Food Control 57 (2015) 1–8

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

An event-specific qualitative and real-time PCR detection of 98140 maize in mixed samples

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ARTICLE INFO

Article history:
Received 10 January 2015
Received in revised form
2 April 2015
Accepted 3 April 2015
Available online 11 April 2015

Keywords:
98140 maize
Event-specific method
Genetically modified organism
Herbicide-resistant maize

ABSTRACT

The purpose of this study is to establish a reliable detection method specific for event 98140, a type of multiple herbicide-resistant corn. Based on the 3′- flanking sequence of event 98140, qualitative and quantitative PCR detection assays were developed. The results revealed the LOD of 0.05% of GM maize for qualitative PCR assay and 4 transgenic haploid genome copies for quantitative PCR assay used in this study. The LOQ was 20 transgenic haploid genome copies, and based on this, as low as 0.05% of 98140 genomic DNA could be accurately and quantitatively detected by means of the quantitative method. Two mixed corn samples, with known 98140 contents (2% and 0.5%), were used to verify the developed real-time PCR system, and the expected results were observed. The results indicated that the developed event-specific PCR methods could be used for the identification and quantification of maize line 98140.

1. Introduction

Genetically modified (GM) crops are considered as the fastest adopted crop in the history of modern agriculture, and the global area of GM crops has reached 181.5 million hectares in 2014, with a remarkable 100-fold increase since the commercialization began in 1996 (James, 2014). Although most studies have shown that GM crops were secure, given the potential risks and harms of genetically modified organisms (GMOs) to human health and the ecological environment (Saladin, 2000), at present the commercialization of GM crops has also raised great concerns regarding bio-safety worldwide and has been an important issue that causes worldwide controversy (Aleksejeva, 2014; Ruane & Sonnino, 2011). Many countries and regions introduced some relevant policies and norms to manage GM products in international trade. Testing for GMOs has become important and necessary in light of the legislation imposed by the European Union, Japan, South Korea, Australia, New Zealand and China, as well as an increasing number of other countries. Labeling laws were established for approved bio-engineered crops to protect the consumers' rights, while at the same time prohibiting the import of unapproved varieties. Material from unauthorized GM organisms in food or feed is not permitted at any level (European Commission, 2003a, 2003b, 2008; Zhu, Zhao, Jia, Sun, & Zhao, 2008). However, most likely influenced by consumer perception, the regulations and labeling thresholds are different from one country to another. For instance, the labeling thresholds are defined as 0.9% in the European Union, 3% in South Korea, and 5% in Japan (http://www.eurofinsus.com/gmotesting/GMO_Testing.html). In China, zero threshold regulation was carried out. Including maize seeds, maize oil, tomato seeds, soybean oil, rapeseed seeds and cotton seeds, a total of 17 different food products required labeling in China (Pan et al., 2006). These mandatory regulations have encouraged the development and application of technologies aimed to precisely quantify the presence of biotechnology derived material in food or feed. At present, several methods based on nucleic acids or proteins have been developed to detect transgenic ingredients (Singh, Ghai, Paul, & Jain, 2006), and polymerase chain reaction (PCR) has been one of the most widely used techniques (Anklem, Gadani, Heinze,

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http://dx.doi.org/10.1016/j.foodcont.2015.04.002
0956-7135/

Pijnenburg, & Van den Eede, 2002; Auer, 2003; Zhang & Guo, 2011). Four PCR detection strategies, i.e., screening, gene-specific, construct-specific, and event-specific PCR detection methods have been developed (Anklam et al., 2002). Among them, event-specific PCR, targeting the junctions between the integrated DNA sequence and the host DNA of an event, is very popular for its unique specificity.

