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Monitoring the prevalence of genetically modified (GM) soybean in Turkish food and feed products

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ABSTRACT

Soybean is the most widely cultivated genetically modified (GM) crop, and an ingredient in many foodstuffs worldwide. Legislation in the EU and Turkey only allows approved GM events to be imported, and requires labelling of food products containing >0.9% GM ingredients. In order to assess compliance with this legislation, 75 soy-containing Turkish food and feed products (none of which were labelled as GM) were successfully screened for the presence of four GM elements (CaMV 35S/tNOS/*bar*/FMV 35S). All positive samples were then tested for the 3GM soybean events approved for use in animal feeds in Turkey (RRS, MON89788 & A2704-12) by real-time quantitative PCR (qPCR). GM soybean was a major ingredient in 15 out of 19 animal feeds tested; it was also detected in some food samples (6/56), although at low levels. These findings provide the most comprehensive study to date of the penetration of GM soybean into the Turkish market.

1. Introduction

By 2013, the total global area of genetically modified (GM) crops cultivated in 27 countries had reached 175.2 million hectares, from which maize and soybean accounted for 32.3% and 48.2% respectively (James, 2013).

Soybean (*Glycine max* L.) continues to be the principal GM crop (James, 2013) and soya and soy derivatives are used as additives in a wide range of food and feed products worldwide (Singh, Kumar, Sabapathy, & Bawa, 2008). In particular, meal derived from GM soybean is increasingly used as a source of protein in animal feed, with its usage reaching about 70 million tons annually (James, 2014).

The growth of GM crops has provoked fierce debate, especially in Europe, as to whether they present unusual risks for health and the environment (Romeis, Meissle, Brunner, Tschamper, & Winzeler, 2013). The rapid adoption of biotech crops in the Americas and Asia has resulted in increasing demands for safety assessments of GM crops, and for the regulation of their cultivation and trade.

As a result, several countries have imposed different biosafety laws and surveillance programmes to regulate GMO use (De Jong, 2010). Within the European Union (EU), current laws and regulations on the traceability and labelling of GMOs require mandatory labelling of food and feed containing any GM ingredients above a certain threshold (>0.9% GMO content) and the identification of GM products throughout the supply chain (The Commission of the European Communities, 2003a, 2003b). Furthermore, the EU has recently adopted a zero tolerance policy towards low level presence of unauthorized GMOs in foodstuffs, with a 0.1% threshold for permissible presence of unauthorized GMO events in animal feed (The Commission of the European Communities, 2011). Turkey has adopted similar labelling thresholds in compliance with EU legislation, with Turkish government directives outlining a 0.9% threshold for mandatory labelling of authorized GMOs in foods and feeds, and 0.1% for unapproved GM material in feeds. Furthermore,

Abbreviations: CaMV, Cauliflower Mosaic Virus; CRM, Certified reference material; CTAB, Cetyltrimethylammonium bromide; EDTA, Ethylenediaminetetraacetic acid; FMV, Figwort Mosaic Virus; GM, Genetically Modified; GMO, Genetically Modified Organism; tNOS, Nopaline synthase terminator; PCR, Polymerase Chain Reaction; RRS, Roundup Ready Soya; SDS, Sodium dodecyl sulfate.

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the production of biotech crops is not permitted in Turkey, but the import of agricultural products is permitted for GM events approved by the Ministry of Food, Agriculture & Livestock. In Turkey to date, only 14 corn events and 3 soybean events (of 22 and 4 applications respectively) have been approved for feed use; none are approved for use in food (Global Agricultural Information Network, 2014).

Turkey imports significant quantities of feed crops for the poultry and livestock sectors each year (Global Agricultural Information Network, 2014), therefore the presence of GM soya in feeds is highly likely. On the other hand, GM soya may also be present in foodstuffs due to its increasing prevalence in world food production.

Seamless monitoring of GMO content in food and feed is challenging, as robust GMO detection methods are required along with the enforcement of analytical tests at early steps in the food and feed supply chain. The polymerase chain reaction (PCR) and especially quantitative real-time PCR (qPCR) has become the method of choice for GMO quantification in accordance with EU legislation for identification, detection and monitoring of GMO products throughout the supply chain (Gruère & Rao, 2007; Querci, Van Den Bulcke, Žel, Van Den Eede, & Broll, 2010).

