Food Sci. Biotechnol. Vol. 18, No. 4, pp. 823 ~ 832 (2009)

RESEARCH REVIEW

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Applications of Capillary Electrophoresis and Microchip Capillary Electrophoresis for Detection of Genetically Modified Organisms

Longhua Guo, Bin Qiu, Xueyang Xiao, and Guonan Chen*

Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University), Department of Chemistry, Fuzhou University, Fuzhou 350002, PR China

Abstract In recent years, special concerns have been raised about the safety assessment of foods and food ingredients derived from genetically modified organisms (GMOs). A growing number of countries establish regulations and laws for GMOs in order to allow consumers an informed choice. In this case, a lot of methods have been developed for the detection of GMOs. However, the reproducibility among methods and laboratories is still a problem. Consequently, it is still in great demand for more effective methods. In comparison with the gel electrophoresis, the capillary electrophoresis (CE) technology has some unique advantages, such as high resolution efficiency and less time consumption. Therefore, some CE-based methods have been developed for the detection of GMOs in recent years. All kinds of CE detection methods, such as ultraviolet (UV), laser induced fluorescence (LIF), and chemiluminescence (CL) detection, have been used for GMOs detection. Microchip capillary electrophoresis (MCE) methods have also been used for GMOs detection and they have shown some unique advantages.

Keywords: genetically modified organism, capillary electrophoresis, microchip capillary electrophoresis, exogenous DNA, novel protein, food safety

Introduction

Genetically modified food (GMF), also called transgenic food, is most commonly used to refer to food or food additives created for human or animal consumption, which are made by GMOs. In the latest decade, with the rapid development of molecular technology and biotechnology, growing countries in the world are devoting to the research of GM techniques. The production of GMOs has now formed a considerable industry around the world. In comparison with the traditional food, GMF has some unique advantages, such as enhancing nutrition, prolonging shelf life, reducing cost and improving yield. The production of GMF brings great profits to the producers. However, public concerns for the use of genetic engineering in the production of foods have been growing during the past few years. Most concerns about GMF fall into two categories: environmental hazards and human health risks (1). Environmental activists, religious organizations, public interest groups, professional associations and other scientists, and government officials have all raised concerns about GMF, and the debate on its benefits and risks are going on. In this context, most governments have dictated regulations on the use, spreading and marketing of GMOs. A number of countries have adopted labeling approaches for GMOs or the products derived thereof.

The need to monitor and verify the presence and the amount of GMOs in food has generated a demand for analytical methods capable of detecting, identifying, and quantifying GMOs. Generally, the detection of GMOs can be accomplished by detecting either the exogenous DNA or the novel proteins (2-5). Proteins specific for a given GMOs are usually detected by enzyme-linked immunosorbent assay (ELISA), Western blot, lateral flow, and test paper (6-9). Most DNA-based detection methods for GMOs rely on the use of polymerase chain reaction (PCR). All kinds of PCR-based methods include qualitative PCR, half-quantitative PCR, quantitative-competitive PCR, nest and half-nest PCR, real-time quantitative PCR, and multiplex PCR are developed for GMOs detection (10-14). However, the reproducibility between different methods and laboratories is still a problem. New and more effective methods are still in great demand.

In recent years, capillary electrophoresis (CE)-based methods have frequently been reported for GMOs detection. In comparison with the agarose gel electrophoresis (AGE), CE has some unique advantages, such as high resolution efficiency, easy for automation, and less time consumption. Therefore, the current trend is towards the replacement of AGE by capillary electrophoresis (15). Till present, some CE-based methods have successfully used for GMOs detection. This review focuses on CE-based methods for the detection of GMOs which are reported since the year of 2000.

CE-based Methods for the Detection of Exogenous DNA in GMOs

Although detection of GMOs may be accomplished by detecting either the exogenous DNA or the novel proteins, the detection of specific DNA sequences by PCR is the most widely used technique because of its high stability and traceability of DNA in both raw material and processed food matrices. The PCR amplicons are usually identified by AGE and ethidium bromide (EtBr) staining.

