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Universal Primer-Multiplex-Polymerase Chain Reaction (UP-M-PCR) and Capillary Electrophoresis−Laser-Induced Fluorescence Analysis for the Simultaneous Detection of Six Genetically Modified Maize Lines

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ABSTRACT: To meet the labeling and traceability requirement of genetically modified (GM) maize and their products for trade and regulation, it is essential to develop a specific detection method for monitoring the presence of GM content. In this work, six GM maize lines, including GA21, Bt11, NK603, Bt176, Mir604, and Mon810, were simultaneously detected by universal primer-multiplex-polymerase chain reaction (UP-M-PCR), and the amplicons for the six event-specific genes as well as the endogenous *Ivr* gene were successfully separated by the method of capillary electrophoresis−laser-induced fluorescence (CE−LIF). The UP-M-PCR method overcame the disadvantages in conventional M-PCR, such as complex manipulation, lower sensitivity, amplification disparity resulting from different primers, etc., and in combination with CE−LIF, it obtained a high sensitivity of 0.1 ng for both single and mixed DNA samples. The established method can be widely used for the qualitative identification of the GM maize lines.

KEYWORDS: Genetically modified maize, universal primer-multiplex-polymerase chain reaction (UP-M-PCR), capillary electrophoresis−laser-induced fluorescence (CE−LIF)

INTRODUCTION

In the past decade, development and commercialization of genetically modified organisms (GMOs) has rapidly progressed.[1] According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA) statistics, commercialized transgenic crops have been planted since 1996 and, from 1996 to 2009, the global area dedicated to genetically modified (GM) crops increased 80-fold, from 1.7 million hectares in 1996 to 134.0 million hectares in 2009. Countries growing transgenic crops have steadily increased from 6 in 1996 to 25 in 2009.[2] There have been a total of 14 million growers benefiting from the transgenic crops all over the world in 2009.[2] In 2009, the planted area of GM maize was 41.7 million hectares, with the ratio of 31% in global biotechnology areas, more than 37.3 million hectares in 2008.[2]

With the rapid development of transgenic technology to the agricultural, food, and pharmaceutical fields, the safety of GM foods has become a hot topic. To execute the labeling requirement, the polymerase chain reaction (PCR) technique has become the main technique for GMO detection.[3] The multiplex-polymerase chain reaction (M-PCR) technique has been demonstrated to be more effective in reducing the cost and time of analysis compared to conventional PCR. This technique involves the simultaneous amplification of more than one target sequence per reaction by mixing multiple primer pairs in the same reaction.[4] M-PCR-based methods allow for a reduction of the number of reactions that are required to investigate a sample for the presence of several GMO-derived DNA sequences.[5] However, the shortcomings of M-PCR are also very obvious, including complex manipulation, lower sensitivity, amplification disparity resulting from different primers, etc.[6] This investigation was designed to develop an improved universal primer-multiplex-polymerase chain reaction (UP-M-PCR) technique for event-specific detection of six GM maize lines, which simplified traditional M-PCR, avoided the disparity of different primers in traditional M-PCR, and resulted in a higher specificity and sensitivity.

Since the 1990s, capillary electrophoresis (CE) has emerged as an important and powerful technique for the rapid and effective separation of DNA because of its advantages of simple instrumentation, high separation efficiency, less time-consuming, and potential for low costs.[7] The combination of PCR with CE has many advantages for the detection of genetic modification via the amplification, separation, and detection of DNA.[8] Molecular techniques combined with capillary electrophoresis−laser-induced fluorescence (CE−LIF) can be proposed as a good alternative for qualitative analysis because this combination gives rise to a novel and relatively inexpensive methodology.[9,10] In this study, we developed a UP-M-PCR procedure combined with CE−LIF detection that allows for rapid, sensitive, and simultaneous analysis of six GM maize lines. With this procedure, smaller amounts of reagents and shorter analysis times than

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Table 1. Information of Compound-Specific Primer Used in UP-M-PCR[a]