A large number of studies using PCR techniques for GMO detection have been performed to monitor the presence of materials containing GMOs in the food and feed industries of several countries. But much of this research, especially on soy and maize-containing products, has focused on the qualitative detection of GM events (Andréia Z. Dinon, Bosco, & Arisi, 2010; Arun, Yilmaz, & Muratoğlu, 2013; Bergerová, Hrnčírová, Stankovská, Lopašovská, & Siekel, 2010; Cardarelli, Branquinho, Ferreira, da Cruz, & Gemal, 2005; Elsanhoty, Al-Turki, & Ramadan, 2013; Gürakan, Aydin, & Yilmaz, 2011; Kakihara, Matsufuji, Chino, & Takeda, 2006; Premanandh, Maruthamuthu, Sabbagh, & Al Muhairi, 2012; Zdjelar et al., 2013). The quantitative detection of GM soy events by qPCR has been reported in relatively few countries (Andréia Zilio Dinon, Treml, de Mello, & Arisi, 2010; Herzallah, 2012; Kim et al., 2013; Köppel, van Velsen, Felderer, & Bucher, 2012; Premanandh et al., 2012; Ujhelyi et al., 2008). In this regard, it is clear that further quantitative analysis of GM soybean presence in foodstuffs and feeds is still required to facilitate accurate labelling globally.

To our knowledge there is no available quantitative data on the prevalence of GM soybean in foodstuffs and feeds commercialized in Turkey. Hence, the aims of the present study were the monitoring of GMO content in soy-containing foodstuffs and feeds, and its quantitation to evaluate compliance with current labelling requirements.

2. Materials and methods

2.1. Samples, reagents and equipment

A total of 56 soy-containing foodstuffs including soy sauce, soya flour, soya milk, biscuits, snacks, chocolate, infant formula, soya mince, and tofu were acquired in local supermarkets in Istanbul and Bursa in 2015. Additionally, 19 soya animal feed samples (in the form of flakes, pellets, small particles or whole grains) were obtained from feed manufacturing companies located in different regions of Turkey (Aegean, Anatolian, Central Anatolian, Marmara, and Mediterranean). Certified reference materials (CRMs) for Roundup Ready Soya (RRS; also called gts40-3-2) at 0%, 0.1%, 1% and 10% GMO content were obtained from Sigma–Aldrich (St. Louis, MO, USA), while those for MON89788 and A2704-12 (100% GMO) were obtained from the American Oil Chemists' Society (AOCS; Urbana, Il, USA). The Foodproof GMO Sample Preparation Kit, Foodproof GMO Screening Kit, and Color Compensation Set 3 were purchased from Biotecon Diagnostics Gmbh (Potsdam, Germany). Other chemicals were obtained at molecular biology grade from Sigma–Aldrich unless otherwise stated. Qualitative PCR was carried out using a Mastercycler 384 Gradient thermocycler (Eppendorf AG, Hamburg, Germany) and real-time PCR experiments were carried out using a LightCycler 480 II instrument (Roche Diagnostics Gmbh, Mannheim, Germany).

2.2. gDNA extraction

All solid samples were homogenized by grinding in liquid nitrogen, used immediately, or stored at −80 °C prior to DNA extraction. The total DNA extraction from 200 mg of each of the less processed samples (feeds, flour, mince, biscuits, snacks & tofu) was carried out using the Foodproof GMO Sample Preparation Kit according to the manufacturers' instructions, except that the initial extraction step was routinely extended from 30 to 60 min, and at the final step 40 μl Elution Buffer was used in 2 × 20 μl elutions, to recover DNA at the highest possible concentration. For the more difficult samples (soy sauce, milk, infant formula & chocolate) a modified version of the cetyltrimethylammonium bromide (CTAB) precipitation method was used. Specifically, 200 mg or 200 μl of homogenized sample was incubated with 1 ml Edward's buffer (0.5% (w/v) SDS, 250 mM NaCl, 25 mM EDTA, 200 mM Tris pH 8.0) at 95 °C for 5 min (Cold Spring Harbor Laboratory, 2005). They were then spun down at relative centrifugal force of 16,000 g in a microcentrifuge for 10 min, and the supernatant was extracted twice with chloroform to remove protein. The aqueous (upper) phase was then incubated with 2 volumes of CTAB precipitation solution, after which the CTAB protocol was followed as previously described (Mafra et al., 2008). When necessary two or more repeat DNA extractions from each sample were carried out to increase DNA yield. DNA yield and purity were evaluated by UV spectrophotometry at 230, 260 & 280 nm using a NanoDrop 2000c instrument (Thermo Scientific, Wilmington, DE, USA). DNA integrity was assessed by agarose gel electrophoresis, in which 25–100 ng of purified DNA samples were separated on 1% agarose gels containing GelRed nucleic acid stain (Biotium, Hayward, CA, USA) in 0.5× TBE buffer.