*Corresponding author: Tel: +86-591-87893315; Fax: +86-591-83713866
E-mail: gnchen@fzu.edu.cn
Received December 1, 2008; March 26, 2009;
Accepted March 27, 2009

Table 1. Diverse CE-based methods for the detection of exogenous DNA of GMOs

GMOs	PCR method	No. amplicons	CE method	Qualitative or quantitative	LOD[1)]	Reference
Bt-176 maize	Multiplex PCR	2	CGE-LIF and CGE-UV	Qualitative	1% for UV and not mentioned for LIF	(16,17)
Bt-176 maize	Multiplex PCR	2	CGE-LIF	Qualitative	0.01%	(18)
Bt-176 maize	QC-PCR	4	CGE-LIF	Quantitative	0.04 ng Bt-176 DNA	(19)
Bt11, T25, MON810, GA21, Bt176 maize	Hexaplex PCR	6	CGE-LIF	Qualitative	0.054% of Bt11, 0.057% of T25, 0.036% of MON810, 0.064% of GA21, and 0.018% of Bt176	(20)
Bt11 maize, Mon810 maize Roundup Ready soy	Multiplex PCR	5	CGE-LIF	Qualitative	0.9% (S/N=542)	(21)
Roundup Ready soy	Duplex PCR	3	CGE-LIF	Qualitative	Not mentioned	(22)
8 Maizes (TC1507, MON863, MON810, T25, NK603, GA21, BT11, BT176)	2 Stages PCR	9	Capillary array sequencer	Quantitative	0.10%	(23)
MON810 maize, Roundup Ready soya	MLPA	4	Capillary sequencer	Quantitative	0.10%	(25)
Bt11, GA21, MON810, NK603 maize	Multiplex PCR	5	CGE-SC	Qualitative	0.10%	(26)
Transgenic oilseed rape	Normal PCR	1	CE-UV	Qualitative	Not mentioned	(27)
Roundup ready maize, Roundup ready soy	QC-PCR	4	CE-UV	Quantitative	<0.5%	(28)
Roundup ready soy	Multiplex PCR	5	CE-CL	Qualitative and Quantitative	0.10%	(29)

[1)]Limit of detection.

In recent years, diverse capillary electrophoresis approaches have also shown applicability in the analysis of GMOs. Table 1 listed the diverse CE-based methods for the detection of exogenous DNA of GMOs.

Applications of CE-laser induced fluorescence (LIF)