gene	primer	primer sequence (5′ → 3′)	fragment length (bp)	reference
GA21	GA21-F	*CCTACGACTCGAGCAACCCCCTTATCGTTATGCTATTTGCAACTT*	152	13
	GA21-R	*CCTACGACTCGAGCAACCCCTGGCTCGCGATCCTCCTCGCGTTTC*		
Bt11	Bt11-F	*CCTACGACTCGAGCAACCCC*TATCATCGACTTCCATGACCA	247	14
	Bt11-R	*CCTACGACTCGAGCAACCCC*AGCCAGTTACCTTCGGAAAA		
IVR	IVR-F	*CCTACGACTCGAGCAACCCCCCGCTGTATCACAAGGGCTGGTACT*	266	15
	IVR-R	*CCTACGACTCGAGCAACCCCGGAGCCCGTGTAGAGCATGACGATC*		
NK603	NK603-F	*CCTACGACTCGAGCAACCCC*GTTGCAAACCCACTGTACGAAT	389	this study
	NK603-R	*CCTACGACTCGAGCAACCCCCCCTCGCCCAGGCTATAAAAAT*		
Bt176	Bt176-F	*CCTACGACTCGAGCAACCCCCTCTACACCCACCTGCTGAA*	450	this study
	Bt176-R	*CCTACGACTCGAGCAACCCCGGAGCGAGAACACGAGAAGA*		
Mir604	Mir604-F	*CCTACGACTCGAGCAACCCCAAAGAAACCACCATTAGCCA*	634	this study
	Mir604-R	*CCTACGACTCGAGCAACCCCT*AGTTAAAGTACCCGTACCACCA		
Mon810	Mon810-F	*CCTACGACTCGAGCAACCCCT*GGGTGTAATTTCATATGGGCT	884	this study
	Mon810-R	*CCTACGACTCGAGCAACCCCC*AAAGGACGGCAAGTAATCAG		
UP		*CCTACGACTCGAGCAACCCC*		this study

[a] The table shows the details of primer sequences, expected DNA fragment length, and the source of primers used in UP-M-PCR. Each primer pair originates from the corresponding specific primer set (sequence in straight matter) and has a common sequence CCTACGACTCGAGCAACCCC (20 bp) at its 5′ end in italic font, which is also the sequences of the UP used in this developed new way.

single PCR are needed, which makes the method more applicable and cost-effective in routine screening of GM maize lines.

MATERIALS AND METHODS

Maize Samples. Six kinds of maize seeds containing the GM maize events, GA21, Bt11, NK603, Bt176, Mir604, and MON810 were used for the study. All six kinds of seeds were kindly provided by the Monsanto Company. Before the extraction of DNA, they were ground into powder with a HR2004 grinder (Philips, Zhuhai, China) with the size of 200 mesh in the fume hood to avoid cross-contamination.

Preparation of the DNA Template. Genomic DNA was extracted from 40 mg of ground sample using the DNeasy 96 Plant kit (QiagenGmbH, Hilden, Germany) according to the protocol of the manufacturer. DNA was quantified with the picogreen dsDNA quantification kit (Molecular Probes, Leiden, The Netherlands). Fluorescence was detected using the FL×800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) and analyzed by KC4 software 2000. DNA purity was evaluated on the basis of the UV absorption ratios of A_{260}/A_{280} and A_{260}/A_{230} and by analysis in 1% agarose gel. Only the samples with the A_{260}/A_{280} ratio ranging from 1.6 to 1.9, the A_{260}/A_{230} ratio ranging from 1.8 to 2.0, and a single band on gel were considered as pure DNA. These templates were stored at −20 °C before amplification to minimize degradation.[6]

Primer Design. Primer pairs for the UP-M-PCR are listed in Table 1. All of the primers were synthesized and purified by the Shanghai Sangon Company. Primer sets used in the UP-M-PCR target for maize *IVR* gene, GA21 event-specific sequence, and Bt11 event-specific sequence were described in previous papers.[13−15] The UP primer, NK603 event-specific sequence, Bt176 event-specific sequence, Mir604 event-specific sequence, and Mon810 event-specific sequence were designed using the ABI PRISM Primer Express, version 2.0, software (Applied Biosystems Company, Foster City, CA), with an optimal melting temperature (T_m) of 60 °C. When the UP primer was designed, the factors including having binding sites with most GM maize genome as little as possible, being rich in GC ("GC" is the accepted abbreviation for the base guanine and cytosine) contents, having a melting temperature (T_m) of about 60 °C, etc. were particularly considered to ensure the suitability of the UP in the novel M-PCR.

UP-M-PCR. In this novel M-PCR approach, a set of primers were designed to amplify the target sequences and the endogenous sequences in GM maize GA21, Bt11, NK603, Bt176, Mir604, and Mon810. All of the primers include a common sequence at their 5′ end,[6] which is also the sequence of the universal primer (UP). The amplification routine of UP-M-PCR is shown in Figure 1. At the initial stage of the reaction (about the former 10 cycles), the compound-specific primers take main action for amplification of target sequences because of their higher annealing temperature, while the UP almost has no amplification. With the compound-specific primers used up and the amplified products incorporating the UP adaptor increasing, the UP begins to play a leading role to take the amplicons as templates and shows its ability to amplify the fragments of seven different targets.

