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Development and primary application of a fluorescent liquid bead array for the simultaneous identification of multiple genetically modified maize

Xueqing Han [a,1,2], Huiyu Wang [b,1,3], Hongjun Chen [a,2], Lin Mei [a,2], Shaoqiang Wu [c,4], Guangle Jia [b,3], Tao Cheng [d,5], Shuifang Zhu [a,*], Xiangmei Lin [e,**]

[a] Chinese Academy of Inspection and Quarantine, Room 614, Building 241, HUIXINLI, Huixinxi Street, Chaoyang District, Beijing 100029, China
[b] Chinese Academy of Inspection and Quarantine, Room 611, Building 241, HUIXINLI, Huixinxi Street, Chaoyang District, Beijing 100029, China
[c] Chinese Academy of Inspection and Quarantine, Room 607, Building 241, HUIXINLI, Huixinxi Street, Chaoyang District, Beijing 100029, China
[d] Chinese Academy of Inspection and Quarantine, Room 812, Building 241, HUIXINLI, Huixinxi Street, Chaoyang District, Beijing 100029, China
[e] Chinese Academy of Inspection and Quarantine, Room 605, Building 241, HUIXINLI, Huixinxi Street, Chaoyang District, Beijing 100029, China

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ABSTRACT

An integration event-specific fluorescent liquid bead array was developed for the simultaneous identification of 10 genetically modified (GM) maize, including Bt176, Bt11, MON810, NK603, GA21, MON88017, MON89034, MIR604, T25 and MIR162, as well as one non-GM maize. The system comprised 11 specific oligonucleotide probes labeled with an amino group and coupled to fluorescence-encoded microspheres. To enable fluorescence detection, 11 pairs of primers labeled with biotin at the 5′ ends were used. The hybridization signal of biotinylated PCR product to the probe-coupled microspheres was then detected. The limit of detection of this assay was 0.1% for GM maize, which is lower than the current labeling threshold levels enforced in the EU (0.9%). The results of the positive and negative controls were consistent with their expected situation, which showed that the method was highly specific. We detected GM maize in 20 of the 1370 commercial food samples tested, which were labeled as containing maize. The overall sensitivity, specificity, rapidity and high throughput capacity of this liquid chip system suggest that it could provide a significant improvement over current methods, and potentially offer an improved platform for further research into the detection of other GM plants.

1. Introduction

The global area dedicated to the cultivation of genetically modified (GM) crops is increasing rapidly. In 2011, the total area amounted to 160 million hectares in 29 countries (Clive, 2011). GM maize is the second-most dominant GM crop, occupying 37.3 million hectares (Clive, 2011). Despite increasing adoption and numerous potential benefits, there is still a great deal of concern about the potential unanticipated impacts of GM crops on the environment, public health, and the economy (José, 2011; Eric et al., 2012). Effects can manifest both in the short term and the long term, inside or outside the GM cultivation area (the latter the result of spreading), and can be observed in both the aboveground and the belowground ecosystems (Eric et al., 2011).

To avoid the potential effects of adventitious contamination by GM material on food safety, several countries, including China, have issued food-labeling laws that incorporate GM threshold limits. Multiplex PCR provides the most straightforward, rapid, and cost-effective approach to the simultaneous detection of genetically modified organisms (GMOs). However, agarose gel electrophoresis does not easily resolve PCR products of similar length and for a large number of different GM loci (Jae-hwan et al., 2010). It is also laborious, time consuming and expensive to perform multiple real-time PCR assays, as the number of GMOs potentially present in food, animal feed and the environment is continually increasing (Sandrine et al., 2009). For high-throughput GMO analysis, alternative methods such as suspension arrays are therefore required. Suspension arrays, also referred to as liquid

* Corresponding author. Tel./fax: +86 10 64912744.
** Corresponding author. Tel.: +86 1064912741; fax: +86 10 64912743.
E-mail addresses: hanxueq@yahoo.com.cn (X. Han), firefox1996@163.com (H. Wang), chenhj1225@263.net (H. Chen), meilin20082008@yahoo.com.cn (L. Mei), sqwu@sina.com (S. Wu), gljiasx@yahoo.com.cn (G. Jia), chengtao0319@163.com (T. Cheng), zhusf@caiq.gov.cn (S. Zhu), xiangmei8lin@yahoo.com.cn, linxm@caiq.gov.cn (X. Lin).
[1] These authors contributed equally to this work.
[2] Tel./fax: +86 10 64912744.
[3] Tel.: +86 1064920322; fax: +86 10 64912740.
[4] Tel./fax: +86 10 64927408.
[5] Tel.: +86 1064967143; fax: +86 10 64912744.

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bead arrays, are known to provide precise analysis due to the elimination of human optical error (Choi, 2011). The suspension arrays technology was successfully applied for GMO detection. Anna et al. (2008) developed a multiple screening protocal allowing the detection of specific DNA sequences of p35S and epsps in GM soya flours simultaneously. Choi et al. (2010) and Choi (2011) developed the detection system using multiplex PCR and suspension arrays for stacked genetically modified rice event (LS28 × Cry1 Ac) and cotton event 281-24-236 × 3006-210-23 respectively. These arrays provide an innovative multi-target platform for the simultaneous detection of the various types of GMO that can be present in either raw samples or finished food products and feedstuffs (Roberta et al., 2005). However, event-specific detection methods are preferred to eliminate the possibility of false positive.