CE with LIF detection is ultrasensitive, and it is one of the earliest CE-based methods used for GMOs detection. Cifuentes and coworkers have done a lot of works in this area. In 2002, they used capillary gel electrophoresis with LIF detection (CGE-LIF) for the detection of transgenic maize in flours (16,17). LIF EnhanCE (Beckman Instruments, Fullerton, CA, USA) was added as an intercalating dye to the CE running buffers. In a following work (18), 4 different fluorescent intercalating dyes, YOPRO-1, SYBR-Green-I (Molecular Probes, Leiden, Holland), EtBr (Sigma-Aldrich, Madrid, Spain) and EnhanCE (Beckman Instruments) are used for CGE-LIF detection of DNA from transgenic maize in flours. Results showed that the limits of detection (LODs) for the detection of 200-bp DNA fragment are 700, 1,000, 11,300 and 97,400 zmol for YOPRO-1, SYBR-Green-I, EtBr, and EnhanCE, respectively. SYBR-Green-I and YOPRO-1 provide better LODs than EnhanCE or EtBr. Further investigation revealed that separations using YOPRO-1 were faster than those using SYBR-Green-I. Also, separations using YOPRO-1 were more efficient than those using SYBR-Green-I. Therefore, YOPRO-1 was selected as the preferred intercalating dye for the detection of transgenic maize in flours. The corresponding LOD was 0.01%. In 2004, they reported a quantitative approach for the detection of transgenic Bt Event-176 maize using double QC-PCR and CGE-LIF (19). Recombinant DNA techniques were used for construction of 2 internal standards (*cryIA(b)* system and *dull1* system) for QC-PCR. Figure 1 shows the scheme for the construction of the internal standard for the *cryIA*(b) QC-PCR system. The QC-PCR products were quantitatively analyzed using homemade CGE-LIF equipment. This approach allowed the use of internal standards differing by only 10 bp from the original target fragments because of the high resolution of CE. Reproducibility of analysis times and corrected peak areas for the CGE-LIF separations were determined to be better than 0.91 and 1.93% (RSD, n=15) respectively, for 3 different days. In comparison with the AGE method, CGE-LIF provides better resolution and a signal/noise ratio improvement of ca. 700 folds. The proposed method was used to determine the Bt Event-176 maize content in certified reference maize powder and food samples of known composition and the results showed good consistent. In the same year, they also reported the other CE-LIF method for the detection of 5 transgenic maizes (Bt11, T25, MON810, GA21, and Bt176) (20). Five mentioned transgenic amplicons plus the zein gene used as reference were amplified using a hexaplex PCR protocol, followed by a CGE-LIF method to analyze the 6 DNA fragments. CGE-LIF was demonstrated very useful and informative for optimizing multiplex PCR parameters. The method developed allowed the simultaneous detection in a single run of percentages of transgenic maize as low as 0.054% of Bt11, 0.057% of T25, 0.036% of MON810, 0.064% of GA21, and 0.018% of Bt176 in flour. The corresponding RSD of migration times and peak areas were lower than 0.67 and 6.80%, respectively, for the same sample and 3 different days (n=12). In 2007, the group

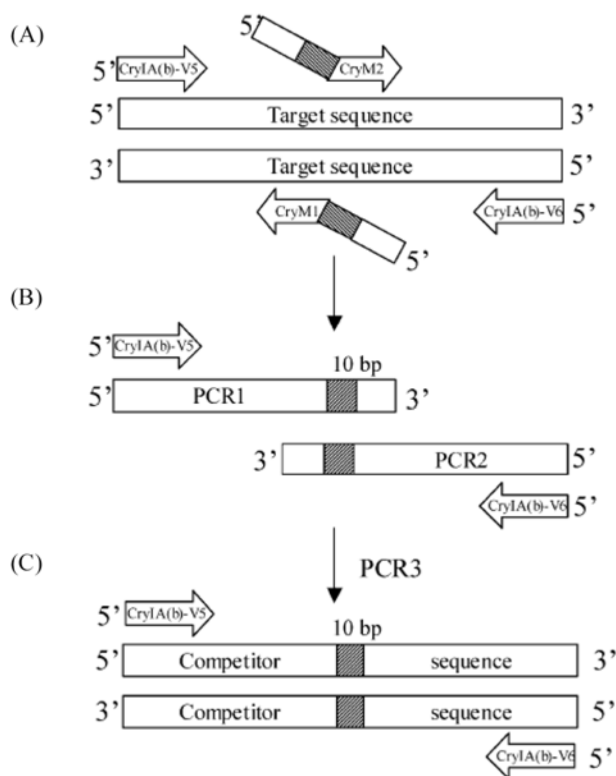

Fig. 1. Scheme for the construction of the internal standard for the cryIA(b) QC-PCR system. (Reprinted with permission from ref. 19)

reported another approach for simultaneous detection transgenic soy and maize using bare fused-silica capillary with LIF detection (21). In this work, they used trisphosphate-ethylenediamine tetraacetic acid (EDTA) and 4.5% of 2-hydroxyethyl cellulose (HEC) as sieving polymer. The separation of 50 to 750 bp DNA fragments was accomplished in less than 30 min using the proposed approach. RSD of migration times for the analyzed DNA fragments were better than 1.0% (n=4) for the same day, 2.2% (n=16) for 4 different days, and 2.3% (n=16) for 4 different capillaries. The proposed approach was then used to analyze of 0.9% of Bt11 maize, Mon810 maize, and Roundup Ready soy in flours and the corresponding signal to noise ratio was up to 542.