On the basis of the conventional M-PCR assay, the annealing temperature of primers was optimized from 56 to 62 °C.[16] The concentration of UP (from 50 to 500 nmol/L at an interval of 50 nmol/L) and compound-specific primers (from 500 to 0.5 nmol/L with a 10-fold gradient dilution) was evaluated. All of the PCRs were carried out using a Peltier thermal cycler controller (MJ Research, BioRad Laboratories, Waltham, MA) in a 50 μL reaction volume, containing 5.0 μL of 10× PCR buffer, 4.0 μL of 2.5 mmol/L dNTP, 0.6 μL (5 units/μL) of Taq DNA polymerase, 1 μL of template DNA (50 ng/μL), 10 nmol/L IVR primer, 15 nmol/L NK603 compound-specific primer, 20 nmol/L GA21, Bt11, and Bt176 compound-specific primers, 30 nmol/L Mon810 compound-specific primer, 40 nmol/L Mir604 compound-specific primer, and 400 nmol/L UP. The thermal cycling program included an initial 5 min of denaturation at 95 °C, then 40 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C, followed by a final extension for 10 min at 72 °C. Each reaction was run in triplicate.

Agarose Gel Electrophoresis. PCR products were initially analyzed by electrophoresis on 2% goldview agarose gel (0.5 μg/mL) for 30 min at 120 V in TAE buffer [40 mM Tris−acetate and 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 8.0].[17] A DL 2000 DNA marker (Tiangen, Beijing, China) was used as the DNA molecular marker.

CE−LIF. The analyses were carried out in a PACE-MDQ (Beckman Coulter, Brea, CA) equipped with an Ar^+ laser working at 488 nm (excitation wavelength) and 520 nm (emission wavelength). Bare fused-silica capillaries (50 μm inner diameter) were used for separation of DNA fragments with 40 cm to the detector and a full length of 50 cm

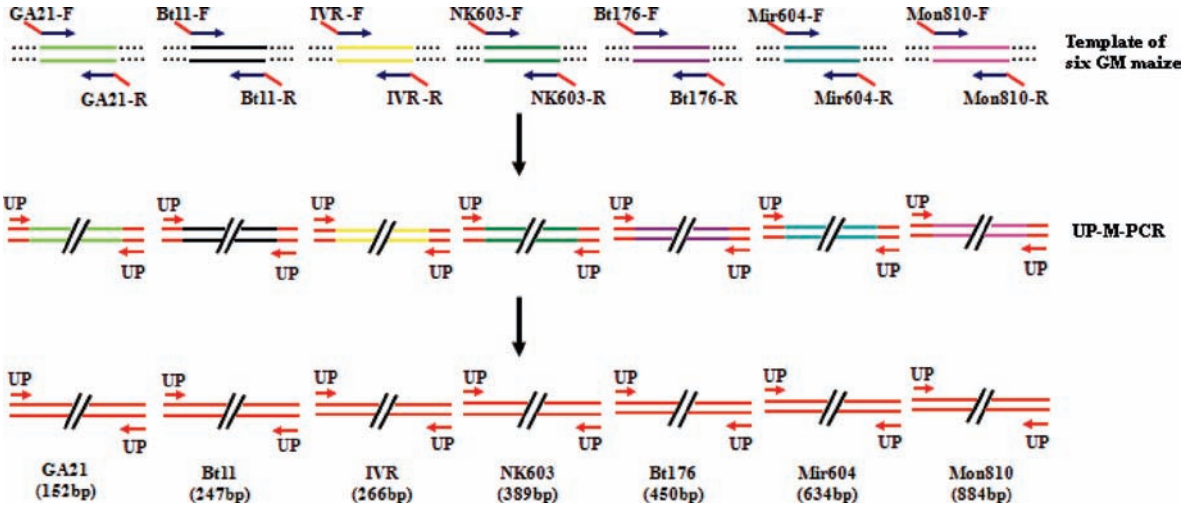

Figure 1. Amplification routine of UP-M-PCR. Each compound-specific primer contained a specific primer at the 5′ end (sky blue) and the universal sequence at the 3′ end (red). The amplified fragments with the primer pairs of GA21-152-F/R, Bt11-247-F/R, IVR-266-F/R, NK603-389-F/R, Bt176-450-F/R, Mir604-634-F/R, and Mon810-884-F/R are individually marked in pea green, black, yellow, green, lilac, blue, and pink, respectively. The amplified fragments only by the UP are marked in red.