2.3. Screening for GMO content

All samples were screened for GMO content with the Foodproof GMO Screening Kit, according to the manufacturer's instructions, using 80 ng of sample DNA in a final reaction volume of 10 μl. A real-time PCR protocol for the LightCycler 480 II was set up as described previously (Turkec, Kazan, Baykut, & Lucas, 2015) with multi-color detection in 4 channels (FAM; VIC/HEX; Rox; Cy5). A color compensation object was first created using Color Compensation Set 3 (Biotecon Gmbh, Potsdam, Germany) and all samples were then screened at least twice on different days, alongside appropriate CRMs as controls.

Qualitative PCR was used to confirm GMO screening results and detect the presence of RRS, MON89788 and A2704-12 genomic DNA, using soybean Lectin gene (*Le1*) as a taxon-specific control. The PCR primers and amplification conditions used were as described in previous studies (Dong et al., 2008; Liu et al., 2009; Pauli et al., 2001; Tengel, Schüssler, Setzke, Balles, & Sprenger-Haussels, 2001), and are summarized in Table 1. PCR reactions were performed in a final volume of 20 μl with appropriate positive and negative controls (CRMs and no template respectively); PCR products were analyzed by electrophoresis on 1.2–2.0% agarose gels alongside the GeneRuler 100 bp DNA ladder (Thermo Scientific, Waltham, MA, USA) and visualized as described in section 2.2.

Table 1
Primers and Conditions used in qualitative PCR Analyses.

Target	Primer	Sequence (5′ — 3′)	Annealing temp. (°C)	Amplicon size (bp)	Ref.
Lectin *Le1*	Lektin1	GACGCTATTGTGACCTCCTC	60	318	Tengel et al., 2001
	Lektin6	GAAAGTGTCAAGCTTAACAGCGACG			
RRS (gts40-3-2)	RRS-F	GCCATGTTGTTAATTTGTGCCAT	58	83	Pauli et al., 2001
	RRS-R	GAAGTTCATTTCATTTGGAGAGGAC			
MON89788	M1F	TTCCTGCTCCACTCTTCCTT	58	205	Liu et al., 2009
	M2R	TGAGGCTTTGGACTGAGAA			
A2704-12	A2704-12-F	TGAGGGGGTCAAAGACCAAG	58	239	Dong et al., 2008
	A2704-12-R	CCAGTCTTTACGGCGAGT			

2.4. Quantification of GM soy events

Quantification of GM content was carried out by real-time PCR using fluorescent hydrolysis probes specific for soybean Lectin and each GM event. Primers and hydrolysis probes were synthesized by Macrogen (Seoul, Rebublic of Korea). The qPCR assays were carried out in a final volume of 10 μl containing 100 ng template DNA, LightCycler 480 Probes Master reaction mix (Roche Diagnostics Gmbh, Mannheim, Germany) and primers and probes with sequences and final concentrations listed in Table 2. The concentrations of each primer/probe set were validated in previous studies (Delobel, Bogni, Pinski, Mazzara, & van den Eede, 2013; Mazzara, Delobel, et al., 2007; Mazzara, Munaro, et al., 2007; Pauli et al., 2001), except for the RRS primers which were optimized to the concentrations given in the table. PCR conditions were as follows: 10 min at 95 °C, followed by 45 cycles of: 10 s at 95 °C, 30 s at 60 °C (reduced to 55 °C for RRS), 10 s at 72 °C. Data acquisition took place in a single channel (FAM) during the elongation step of each cycle. For real-time quantifications, the double standard curve method was used, as this is typically more robust than Ct comparison (Cankar, Stebih, Dreo, Zel, & Gruden, 2006). The standard curve for soybean Lectin was set up using DNA from the CRM containing 10% RRS DNA, starting from 100 ng DNA/reaction and carrying out 5 serial 4-fold dilutions. A second Lectin standard curve prepared in the same way from MON89788 DNA gave essentially similar results, but with slightly higher error, so the former curve was used in subsequent calculations.