As an important raw material for the processing of food or other products, maize has been a frequent export and import good, including some approved GM maize. Currently, increasing demand has propelled China to become the world's fifth largest corn buyer, thus strengthening the necessity for scrutiny of foreign grain. Until 2013, about 15 types of GM maize strains have been approved for import into China only as raw and processed materials for food or feed (http://www.moa.gov.cn/ztzl/zjyqwgz/spxx/.). And China has not yet approved any GM corn for commercial sale. The need to monitor and verify the potential presence of GM ingredients in foods and track their movement has prompted the development of numerous studies on detection methods. Currently, some event-specific, quantitative PCR detection methods for GM maize have been published, such as Bt11 (Rønning, Vaïtilingom, Berdal, & Holst-Jensen, 2003; Zimmermann, Lüthy, & Pauli, 2000), MON810 (Hernández et al., 2003; Holck, Vaitilingom, Didierjean, & Rudi, 2002), NK603 (Kim, Zhang, & Kim, 2014; Nielsen, Berdal, & Holst-Jensen, 2004), MON863 (Pan et al., 2006; Zhu et al., 2008), DAS-59122-7 (Xu et al., 2009) and LY038 maize (Zhang et al., 2011). However, no paper has reported on the detection of event 98140 maize. Event 98140, a multiple herbicide-resistant corn which contains the glyphosate acetyltransferase (*gat4621*) gene and optimized form of the endogenous *als* gene (*zm-hra*) cassettes, was developed by Pioneer Hi-Bred International, Inc. (Chicoine et al., 2009, 2011). It has tolerance to herbicide glyphosate and ALS-inhibiting herbicides, such as chlorimuron and thifensulfuron (Green, Hale, Pagano, Andreassi, & Gutteridge, 2009). It contributes to environmentally and agronomically sustainable weed control measures, and has just recently been approved in the USA, Korea and Canada, as published by the Center for Environmental Risk Assessment (http://cera-gmc.org/index.php?evidcode%5B%5D=Event+98140&auDate1=&auDate2=&action=gm_crop_database&mode=Submit). Due to zero tolerance against unauthorized GM plants in Switzerland and the EU and its unlicensed state in China, a validated detection method to specifically detect maize line 98140 is urgently required. Based on this background, the current study was launched to establish effective methods for the event-specific detection of event 98140 lines. Qualitative and quantitative PCR assays for this GM event line and its concrete development proposals are described in this paper, the results of which are certain to aid in convenient and precise detection of GM content in food or additives.

2. Materials and methods

2.1. Materials

GM Maize 98140 was purchased from the JRC Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) by agent. GM Maize (MIR604, NK603, TC1507, Bt176, GA21, MON863 and MON 810), GM Soya (GTS40-3-2, A2704-12 and CV127), GM Cotton (LLcotton 25) and GM Rape (RF3) and GM rice (TT51-1) was collected by our laboratory. Non-transgenic seeds of *Zea mays*, *Glycine max*, *Oryza sativa*, *Brassica napus* and *Gossypium hirsutum* were purchased from a local market in Chengdu, China.

2.2. DNA extraction

Genomic DNA was extracted and purified from ground plant material using a Plant Genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. The quantity of DNA in the sample was calculated using absorbance measurements at 260 nm with a NanoDrop ND-1000 UV Spectrophotometer (USA), and further characterized by agarose gel electrophoresis, ethidium bromide staining and quantification with Quantity One software (Bio-Rad, USA). The different GMO concentrations tested were purchased from IRMM or prepared by mixing the DNA of 0.5% GM 98140 maize with increasing amounts of non-transgenic maize DNA.

2.3. Determination of flanking region DNA

Transgenic maize event 98140 was obtained by transformation of the plasmid PHP24279, which comprises an expression cassette containing *glyat4621* gene and *zm-hra* gene (Chicoine et al., 2011). Based on the figure of the plasmid PHP24279 and the obtained sequence of *zm-hra* gene published in US Patent (Chicoine et al., 2009, 2011), two primers (6P1, 6P2) were designed to amplify the 3′-flanking sequences. Details of the primers are shown in Table 1. The 3′-integration sequence of the 98140 event was isolated and determined using a GenomeWalker Universal Kit (Clontech, Mountain View, CA, USA). The 98140 genomic DNA libraries were constructed according to the manufacturer's manual.

The primary PCR was carried out in a volume of 25 μL containing 2.5 ng genomic DNA libraries, 1 × Quick Taq® HS DyeMix (Toyobo Co., Ltd., Osaka, Japan), and 0.8 μM each of kit-provided adopter primer AP1 and the gene-specific primer 6P1. The PCR reactions were performed in an ABI Veriti 96 thermal cycler (Applied Biosystems, USA) with the program as follows: one step of 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 1 min at 58 °C, 2 min at 72 °C; and one step of 7 min at 72 °C. Each of the primary PCR products was diluted 10 times with water, and then 1 μL each of the diluted

Table 1
Primers and probes used for PCR system.

Name	Sequence (5′-3′)	Amplicon size (bp)	Purpose	Specificity
6P1	GCTGCGTCTTGTTGGTGAATC	/	To obtain the 3′- flanking sequence of event 98140	/
6P2	CTGTTCTTTATGTGGGCGGTG			
AP1	GTAATACGACTCACTATAGGGC			
AP2	ACTATAGGGCACGCGTGGT			
zSSIIb-F	CTCCCAATCCTTTGACATCTGC	151	PCR analysis of endogenous gene	Maize genome
zSSIIb-R	TCGATTTCTCTCTTGGTGACAGG			
zSSIIb-P	FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA			
98140DX-F	ATGTCTCTTTGCTTGGTCTTTCT	168	Event-specific PCR analysis of 98140	Junction site of transgenic maize genome
98140DX-R	GTTGCGGTTCTGTCAGTTCC			
Event98140-F	GCGTTTTTTTGTGTGTGTATGTCTCT	86		
Event98140-R	CGTTTCCCGCCTTCAGTTTA			
Event98140-P	FAM-TGCTTGGTCTTTCTCTATCGATCCCCCTC-TAMRA			