(Yongnian Optical Fiber Co., Hebei, China). Data acquisition and integration were performed with 32 Karat software (Beckman Instruments, Inc., Fullerton, CA).

Before first use, any uncoated capillary was preconditioned by rinsing with 0.1 mol L^{-1} HCl for 20 min and then rinsed with deionized water for 10 min, methanol for 10 min, and deionized water for 10 min.[18] The following conditions were used for CE−LIF analysis of the PCR products. Separation buffer TBE (89 mmol/L Tris-HCl, 89 mmol/L boric acid, and 2 mmol/L Na_2EDTA at pH 8.3) was used to rinse the capillary for 10 min, and then the capillary was physically coated using polyvinyl pyrrolidone (PVP) (M_w, 1 300 000; Sigma-Aldrich, St. Louis, MO) with the condition of 40 psi (1 psi = 6894.76 Pa) for 5 min. The fluorescent dye used was 1× GeneFinder soluble in PVP. The DNA sample was injected at the cathodic end by using electrokinetic mode of −5.0 kV for a given time (the default setting in the 32 Karat software, generally 10 s), and the sample volume for analyzing is about 5 nL under this condition. A pGEM DNA marker (Promega, Madison, WI) was used as the DNA molecular marker in CE−LIF. Each sample was measured in triplicate.

RESULTS AND DISCUSSION

Feasibility of UP. When the specific primers for the *IVR* gene are taken for an example, keeping the concentration of templates at 50 ng, with the amount of the specific primers for the *IVR* gene decreasing (from 500 to 50 to 5 nmol/L), the intensity of the band was lessened gradually (primer 500 nmol/L) until nothing (primer 5 nmol/L) in conventional single PCR (lanes 2−4 of Figure 2), which showed that the concentration of amplified fragments was decreased relevantly. While in the novel singlet PCR, for the addition of UP (400 nmol/L), although there is a down-gradient concentration of compound-specific primers IVR-266-F/R from 500 to 5 nmol/L, the PCR system above worked efficiently and resulted in an nearly equivalent amount of amplified products (lanes 5−7 of Figure 2). Similar results were achieved from the compound-specific primers GA21-152-F/R, Bt11-247-F/R, NK603-389-F/R, Bt176-450-F/R, Mir604-634-F/R, and Mon810-884-F/R (data not shown) with UP in novel singlet PCR. The sharp contrast showed that the UP was well-designed

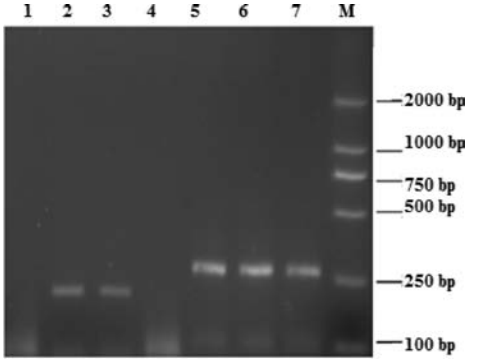

Figure 2. Feasibility of UP in single PCR. Lane M, DNA marker DL 2000; lane 1, no template control; lanes 2−4, specific primers for the *IVR* gene with the final concentration of 500, 50, and 5 nmol/L; and lanes 5−7, compound-specific primers for the *IVR* gene with the final concentration of 500, 50, and 5 nmol/L. The final concentration of UPs is 400 nmol/L.

to work efficiently for the PCR amplification and had a high feasibility to amplify the amplicons produced by compound-specific primers.

Optimization of the UP-M-PCR. The concentrations of primers strongly influence the efficiency and disparity of the PCR, which is very important for the PCR especially in M-PCR. The concentrations of each primer were optimized in the single and M-PCR system, ranging from 400 to 4 nmol/L with the series of dilutions, while the UP always had a normal concentration of 400 nmol/L. The final optimized concentration of UP was 400 nmol/L in UP-M-PCR, which is the same as in normal single PCR, while the other seven compound-specific primers GA21-152-F/R, Bt11-247-F/R, IVR-266-F/R, NK603-389-F/R, Bt176-450-F/R, Mir604-634-F/R, and Mon810-884-F/R were 10−50 nmol/L (about $^1/_{10}$ of the normal concentration). To find the best annealing temperature, a gradient temperature PCR from 56 to 65 °C was performed (data not shown). Finally, the optimum annealing temperature of 60 °C was chosen, using the following PCR program: preincubation at 95 °C for 5 min,