In this study, we developed a suspension array method for the simultaneous detection of 10 g maize. Our method comprised amplification of DNA, event-specific detection, and a multiplex assay. We also systematically tested this method for reproducibility, specificity and sensitivity.

2. Material and methods

2.1. Plant material

Maize GMO Standards (ERM-BF412 BioChemika, 5% Bt-11; ERM-BF411 BioChemika, 5% Bt-176; ERM-BF414 BioChemika, 4.3% GA21; ERM-BF413 BioChemika, 5% MON 810; ERM-BF415 BioChemika, 5% NK603; ERM-BF423 BioChemika, 10% MIR604) were purchased from Sigma-Aldrich (MO, USA). AOCS 0406-D MON 88017, AOCS 1208-A MIR162, AOCS 0906-E MON 89034, and T25 maize national certified reference materials (# GSB11-2269-2008), as well as non-GM maize powder, cotton seed powder, and soybean powder were provided by the Chinese Certified Reference Materials (CRM) Information Center.

2.2. Genomic DNA extraction

Genomic DNA was extracted from 50 ng powder samples using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Isolated DNA was dissolved in double distilled H_2O. The concentration of genomic DNA was estimated using a UV spectrophotometer (UV1700, Shimadzu, Kyoto, Japan) and then adjusted to 50 ng/μL.

2.3. Primer design

Eleven specific primer pairs were designed to analyze 10 different GM maize and one non-GM maize. The target sequences selected were the endogenous reference gene present in both GM and non-GM maize (the zein gene), and each of the 10 specific integration junction sequences between the host plant genome and the integrated genes present in the GM maize. Primers were designed using Primer Premier software, based on published DNA sequences. Primers were designed to meet the following conditions: (1) ability to detect degraded genomic DNA in processed food (amplification segments were between 100 and 300 bp); (2) melting point between 55 and 60 °C; and (3) compatibility in a multiplex-PCR system. The reverse primers were labeled with biotin at the 5′ end. All primers were synthesized by Invitrogen (Shanghai, China). A list of the primers used in this study is presented in Table 1.

2.4. Probe design

Eleven probes were designed using the Primer Premier software according to the target nucleotide sequences. The sequence specificity of the probes was first evaluated for each transgene using the BLAST homology search system from DDBJ (http://www.ddbj.nig.ac.jp/E-mail/homology.html). Each probe was then checked for potential hybridization to any other, non-target sequences, amongst those amplified during multiplex PCR, by aligning its sequence to the sequences of all of the amplified products. The HPLC-purified probes were synthesized with an amino-modified 12 carbon spacer at the 5′ end (Invitrogen). A list of probes used in this study is presented in Table 2.

2.5. Single-target PCR

PCR was performed in a Mastercycler Gradient (Eppendorf, Germany). The reaction mixture was prepared as follows: 10 × buffer including final concentrations of 2 mM $MgCl_2$; dNTP, 0.25 mM each; TaKaRa Ex Taq (TaKaRa, Japan), 2.5 U; forward primer (f), 0.5 μM; reverse primer (r), 0.5 μM; template DNA

Table 1
Sequences of primer sets for PCR detection of genetically modified maize.

Target	Reference; Genbank accession no.	Primer name	Sequence (5′–3′)	Expected amplicon size (bp)
Maize zein	X07535	Zein-F	TGATGGCGTGTCCGTCCC	380
		Zein-R	CTAGAATGCAGCACCAACAAAGG	
MON89034 event-specific	FV532179	MON89034-F	ACTCCAGCCACTGAAATGTTG	455
		MON89034-R	GTGACAGGTAGGATCGGAAAG	
MON810 event-specific	AY326434	MON810-F	CAAGTGTGCCCACCACAGC	328
		MON810-R	GCAAGCAAATTCGGAAATGAA	
MIR604 event-specific		MIR604-F	GCGCACGCAATTCAACAG	308
		MIR604-R	GGTCATAACGTGACTCCCTTAATTCT	
MON88017 event-specific	HV702026	MON88017-F	TTGTCCTGAACCCCTAAAATCC	299
		MON88017-R	CCCGGACATGAAGCCATTTA	
Bt176 event-specific	AJ878607	Bt176-F	GGCATGACGTGGGTTTCTGG	210
		Bt176-R	AGAACTCCGTGGGCGTGGTAT	
Bt11 event-specific	AY123624	Bt11-F	TATCATCGACTTCCATGACCA	207
		Bt11-R	AGCCAGTTACCTTCGGAAAA	
T25 event-specific		T25-F	GCCAGTTAGGCCAGTTACCCA	149
		T25-R	TGAGCGAAACCCTATAAGAACCCT	
GA21 event-specific	AJ878608	GA21-F	AGAGCTGTAGTTGTTGGCTGTG	145
		GA21-R	GCTGGGGGATCCACTAGTTCT	
MIR162 event-specific	HI203339	MIR162-F	TATAGCGCGCAAACTAGGAT	140
		MIR162-R	CTACCACAAGGCCCAGTATG	
NK603 event-specific	AX342368	NK603-F	CGGCCAGCAAGCCTTGTAGC	130
		NK603-R	TGTTTTATTTTGGACTATCCCGAC	