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A survey on genetically modified maize in foods commercialised in Portugal

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ABSTRACT

Maize, the second most important genetically modified (GM) crop, has the highest number of authorised GM events for food and feed in the EU. To provide consumer's information, labelling for food products containing more than 0.9% of GM material is demanded by the actual EU legislation. Analysis of foods is then essential to detect and quantify GM maize material and verify the compliance with labelling information. The aim of the present work was to assess the presence of GM maize in a range of processed foods commercialised in Portugal between 2007 and 2010. For this purpose, screening of GM material was carried out by qualitative PCR targeting the 35S promoter and the NOS terminator, followed by the specific detection of Bt11, MON810, Bt176, GA21, MON863, NK603, TC1507 (also known as DAS1507), DAS59122 and MIR604 events. The identified maize events were confirmed and quantified by real-time PCR with hydrolysis probes. The overall results of GMO screening were 30% for 35S promoter, 10% for NOS terminator and 25% for identified events. The most frequently detected events were MON810, TC1507 and NK603, with one sample containing GA21, while the other events were not detected in any of the analysed foods. The quantitative results suggest the need for a more severe control since 4% of the analysed foods contained more than the threshold for labelling and none of them declared the presence of GMO.

1. Introduction

During the past two decades, the progress of biotechnology has rendered the farmers to adopt the cultivation of genetically modified (GM) crops agronomic characteristics of interest. Despite the controversies surrounding genetically modified organisms (GMO), their cultivation has been steadily increasing since the first commercialised crop in 1996. In 2012, 170 million hectares were occupied by biotechnological crops, from which maize accounted for 33% (James, 2012). Portugal was included in the 28 countries planting GM crops in 2012, ranking the 22nd position (James, 2012) with approximately 5 thousand hectares of GM maize produced, for both food and feed, in 2011 (Agência Portuguesa do Ambiente, 2013). Maize, the second most important GM crop, has the highest number of authorised transgenic events for food and feed in EU (GMO Compass, 2013).

Ever since the GMO entered the food chain, the EU has dedicated special attention to consumer information by requiring compulsory labelling for food products containing more than 0.9% of GM material (Regulation (EC) No. 1829/2003). Thereby, to guaranty the implementation of legislation, analytical methodologies allowing accurate determination of GMO are demanded. The most accepted methods for GMO detection rely on DNA analysis using polymerase chain reaction (PCR) because of the superior stability of nucleic acids compared to proteins. The high specificity, sensitivity and reliability of PCR techniques, enabling the quantification of minute amounts of GM material even in highly processed foods are also main advantages (Gryson, 2010; Mafra, Ferreira, & Oliveira, 2008). Real-time quantitative PCR represents the most powerful means of quantifying GM material in agricultural and food products. It allows the continuous monitoring of the amplification products by the measurement of fluorescent signals. The use of specific-sequence fluorescent probes, such as the hydrolysis TaqMan probes, is the most frequently applied approach for GMO analysis as recently reviewed by Mafra (2011).

The need to monitor and verify the presence of biotechnology-derived material in foods has prompted the development of

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numerous studies. In this regard, several reports aiming at detecting the presence of GMO in foods and feeds have been described in relation to some markets/regions around the globe, namely Brazilian (Branquinho, Ferreira, & Cardarelli-Leite, 2010; Cardarelli, Branquinho, Ferreira, da Cruz, & Gemal, 2005; Dinon, Bosco, & Arisi, 2010; Dinon, de Melo, & Arisi, 2008; Greiner & Konietzny, 2008; Greiner, Konietzny, & Villavicencio, 2005), Egyptian (El Sanhoty et al., 2002), Serbian (Taski-Ajdukovic et al., 2009; Zdjelar et al., 2013), South African (Viljoen, Dajee, & Bothal, 2006), Hungarian (Ujhelyi et al., 2008), Italian (Germini, Salati, Quartaroli, & Marchelli, 2005), Malaysian (Kaur, Radu, Ghazali, & Kqueen, 2010), Arabian (Premanandh, Maruthamuthu, Sabbagh, & Al Muhairi, 2012), Jordanian (Herzallah, 2012), Iranian (Rabiei, Mehdizadeh, Rastegar, Vahidi, & Alebouyeh, 2013) and Turkish (Arun, Yilmaz, & Muratoglu, 2013; Gurakan, Aydin, & Yilmaz, 2011). All those studies target GMO detection by PCR techniques, but quantitative assessment by real-time PCR of GM maize events in foods was only effectively reported in the cases of the Brazilian and Jordanian markets (Greiner et al., 2005; Greiner & Konietzny, 2008; Herzallah, 2012). It also should be stressed that most research works have focused mainly soybean detection and the targeted maize events understudy have been mainly restricted to Bt11, MON810 and/or Bt176. In this sense, there is still a need for quantitative data and prevalence information about maize events in commercialised foodstuffs worldwide.