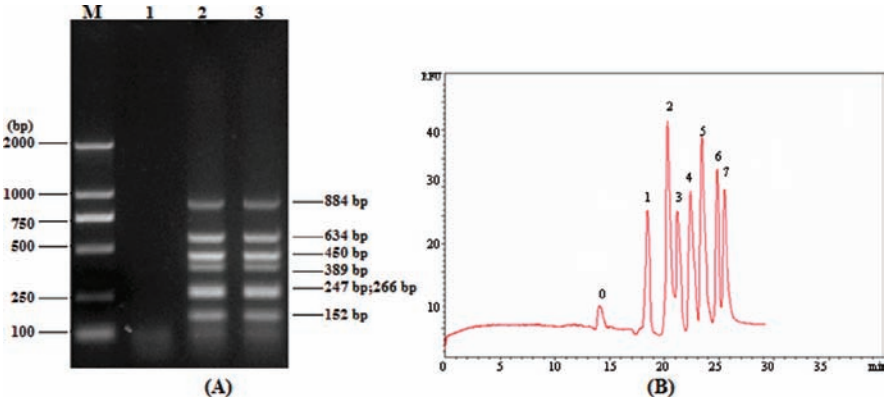

Figure 3. Detection of GM maize by UP-M-PCR. (A) M-PCR products analyzed by agarose gel electrophoresis. Lane M, DNA marker DL 2000; lane 1, no template control; and lanes 2 and 3, M-PCR products. (B) M-PCR products analyzed by CE−LIF: 0, dimer; 1−7, PCR products of compound-specific primers for GA21, Bt11, endogenous *Ivr* gene, NK603, Bt176, Mir604, and Mon810. The length of the fragments were 152, 247, 266, 389, 450, 634, and 884 bp.

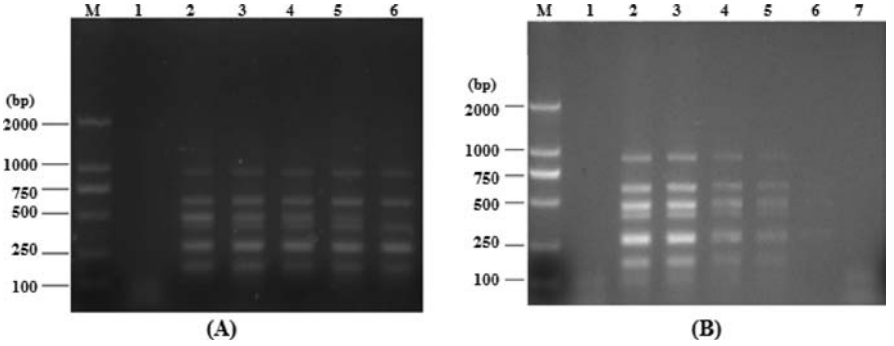

Figure 4. Sensitivity detection of UP-M-PCR by agarose gel electrophoresis. (A) Sensitivity of Bt176 alone. Lane M, DNA marker DL 2000; lane 1, no template control; and lanes 2−6, M-PCR products with Bt176 template of 50, 10, 2, 0.5, and 0.1 ng. The other five kinds of GM maize in the mixed sample stayed at 50 ng. (B) Sensitivity of the template mixture. Lane M, DNA marker DL 2000; lane 1, no template control; and lanes 2−7, M-PCR products with a mix template of 50, 10, 2, 0.5, 0.1, and 0.02 ng.

40 cycles consisting of denaturation at 95 °C for 0.5 min, annealing at 60 °C for 0.5 min, and extension at 72 °C for 0.5 min, followed by a final extension at 72 °C for 7.0 min. Figure 3A shows the amplification results by UP-M-PCR on 2.0% agarose gel. All of the reactions were performed with the same amount of template (50 ng). Each compound-specific primer pair in the mixture was sensitive and specific enough to amplify the corresponding sequence and generated the expected length of amplicons, the same as in the single PCR, and no unexpected PCR product was detected. There was less or even no disparity between various primers as in UP-M-PCR. Similar duplex, triplex, and four-plex PCR results were achieved with an arbitrary combination of compound-specific primer pairs (data not shown). However, because of the resolution limit of the agarose gel electrophoresis, the amplicons of 266 and 247 bp targeted for the *Ivr* gene and Bt11 event-specific sequence, respectively, could not be separated effectively; thus, CE−LIF with a higher resolution were used in the subsequent analysis.

In the optimized UP-M-PCR system, the UP has a concentration of 400 nmol/L just as the primer concentration in the normal PCR, while the concentration of each compound-specific primer was low from 10 to 40 nmol/L; therefore, the total amount of all of the primers was almost equal to that of conventional single PCR, in which all of the primers are mixed with a normal concentration of about 400 nmol/L. Therefore, it really simplified the M-PCR system, which was also the reason why it could avoid the amplification disparity resulting from different primers in traditional M-PCR.