The standard curve for RRS was prepared similarly using the CRM containing 10% RRS DNA. The MON89788 and A2704-12 CRMs contained 100% GMO material, so for these an initial 10-fold dilution was followed by 5 serial 4-fold dilutions. All standard curve dilutions were prepared and assayed in duplicate on at least two separate occasions.

Test samples were assayed in triplicate for soybean Lectin and each GMO event; every qPCR plate also included the CRM of each event at 2% GMO concentration as a positive control/calibrator. The crossing point (Cp/Ct value) of each amplification curve was calculated using the Second Derivative Maximum method (Rasmussen, 2001), and the copy number of each element was estimated by comparison with the relevant standard curve. The copy number of each GMO event was divided by that of soybean lectin to derive the % GMO content.

3. Results

3.1. Sample collection and DNA isolation

In the present study, 77 samples of soy foods and feeds commercially available in the Turkish retail market were obtained in 2015. As reported in our previous work, successful GMO screening of food products by PCR depends on selection of the appropriate DNA isolation method (Turkec et al., 2015). Accordingly, in this study DNA from less processed materials (soya feeds, flour, mince, tofu and soya-containing snacks & biscuits) was isolated using the Foodproof GMO Sample Preparation kit, giving excellent yield and purity (A260/280 ratio > 1.8) for these samples. However, this method gave low DNA yields with highly processed and oily samples (soy sauce, soya milk, infant formula, and chocolate), for which the established CTAB precipitation method was preferred. Among modifications tested to optimize the procedure for these samples, using Edwards' buffer instead of 2% (w/v) CTAB buffer in the initial lysis step was empirically found to improve the DNA yield and/or purity. This suggests that the negatively charged SDS detergent included in Edwards' buffer is more effective than positively charged CTAB at separating soya gDNA from the other components of these foodstuffs.

The integrity of the DNA isolated from each sample was also checked by agarose gel electrophoresis. CRMs gave a single high-molecular weight band, while food samples showed varying degrees of smearing (data not shown). Generally, in more highly processed samples the smear shifted to lower molecular weights, giving evidence of increased DNA degradation.

Table 2
Primers and hydrolysis probes used in quantitative PCR Analyses.

Target	Primer/probe	Sequence (5′ — 3′)	Final conc. (nM)	Amplicon size (bp)	Ref.
Lectin *Le1*	Lectin-F	TCCACCCCCATCCACATTT	900	81	Pauli et al., 2001
	Lectin-R	GGCATAGAAGGTGAAGTTGAAGGA	900		
	Lectin-TMP	FAM-AACCGGTAGCGTTGCCAGCTTCG-TAMRA	200		
RRS (gts40-3-2)	40-3-2 AF	TTCATTCAAAATAAGATCATACATACAGGTT	200	84	Mazzara, Munaro, et al., 2007
	40-3-2 AR	GGCATTTGTAGGAGCCACCTT	200		
	40-3-2 AP	FAM-CCTTTTCCATTTGGG-BHQ1	200		
MON89788	MON89788-F	TCCCGCTCTAGCGCTTCAAT	150	139	Delobel et al., 2013
	MON89788-R	TCGAGCAGGACCTGCAGAA	150		
	MON89788-P	FAM-CTGAAGGCGGGAAACGACAATCTG-TAMRA	50		
A2704-12	KVM175	GCAAAAAAGCGGTTAGCTCCT	400	64	Mazzara, Delobel, et al., 2007
	SMO001	ATTCAGGCTGCGCAACTGTT	400		
	TM031	FAM-CGGTCCTCCGATCGCCCTTCC-TAMRA	200		