Zhou *et al.* (22) also did some useful work for GMOs detection using a duplex PCR-CE system with LIF detection. A duplex PCR was performed with primers designed to simultaneously amplify 3 hetero-geneous genes in transgenic soybean: CaMV-35S promoter, NOS terminator, and CP4-EPSPS gene. The PCR products were sequenced with a DNA sequencer and results showed that the measured sequences were identical with that of the original genes. Homemade CE-LIF system was applied to the rapid analysis of the above PCR products by using a capillary coated with linear polyacrylamide. HPMC-4000 (8 g/L) was used as sieving buffer. Results showed that their method was able to simultaneously detect the 3 heterogeneous genes existing in genetically modified soybean under the optimum conditions of PCR and CE. The corresponding RSD of the migration times for the detection of PCR products were ≤3.2%.

Applications of capillary array sequencer Heide *et al.* (23) developed a method for quantitative detection of 8 maizes (TC1507, MON863, MON810, T25, NK603, GA21, BT11, and BT176) using 9-plex PCR followed by capillary array sequencer detection. It was interesting that PCR was carried out in 2 stages in their approach. In the first stage, bipartite primers containing a universal 5'-sequence and a GMO specific 3'-sequence were used and only 6 PCR cycles were performed. In the second PCR stage, only a universal primer was used and 40 PCR cycles were performed. This created a competitive PCR where all fragments were amplified with the same efficiency, and thus the initial ratios of amplicons were conserved throughout the reaction. Trypsin was used to degrade the excessive primers between the 2 stages and results showed that the trypsin digestion enhanced signal strength and reproducibility. After PCR, the amplicons were then hybridized to fluorescence-labeled specific probes and analyzed with the ABI GA3100 capillary array sequencer (Applied Biosystems). This approach was proven to be robust and quantitative. It was suitable for quantification in the 0-2% range with a detection limit of approximately 0.1% of GMOs. Good agreement was observed in 76 of 80 determinations when 10 food and feed samples were analyzed using the proposed assay and compared to the results from quantitative real-time 5'-nuclease PCR.

More recently, multiplex ligation-dependent probe amplification (MLPA) coupled with capillary array sequencer detection was used for event specific detection and relative quantification of GMOs. MLPA was first reported by Schouten *et al*. (24) in 2002. It is a new, high resolution and simple method for relative quantification of up to 40 nucleic acid sequences in a single reaction. Figure 2 shows the detail principle of MLPA technique. It can be seen from Fig. 2 that in MLPA, not sample nucleic acids but probes added to the samples are amplified and quantified. The other unique character of this technique is all ligated probes have identical end sequences, permitting simultaneous PCR amplification using only 1 primer pair, which is helpful to obtain identical PCR efficiency, and thus to conserve the initial ratios of amplicons throughout the reaction. MLPA technique has been widely applied to the relative quantification of DNA in the field of medical diagnostics soon after its birth. In 2006, Moreano *et al.* (25) used this technique for GMOs detection for the first time. Two GMOs, transgenic maize line MON810 and Roundup Ready soya (RRS) were detected. Respective probes were designed to allow the detection of reference genes in the genomes from maize and soya, as well as of event-specific junction regions in the transgenic maize line MON810 and in RRS. The amplicons were analyzed by a capillary sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems). Sensitivity testing was carried out to confirm that the detection of traces of a GMO-specific target was not restrained by the presence of high levels of other target sequences. Mixtures containing 75 ng DNA from maize or soya standards (0.1% maize MON810 and 5% RRS, and *vice versa*) were tested positively in the presence of both GMOs target sequences.