Sensitivity Test of M-PCR by Agarose Gel Electrophoresis. Sensitivity assays were carried out to determine the limit of detection of each GM maize event in the mixed sample and all six kinds of GM maize template mixtures in M-PCR by agarose gel electrophoresis. For example, DNA samples containing five different levels of Bt176 ranging from 50 to 0.1 ng with a 5-fold gradient dilution were prepared, while the content of the other five kinds of GM maize in the mixed sample stayed at 50 ng. The target-specific amplicons of GM maize Bt176 could be detected from 50 to 0.5 ng (lanes 2−5 of Figure 4A) in the optimized M-PCR system by agarose gel electrophoresis, which suggested that the detection limit of GM maize Bt176 in this method is 0.5 ng.

Similar to this, six levels of simulated GM maize template mixture ranging from 50 to 0.02 ng with a 5-fold gradient dilution were prepared. When the GM maize template mixture was from 50 to 0.5 ng (lanes 2−5 of Figure 4B) in the optimized M-PCR system, all target-specific amplicons could be detected, while almost nothing could be seen when the GM maize template mixture was decreased to 0.1 and 0.02 ng (lanes 6 and 7 of Figure 4B). All target-specific amplicons could be simultaneously detected from GM maize samples when the concentration of the GM maize template mixture was 0.5 ng by agarose gel electrophoresis,