3.2. Screening for GMO presence and events by qualitative PCR

For PCR analysis, all samples were first tested for the presence of PCR-amplifiable soybean DNA using primers for the taxon-specific gene *Le1* (Soybean Lectin) and then screened using the Foodproof GMO Screening kit, which provides a multiplex qualitative real-time PCR assay targeting four elements of non-plant origin widely used in GM constructs: Cauliflower Mosaic Virus 35S promoter (CaMV 35S), *Agrobacterium tumefaciens* Nopaline synthase terminator (tNOS), Figwort Mosaic Virus 35S promoter (FMV 35S) and the *bar* gene from *Streptomyces hygroscopicus*. All samples tested positive for the plant gDNA-specific control gene included in the screening kit apart from 2 of the chocolate samples; therefore for these 2 cases, no conclusions can be drawn about GMO content. Of the remaining 75 samples, 15/19 animal feeds (79%) and 6/56 soya-containing food samples (10.7%) tested positive for one or more GMO elements (Table 3). Of these, the GMO-positive foodstuffs included 2 different soya milk samples, 2 soya-containing biscuits, 1 soya-containing snack, and 1 tofu sample. Notably, Biscuit#02 was marketed as a baby biscuit; however, no GMO material was found in any of the infant formula samples tested, nor in the soya flour, soy sauce, or soy-containing chocolate samples.

Only 3 soya GMOs are approved for import into Turkey, and each contains different GM constructs. Of the elements screened for by the Foodproof kit, A2704-12 includes only CaMV 35S, RRS contains CaMV 35S & tNOS, and MON89788 contains only FMV 35S. Therefore, the element screening results provide clues as to which GMO(s) may be present in each sample. All samples that screened positive for GM elements were verified by qualitative PCR with primers specific for each of these three GMO events. As shown in Table 3, RRS was the predominant GMO detected, being present in 19/21 of the GMO-positive samples, followed by 12/21 containing MON89788. A2704-12 was only detected in 4 samples, all of which also contained RRS. The GMO-positive snack sample consistently tested positive for the *bar* gene, which is not present in any of the GM soya varieties approved for use in Turkey; it was also detected at a relatively high cycle number, suggesting that it is only present in trace amounts. The event-specific PCR results correlated almost perfectly with the element-specific screen, with the exception of one feed sample which tested positive for FMV 35S but not MON89788, and the tofu sample where the reverse was true. Possible reasons for this discrepancy are discussed below.

3.3. Quantification of GM soybean events

In order to comply with the EU and Turkish labelling thresholds (≥0.9% total approved GMO), quantification of GM content must be carried out. Therefore, the amount of each GM soya event in all GMO positive samples was determined by quantitative real-time PCR with event-specific hydrolysis probes (see Materials and Methods). The soybean-specific *Le1* gene was used to determine the amount of soya genomic DNA in each sample. For the evaluation of quantitative data, dilution series of each element were amplified and standard curves created by plotting the Crossing Point (Cp) against the log of the concentration of each standard (Table 4). All of these curves were linear across the concentration range tested and were within recommended limits for amplification efficiency (90%–105%) and error (<0.2), indicating their suitability for quantification of our samples. The quantitation results were also calibrated/confirmed by including 2% CRM samples as positive controls and GMO-free soya genomic DNA as negative controls on each reaction plate.

The relative GMO content of GM soybean detected in the soya-containing products tested is presented in Table 5. In some samples a GM event was consistently detected but below the range of the standard curve, in which case this is reported as 'Trace' (<9 copies of the GM target sequence in the quantification reaction).

All the samples that tested positive in qualitative screens were confirmed by the quantitation results. As negative controls, several samples (Feed #17, Biscuit #01, Snack #09) that were GMO negative in the initial screen were also tested in the quantitation assay, and no GMO was detected, again showing consistency between the assays. Both the tofu sample and the feed sample (Feed#02) that gave conflicting results for FMV 35S/MON89788 in the qualitative tests were shown to be positive for MON89788, but at low concentrations (<0.5%). Additionally, very low levels (<0.1%) of MON89788 and A2704-12 were detected in 5 and 3 samples respectively that were not positive in the qualitative tests. These observations suggest that quantitation with hydrolysis probes offers greater sensitivity than normal PCR amplification for these elements. In the feed samples RRS was the predominant GMO detected, comprising between 16 and 100% of

Table 4
Standard Curve statistics for quantitative real-time PCR assays.