To our knowledge, no data is available regarding the prevalence of GM maize in foods commercialised in Portugal. Thus, the aim of the present work was to assess the presence of GM screening elements in processed maize foods commercially available in the Portuguese markets in 2007, 2009 and 2010, and to detect and quantify the events Bt11, MON810, Bt176, GA21, MON863, NK603, TC1507 (also known as DAS1507), DAS59122 and MIR604.

2. Materials and methods

2.1. Reference materials

Certified reference materials (CRM) from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) were acquired to Fluka (Buchs, Switzerland) and used as standards containing 0%, 0.1%, 1% and 5% for the evaluation of maize events Bt11, MON810, Bt176, GA21 and NK603, and containing 0%, 0.1%, 1% and 10% for events MON863, TC1507, DAS59122 and MIR604.

2.2. Sampling

A total of 119 food samples including maize grains, maize flours, maize cobs (grouped in grains/flours), snacks, bakery foods, cornflakes, cereals foods, cereal bars, sweet maize, frozen maize, popcorn and infant formula, were acquired in local supermarkets in 2007, 2009 and 2010. All the samples were triturated and homogenised using different blender containers previously treated with DNA decontaminator solution, and stored at −20 °C prior to DNA extraction.

2.3. DNA extraction

DNA was extracted by means of the Wizard or CTAB methods as described by Mafra, Silva, Moreira, Silva, and Oliveira (2008) using 100-200 mg of sample, depending on the matrix. All the extractions were performed in duplicate assays, though some needed more repetitions to improve DNA yield.

The quality of extracted DNA was analysed by electrophoresis in a 1.0% agarose gel carried out in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) for 40 min at 120 V, stained with ethidium bromide (0.4 μg mL^{-1} for 5 min) and destained in distilled water for 20 min. The agarose gel was visualised under UV light and a digital image was obtained using a Kodak Digital Science™ DC120 (Rochester, NY, USA).

The DNA was quantified by spectrophotometry using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The DNA concentration was determined by UV absorbance at 260 nm (1 absorbance unit corresponds to 50 μg mL^{-1} of dsDNA). The purity of the extracted DNA was determined by the ratio of the absorbance at 260 and 280 nm.

2.4. Oligonucleotide primers and probes

For qualitative PCR analysis, the oligonucleotide primer pairs targeting taxon-specific sequences were IVR1-F/IVR1-R or Mz-F/Mz-R for maize *invertase* and *zein* genes, respectively (Table 1). For GMO screening, fragments of P-35S and T-NOS elements were

Table 1
Oligonucleotide primers used in qualitative PCR.

Target	Primer	Sequence (5 − 3′)	Amplicon (bp)	Reference
Maize invertase gene	IVR1-F	CCGCTGTATCACAAGGGCTGGTACC	226	ISO 21569 (2005)
	IVR1-F	GGAGCCCGTGTAGAGCATGACGATC		
Maize zein gene	Mz-F	CGCCAGAAATCGTTTTTCAT	139	Germini et al. (2004)
	Mz-R	GGTGGTGTCCTTGCTTCCTA		
P-35S	35S-cf3	CCACGTCTTCAAAGCAAGTGG	123	ISO 21569 (2005)
	35S-cr4	TCCTCTCCAAATGAAATGAACTTC		
T-NOS	HA-nos118f	CGATGACGTTATTTATGAGATGGG	118	ISO 21569 (2005)
	HA-nos118-r	GACACCGCGCGCGATAATTTATCC		
MON810	VW01	TCGAAGGACGAAGGACTCTAACG	178	ISO 21569 (2005)
	VW03	TCCATCTTTGGGACCACTGTCG		
Bt11	IVS2-2	CTGGGAGGCCAAGGTATCTAAT	189	ISO 21569 (2005)
	PAT-B	GCTGCTGTAGCTGGCCTACTAATCT		
Bt176	CRY03	CTCTCGCCGTTCATGTCCGT	211	ISO 21569 (2005)
	CRY04	GGTCAGGCTCAGGCTGATGT		
GA21	GA21-F	TCTCCTTGATGGGCTGCA	270	Germini et al. (2004)
	GA21-R	ACGGTGGAAGAGTTCAATGTATG		
MON863	P863−3F	GGCGATGAATAAATGAGAAATA	200	Pan et al. (2006)
	P863-4R	TAGCCAGTTCATTGCGAGTA		
DAS59122	DAS-AF	CGCACCTGTGATTGGCTCAT	116	Kim, Kim, Lee, Kim, and Kim (2010)
	DAS-AR	GATTGTCGTTTCCCGCCTTC		
MIR604	MIR604-AF	CGCTCTGCGCACGCAATTCA	132	Kim et al. (2010)
	MIR604-AR	GGTTCTGTCAGTTCCAAACG		