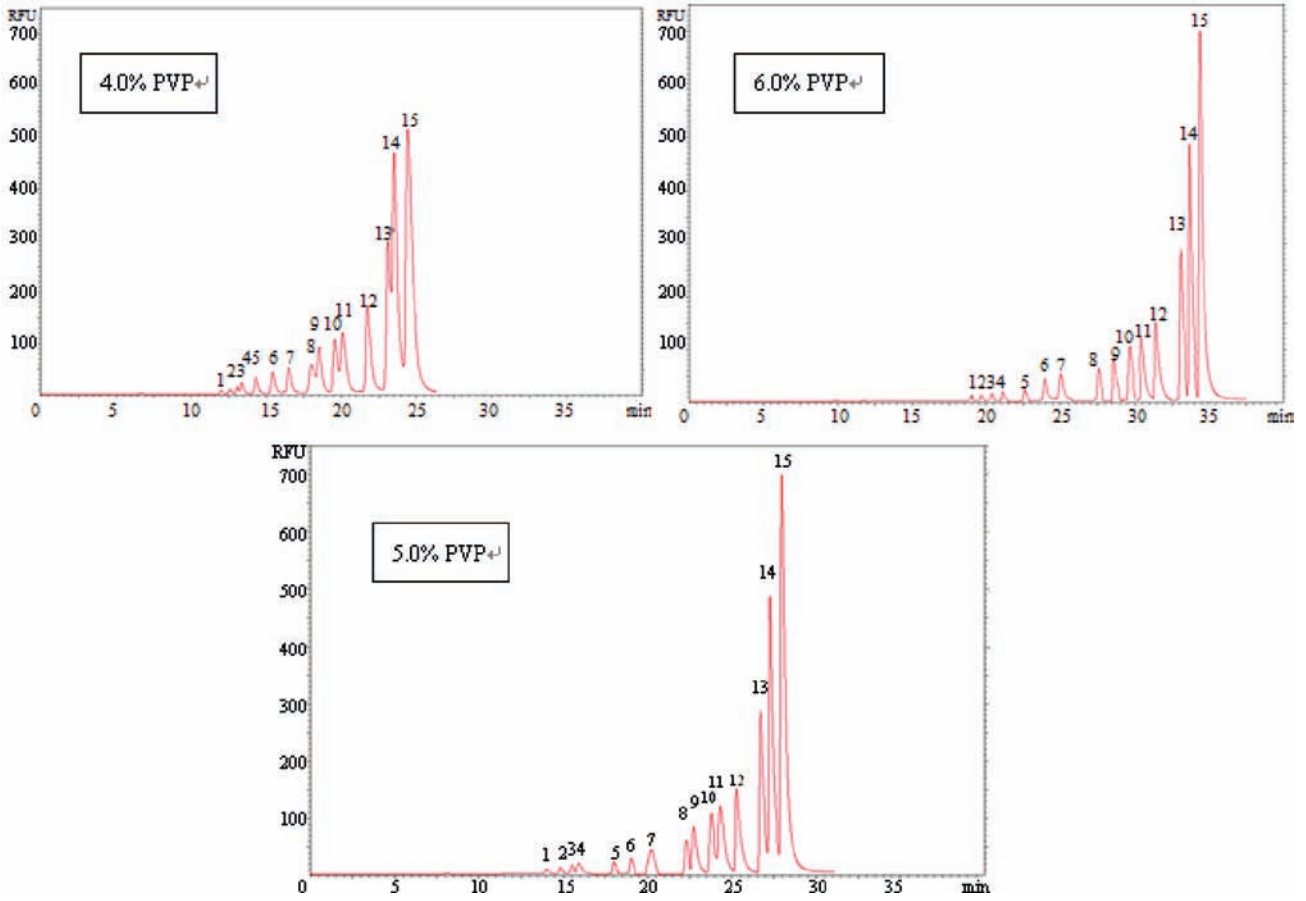

Figure 5. Optimization of the PVP concentration (4.0−6.0%) in CE−LIF. The pGEM DNA markers (Promega) were used to optimize. The concentration of PVP used in CE−LIF was marked on each panel: 1, 36 bp; 2, 51 bp; 3, 65 bp; 4, 75 bp; 5, 126 bp; 6, 179 bp; 7, 222 bp; 8, 350 bp; 9, 396 bp; 10, 460 bp; 11, 517 bp; 12, 676 bp; 13, 1198 bp; 14, 1605 bp; and 15, 2645 bp.

which suggested that the detection limit of the GM maize template mixture in this method is sensitive to 0.5 ng by agarose gel electrophoresis.

Optimization of CE−LIF. To achieve well-resolved and reproducible analysis results, separation of DNA fragments in polymer solutions is usually performed using capillaries with zero electro-osmotic flow (EOF). The inner wall of the capillary is normally covalently coated with a neutral polymer, such as polyacrylamide or polyvinyl alcohol (PVA), to suppress EOF and to reduce or eliminate a DNA−wall interaction that leads to irreproducibility and poor resolution.[11] Moreover, it is impossible to regenerate coatings when resolution degrades. In previous studies, different strategies of dynamic coating have been proposed to suppress the EOF of fused silica capillaries, making them suitable for DNA separations. Some water-soluble linear polymers, including polydimethylacrylamide (PDMA), hydroxypropyl-methyl cellulose (HPMC), polyethylene oxide (PEO), PVP, and poly(*N*-isopropylacrylamide) (PNIPAM), and some novel co-polymers have been investigated to have dynamic coating ability and have been applied as sieving media for electrophoretic separation of DNA fragments in bare capillaries.[12]

The separation conditions of CE−LIF were optimized using pGEM DNA markers. The DNA sample was injected at the cathodic end using an electrokinetic mode of −5.0 kV for 10 s. The separation voltage is optimized at −10 kV. The electropherograms of the DNA marker at different concentrations of PVP from 4.0−6.0% are shown in Figure 5. It was shown clearly that, when the concentration of PVP is 4.0%, the DNA fragments with the size of 350 and 396 bp (marked by 8 and 9, respectively, in Figure 5) cannot be completely separated and, when the concentration of PVP is 6.0%, the time for total separation of the DNA maker is more than 35 min, which needs more time than the ordinary agarose gel separation system. Considering the factors of the separation effect and migration time, 5.0% PVP was chosen as the sieving medium in this study, with which all fragments in the pGEM DNA marker can be separated effectively in 28.5 min.

Specificity and Sensitivity Tests of M-PCR by CE−LIF. Because agarose gel electrophoresis has its limitation in the detection of DNA fragments, CE−LIF was used in this study to detect the products of UP-M-PCR. Optimized conditions were used to separate UP-M-PCR products. Eight peaks could be detected by CE−LIF in 27 min (Figure 3B), and the migration time of the first peak was 14.5 min. In comparison to the results of pGEM DNA markers in the middle of Figure 5, it was considered as a primer dimer in the PCR system because it was less than 51 bp. The other seven peaks were target products after comparing to the migration time of pGEM DNA markers; therefore, these six kinds of GM maize could be simultaneously detected by UP-M-PCR with CE−LIF in this study.