Target	Range (gene copies)[a]	E (%)[b]	Error[c]
Lectin	85–88,500	104.8%	0.0110
RRS (gts40-3-2)	9–8850	90.3%	0.0271
MON89788	9–88,500	100.4%	0.0170
A2704-12	9–88,500	100.0%	0.0058

[a] Calculated using a genome size (*C*-value) of 1.13 pg (Bennett & Leitch, 2012).
[b] E -PCR efficiency, calculated as $[10^{(-1/\text{slope})} - 1] \times 100$.
[c] Mean squared error of the fit of the data points to the standard curve.

Table 3
Results of qualitative screening for GM elements in soya products.

Sample type (total no. of samples)	Foodproof GMO screening kit *No. of samples testing positive*					Qualitative soya GM event-specific PCR *No. of samples testing positive*			
	Plant gene	CaMV 35S	tNOS	FMV 35S	*bar* gene	Lectin	RRS	MON 89788	A2704-12
Animal feed (19)	19	15	15	10	0	19	15	9	2
Soy sauce (4)	4	0	0	0	0	4	[a]	[a]	[a]
Soya flour (2)	2	0	0	0	0	2	[a]	[a]	[a]
Soya milk (4)	4	1	0	1	0	4	1	1	0
Soya mince/tofu (4)	4	1	1	0[b]	0	4	1	1[b]	0
Biscuit containing soya (11)	11	2	2	1	0	11	2	1	2
Snack containing soya (9)	9	0	0	0	1	9	0	0	0
Chocolate containing soya (14)	12[c]	0	0	0	0	12	[a]	[a]	[a]
Infant formula containing soya (10)	10	0	0	0	0	10	[a]	[a]	[a]

[a] Not tested.
[b] The GMO-positive tofu sample gave conflicting results in the qualitative screen for the presence of FMV 35S/MON89788; for discussion, see section 3.3.
[c] Of 14 chocolate samples, 2 failed to amplify plant DNA, and were excluded from the GMO analysis.

Table 5
Results of quantitation of specific GMO content in positive samples.

Sample	RRS (%)[a]	MON89788 (%)[a]	A2704-12 (%)[a]	Total GMO (%)[b]
Biscuit#02	7.0 ± 4.6	8.3 ± 2.6	1.5 ± 1.2	16.7 ± 5.4
Biscuit#09	6.6 ± 1.9	33.9 ± 4.0	2.0 ± 0.5	42.5 ± 4.4
Tofu#01	Trace	0.031 ± 0.004	ND	0.034 ± 0.004
Milk#01	ND	6.6 ± 1.4	ND	6.6 ± 1.4
Milk#02	Trace	ND	ND	Trace
Feed#01	61.5 ± 4.6	32.1 ± 2.1	1.9 ± 0.2	95.4 ± 5.1
Feed#02	23.2 ± 2.4	0.37 ± 0.05	0.037 ± 0.004	23.6 ± 2.4
Feed#03	55.7 ± 10.6	1.9 ± 0.2	0.24 ± 0.07	57.8 ± 10.6
Feed#04	59.2 ± 4.7	4.2 ± 0.5	ND	63.3 ± 4.8
Feed#05	50.9 ± 2.1	1.3 ± 0.1	ND	52.2 ± 2.1
Feed#07	58.1 ± 3.2	ND	ND	58.1 ± 3.2
Feed#08	22.5 ± 0.9	0.10 ± 0.02	ND	22.6 ± 0.9
Feed#09	21.9 ± 2.3	0.09 ± 0.05	ND	22.0 ± 2.3
Feed#10	108.9 ± 17.5	4.6 ± 1.9	Trace	113.5 ± 17.6
Feed#11	16.3 ± 1.9	0.31 ± 0.1	0.093 ± 0.029	16.7 ± 1.9
Feed#12	23.4 ± 2.9	0.11 ± 0.0	ND	23.6 ± 2.9
Feed#13	18.8 ± 2.4	ND	ND	18.8 ± 2.4
Feed#14	24.1 ± 3.6	ND	ND	24.1 ± 3.6
Feed#15	Trace	ND	ND	Trace
Feed#19	69.5 ± 5.4	8.5 ± 0.6	ND	78.0 ± 5.5

