatory elements (Tung Nguyen et al., 2008). In another study conducted in Jordan, 100% of soy and 18.18% of maize used in feed production were found to be positive for GM organisms (Al-Rousan et al., 2010). These results indicate that GM products are frequently being used worldwide in feed material. Although the feed samples analyzed in this study were found to be transgenic, none of them contained Bt176 maize event. There is no permission for using Bt176 event in food or feed in Turkey (Official Gazette of the Republic of Turkey, 2011b), and thus, the results showed compliance with the law. On the other hand, our findings demonstrated that 100% of the feed samples included GTS-40-3-2 soy also appeared to be consistent with the law because this GM event used in the feed samples tested in this study is on the approved list of the Turkish Biosafety Committee. In conclusion, all of our findings showed compliance with the Biosafety Law of Turkey (Official Gazete of the Republic of Turkey, 2010).

ACKNOWLEDGMENTS

Research supported by the Research Fund of the Istanbul University (#19510). The authors thank the Turkish Feed Manufacturers' Association for providing the feed samples.

REFERENCES

Abel PP, Nelson RS, De B, Hoffmann N, et al. (1986). Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232: 738-743.

Ahmed FE (2002). Detection of genetically modified organisms in foods. *Trends Biotechnol.* 20: 215-223.

al-Babili S, Ye X, Lucca P, Potrykus I, et al. (2001). Biosynthesis of beta-carotene (provitamin A) in rice endosperm achieved by genetic engineering. *Novartis Found. Symp.* 236: 219-228.

Al-Rousan H, Al-Hmoud N, Hayek B and Ibrahim M (2010). A study on the occurrence of genetically modified soybean and maize feed products in the Jordanian market. *J. Cell Mol. Biol.* 8: 87-94.

Anklam E, Gadanif F, Heinze P, Pijnenburg H, et al. (2002). Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. *Eur. Food Res. Tech.* 214: 3-26.

Aulrich K, Bohme H, Daenicke R, Halle I, et al. (2001). Genetically modified feeds in animal nutrition. 1st communication: *Bacillus thuringiensis* (Bt) corn in poultry, pig and ruminant nutrition. *Arch. Tierernahr.* 54: 183-195.

Baulcombe D (1994). Novel strategies for engineering virus-resistance in plants. *Curr. Opin. Biotechnol.* 5: 117-124.

Block MD, Botterman J, Vandewiele M, Dockx J, et al. (1987). Engineering herbicide resistance in plants by expression of a detoxifying enzyme. *EMBO J.* 6: 2513-2518.

Cushman JC and Bohnert HJ (2000). Genomic approaches to plant stress tolerance. *Curr. Opin. Plant Biol.* 3: 117-124.

Flachowsky G, Chesson A and Aulrich K (2005). Animal nutrition with feeds from genetically modified plants. *Arch. Anim. Nutr.* 59: 1-40.

Greiner R, Konietzny U and Villavicencio ALCH (2005). Qualitative and quantitative detection of genetically modified maize and soy in processed foods sold commercially in Brazil by PCR-based methods. *Food Control* 16: 753-759.

Holst-Jensen A and Berdal KG (2004). The modular analytical procedure and validation approach and the units of measurement for genetically modified materials in foods and feeds. *J. AOAC Int.* 87: 927-936.

Holst-Jensen A, Ronning SB, Lovseth A and Berdal KG (2003). PCR technology for screening and quantification of genetically modified organisms (GMOs). *Anal. Bioanal. Chem.* 375: 985-993.

James C (2011). Global Status of Commercialized Biotech/GM Crops: 2011. ISAAA Brief no. 43, ISAAA, Ithaca.

Kramer M, Sanders R, Boklan H, Waters C, et al. (1992). Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: processing, firmness and disease resistance. *Postharvest Biol. Technol.* 1: 241-255.

Lipp M, Brodmann P, Pietsch K, Pauwels J, et al. (1999). IUPAC collaborative trial study of a method to detect genetically modified soy beans and maize in dried powder. *J. AOAC Int.* 82: 923-928.

Moellenbeck DJ, Peters ML, Bing JW, Rouse JR, et al. (2001). Insecticidal proteins from Bacillus thuringiensis protect corn from corn rootworms. *Nat. Biotechnol.* 19: 668-672.

Mullis KB and Faloona FA (1987). Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155: 335-350.

Official Gazette of the Republic of Turkey (2010). Number: 27533. Biosafety Law.

Official Gazette of the Republic of Turkey (2011a). Number: 27827, Biosecurity Committee Decisions, decision no.1, 2 and 3.

Official Gazette of the Republic of Turkey (2011b). Number: 28152, Biosecurity Committee Decisions, decision no. 4-16.

Querci M and Mazzara M (2006). Characteristics of RoundUp Ready Soybean, MON810 maize and Bt-176 maize. In: The Analysis of Food Samples for the Presence of Genetically Modified Organisms (Querci M, Jermini M and Eede Den GV, eds.). European Commission Joint Research Centre, Luxemburg, 11-16.

Redenbaugh K, Hatt W, Martineau B, Kramer M, et al (1992). A Case Study of the FLAVR SAVR™ Tomato. In: Safety Assessment of Genetically Engineered Fruits and Vegetables. CRC Press Inc., Boca Raton.

Register III JC and Nelson RS (1992). Early events in plant virus infection: relationships with genetically engineered protection and host gene resistance. *Semin. Virol.* 3: 441-451.

Sieradzki Z, Walczak M and Kwiatek K (2006). Occurrence of genetically modified maize and soybean in animal feedingstuffs. *Bull. Vet. Inst. Pulawy* 51: 567-570.

Somma M (2006). Extraction and Purification of DNA. In: The Analysis of Food Samples for the Presence of Genetically Modified Organisms (Querci M, Jermini M and Eede Den GV, eds.). European Commission Joint Research Centre, Luxemburg, 1-18.

Studer E, Dahinden I, Lüthy J and Hübner P (1997). Nachweis des gentechnisch veranderten 'maximizer'-mais mittels der polymerase-kettenreaktion (PCR). *Mitteilungen aus dem Gebiet der Lebensmittel und Hygiene* 88: 515-524.

Tony MA, Butschke A, Zagon J, Broll H, et al. (2003). Incidence of genetically modified soyabean and maize as animal feed in Egypt. *J. Anim. Feed Sci.* 12: 333-347.

Tung Nguyen CT, Son R, Raha AR, Lai OM, et al. (2008). Detection of genetically modified organisms (GMOs) using molecular techniques in food and feed samples from Malaysia and Vietnam. *Int. Food Res. J.* 15: 155-166.

Ujhelyi G, Vajda B, Beki E, Neszlényi K, et al. (2008). Surveying the RR soy content of commercially available food products in Hungary. *Food Control* 19: 967-973.

Vain P (2006). Global trends in plant transgenic science and technology (1973-2003). *Trends Biotechnol.* 24: 206-211.

Yoke-Kqueen C, Yee-Tyan C, Siew-Ping K and Son R (2011). Development of multiplex-PCR for Genetically Modified Organism (GMO) detection targeting EPSPS and Cry1Ab genes in soy and maize samples. *Int. Food Res. J.* 18: 515-522.

Zhang SZ, Yang BP, Feng CL and Tang HL (2005). Genetic transformation of tobacco with the trehalose synthase gene from *Grifola frondosa* Fr. enhances the resistance to drought and salt in tobacco. *J. Integr. Plant Biol.* 47: 579-587.