Sensitivity assays were carried out again to determine the limit of detection of both GM maize Bt176 in the GM maize mixture

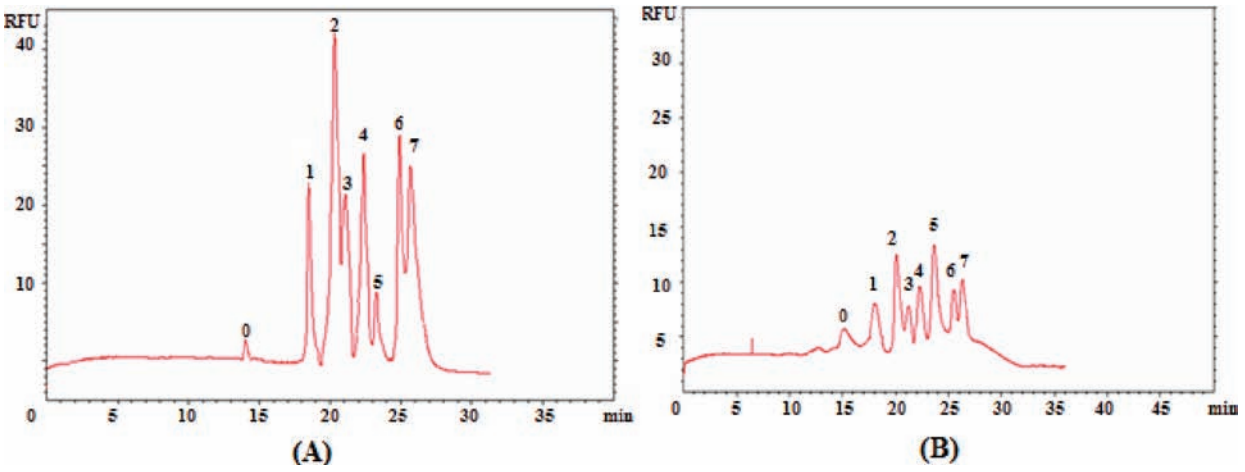

Figure 6. Sensitivity detection of UP-M-PCR by CE−LIF: 0, dimer; 1−7, PCR products of compound-specific primers for GA21, Bt11, endogenous *Ivr* gene, NK603, Bt176, Mir604, and Mon810. The length of the fragments were 152, 247, 266, 389, 450, 634, and 884 bp. (A) Detection of the template mixture consisting of 0.1 ng of Bt176 and 50 ng of other GM maize lines. (B) Detection of the template mixture consisting of six GM maize lines at 0.1 ng.

and six kinds of GM maize template mixtures in M-PCR by CE−LIF. First, CE−LIF was used to detect DNA fragments when the content of Bt176 in the mixed sample was 0.1 ng. Eight peaks could be detected by CE−LIF in 27 min, and the migration time of the first peak was 14 min (Figure 6A). In comparison to the result of pGEM DNA markers in the middle of Figure 5, it was considered as a primer dimer in the PCR system because it was less than 51 bp. The target-specific amplicons of GM maize Bt176 could be successfully detected as the fifth peak, which suggested that the detection limit of GM maize Bt176 in this method is 0.1 ng by CE−LIF.

Similar to this, CE−LIF was used again to detect DNA fragments when the content of the six GM maize template mixture was 0.1 ng. Eight peaks could be detected by CE−LIF in 27 min, and the migration time of the first peak was nearly 15 min (Figure 6B). In comparison to the result of pGEM DNA markers in the middle of Figure 5, it was considered as a primer dimer in the PCR system because it was less than 75 bp. All of the target-specific amplicons could be successfully detected by CE−LIF, which suggested that the detection limit of the GM maize template mixture in this method is 0.1 ng by CE−LIF. In comparison to the published conventional M-PCR system detecting eight GM maize lines at the same time,[4] the limit of detection of which is 0.25% GM in the total 100 ng template, equaling 0.25 ng of GM content in the mixed samples, the novel PCR system has a much higher sensitivity.

An available method for DNA separation that uses uncoated capillaries together with LIF detection was developed.[12] This technique involves a high degree of automation,[19] uses minimum quantities of sample and reagents,[20] is able to produce reproducible separations of PCR products with great efficiency,[21] and has proven to be a good alternative to obtain accurate, precise, and adequate sensitive results in the detection of PCR-amplified DNA fragments from GM maize.[22] In comparison to agarose gel electrophoresis, CE−LIF in this study is efficient, rapid, sensitive, and low-cost.[23] This established that an event-specific detection method could be widely applied in the qualitative identification of the GM maize lines. Because the UP-M-PCR system has the advantage of overcoming the amplification disparity resulting from different primers in conventional M-PCR and the CE−LIF used as the analysis method for PCR products exhibits extraordinary resolution of separating the DNA pieces differing by even a single base pair, they could not restrict the increase of the detected targets. With the increase of GM maize events every year, UP-M-PCR combined with CE−LIF analysis can totally meet the demand of detecting a lot more GM events at the same time in the mixed samples and has a broader application prospect.

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