[a] Results are given as Mean ± Standard Deviation of 3 replicates. Trace = PCR positive, but at lower than the quantitative range tested. ND = None Detected under the conditions used.
[b] Total of the Means ± Combined Standard Deviation of the GMO events tested.

the total DNA content of those feeds that contained it. MON89788 and A2704-12 were also present in 11 and 5 of the feeds respectively, but at lower levels than RRS. In contrast, MON89788 was present at higher levels than RRS in the GMO-positive food samples, for example comprising ~34% of the soya DNA content in Biscuit#09. A2704-12 was found in lower quantities and in fewer samples than the other soya GMOs.

All of the GMO feed samples except Feed#15 had a total GMO content >0.9%, indicating that they should be labelled as containing GMO. Three of the food samples also had >0.9% GMO soya. However, in the case of both of the biscuits, wheat flour was the primary source of DNA, with soya only a minor ingredient. Therefore, the soya GMO is likely to be <0.9% of total DNA present in the samples, and so below the EU labelling threshold, assuming that no other GMO ingredients are present. In contrast, soya is expected to be the only source of DNA in the soya milk samples; Milk#01 contained 6.6 ± 1.4% MON89788, while Milk#02 contained trace amounts of RRS.

4. Discussion

4.1. Presence of soya GMOs in the Turkish retail market

In this study, we have conducted the most comprehensive survey reported to date of the prevalence of soya GMOs in Turkish food and feed products. Turkey presents an interesting case study for the penetration of GM soya into new markets, as it has some of the strictest laws in the world on the use of GMOs, with a complete ban on GM crop production. While a limited number of GM events are approved for import use in animal feed products, it is currently illegal for them to be present in food products. Accordingly, 79% of the feed samples tested here contained GM soya, of which all but one were well above the 0.9% EU labelling threshold and 7/19 comprised more than 50% GM material. In contrast, only 6/56 food samples (10.7%) tested positive for GM elements, in line with our previous study in which 4/34 (13%) food samples tested positive for GMOs (Turkec et al., 2015). In that study only 5/10 feed samples were GMO positive, which may indicate that GM soya use is increasing in Turkish animal feed products. Similarly, Meriç, Cakır, Turgut-Kara, and Arı, (2014) detected RRS in all of 11 feed samples tested. Over the past decade, the incidence of soya GMOs in commercial food and feed products has been reported from several parts of the world. Of 240 soy-containing products sold in Brazil between 2004 and 2007 (Branquinho, Ferreira, & Cardarelli-Leite, 2010), 68 (28.3%) were GM positive. In contrast, only 6/59 (10.2%) Brazilian processed meat products tested (Andréia Zilio Dinon et al., 2010) were positive for RRS. Ujhelyi et al. (2008) showed that out of 251 soy foodstuffs from the Hungarian market, 38% contained RRS. Herzallah (Herzallah, 2012) reported that out of 280 food and feed samples collected in Jordan during 2007–2008, 5.4% contained Bt-176 maize or RRS, whereas a recent report from Serbia (Zdjelar et al., 2013) showed that 8/32 (25%) soy-containing products were positive for RRS during 2009–2010. CaMV 35S, tNOS and soybean lectin were also detected in 6/16 soy-containing products from the United Arab Emirates, indicating the probable presence of RRS (Premanandh et al., 2012). These various studies demonstrate the presence of GM soya in food markets worldwide, with an increasing prevalence over time in parallel with growing GMO cultivation in some markets (Branquinho et al., 2010; Greiner & Konietzny, 2008).

A few other recent reports have also demonstrated the presence of GM soya in foodstuffs in Turkey; Arun et al. (2013) reported the presence of CaMV 35S & tNOS in 11/59 (19.3%) of soy-containing food products collected from 2008 to 2011, all of which also tested positive for RRS. CaMV 35S & tNOS were also found in 5/9 soy samples collected in 2009 (Mandaci, Çakir, Turgut-Kara, Meriç, & Ari, 2014). These two reports form a useful counterpoint to our study in that their samples were mostly collected before the current Biosafety Law prohibiting unapproved GMOs came into effect (September 2010). Therefore, the lower incidence of GMO positive food samples we observed may indicate that the Law has been somewhat effective in reducing the incidence of GM soya in Turkish foodstuffs, although not entirely. Similarly, a study of Turkish processed meat products carried out more recently (Ulca, Balta, & Senyuva, 2014) detected GM elements in only 2/32 soya-containing samples.

4.2. Implications for GMO monitoring strategies

In most of the aforementioned studies, the only GM soya elements tested for were CaMV 35S, tNOS, and RRS-specific sequences. This was a legitimate approach, as RRS (gts40-3-2) was one of the first GMOs to be approved for commercial use, and represented the largest single biotech crop in the world and the great majority of GM soya by 2005–6 (Kluga, Folloni, van den Bulcke, van den Eede, & Querci, 2012). However, since 2008, Monsanto Corporation has been marketing MON89788 under the trade name 'Roundup Ready 2 Yield' as an improved replacement for RRS (Monsanto Asia-Europe, 2008). As MON89788 contains neither CaMV 35S nor tNOS elements, it would not have been detected in most of the previous studies. Our data suggest that RRS is still the predominant GM event found in animal feeds in Turkey, but that MON89788 is more likely to be present in soy-containing foodstuffs – perhaps because it may be overlooked by routine GMO screens if they do not test for FMV 35S or other MON89788-specific sequences. The third soya GMO approved for feed use in Turkey, A2704-12, was also present both foods and feeds, but in fewer samples and at lower levels than either of the other two.

This study is also the first to our knowledge to quantify the GMO content in soya-containing foods and feeds in Turkey, and as such provides a more detailed picture of the current market than previous reports. The regulations for labelling and traceability of GMOs in the food and feed industry outlined by the EU require

compulsory labelling when the content of approved GM elements is above the 0.9% threshold (The Commission of the European Communities, 2003a). In our data, all but one of the GMO-positive feed samples tested were above this labelling threshold, but most of the GMO-positive food samples were not, indicating the importance of quantitative analysis to confirm compliance with labelling regulations. Similarly, recent quantitative studies in Hungary (Ujhelyi et al., 2008) and Serbia (Zdjelar et al., 2013) found that most or all soya GMOs detected in food products were below the labelling threshold, suggesting that GM soya was only a minor ingredient, or possibly a result of cross-contamination in food production lines or transport networks. Contamination from another product is the most likely explanation for the detection of trace amounts of the *bar* gene in one snack sample, as this GM element is not found in any widely used soya GMOs.

Turkey has adopted essentially the same labelling requirements as the EU (Global Agricultural Information Network, 2014) although in practice, as no GM events have yet been approved for food use, presence of any GMOs in food products is currently illegal. Therefore while 5/6 of the soya-containing food products that tested positive for GMOs would be permissible in the EU, none of them are legal in Turkey.

4.3. Conclusions

In summary, 75 soya-containing food and feed samples were successfully screened for GMO content by qualitative and quantitative PCR in the most comprehensive such study carried out in Turkey to date. Of the food samples tested here 10.7% were positive for GM events, of which MON89788 was the most prevalent, but generally below the 0.9% threshold level. The low amounts of GMO quantified in these positive samples are probably explained by unintentional or unavoidable contamination since none of them were labelled as containing GM soya, suggesting their compliance with EU legislation. In contrast, most of the soy-containing feeds were significantly above the 0.9% labelling threshold. As none of these products declared that they contained GM soya at the point of purchase, this suggests that the Turkish feed industry is not in compliance with current labelling requirements.

Finally, these results suggest that legal restrictions on GMO use in Turkey are limiting the presence of GM events in soya-containing food products, but cannot eliminate it entirely. That being the case, there is a need for the Turkish authorities to review the current legislation for its practicability, and take appropriate action to monitor compliance with current labelling legislation in the EU and Turkey. Further investigation should evaluate not only presence of GM soya events in the food and feed supply chain, but also other important crops such as cotton, rice and maize.

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