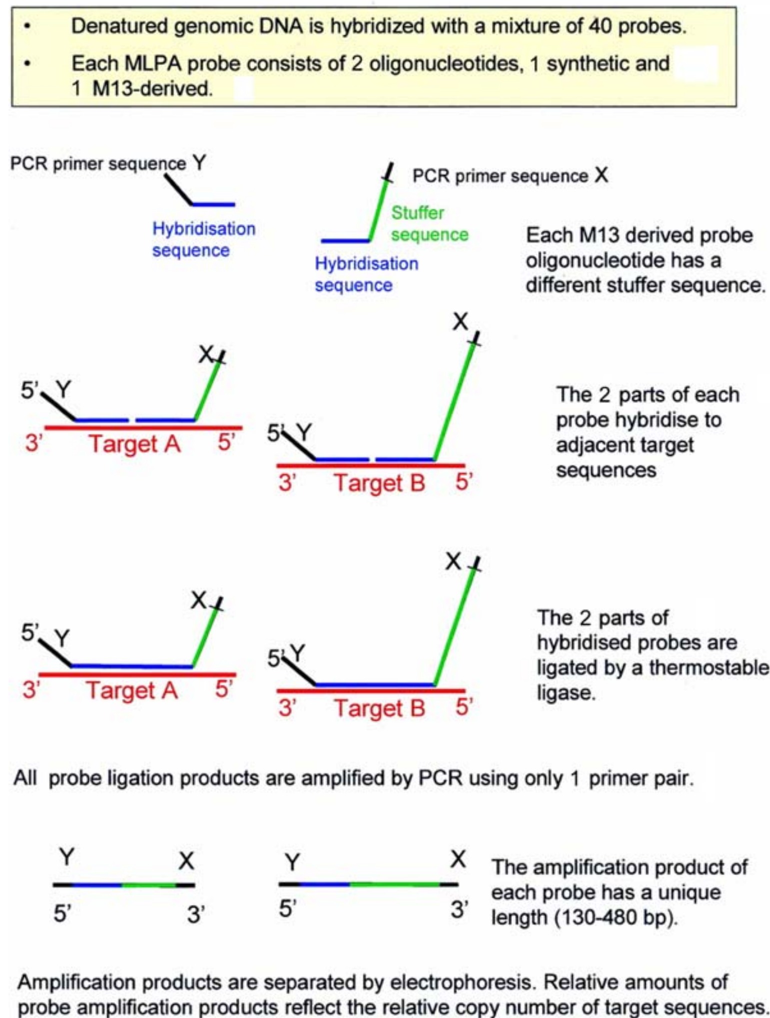

Fig. 2. Schematic representation of MLPA technique. (Reprinted with permission from ref. 24)

Applications of CGE coupled with size and color detection (CGE-SC) Nadal *et al.* (26) reported a novel CGE approach that combined the identification of multiplex PCR amplicons by size and color, and the novel approach was successfully applied for simultaneous detection of multiple transgenic events in maize. Five pairs of PCR primers specific to events Bt11, GA21, MON810, NK603, and *Zea mays* L. (alcohol dehydrogenase) were used for the initial multiplex PCR amplification. The amplicons of the 5 targets were short and similarly sized. In order to achieve a clear distinction, the 5 targets were specifically labeled with 3 different fluorescent dyes and the targets were identified by size and color (SC). Figure 3 is a typical electrophoretogram for GMOs detection with the CGE-SC approach. It can be seen from this figure that 2 couples of the amplicons were similarly sized (the first couple: BT11, 70 bp and GA21, 72 bp; the second couple, MON810, 106 bp and NK603, 108 bp). The separation of these similarly sized amplicons were a big technical challenge for traditional CE approaches. However, they were clearly distinguished with the proposed CGE-SC approach.

Applications of CE-UV CE-UV detection is one of the most developed and widely used CE methods. It was also used for GMOs detection. Besides the applications of CE-LIF for GMOs detection, Cifuentes and coworkers have also done some valuable works in this area (16,17). Using 4.5% HEC polymer as separation buffer and 254 nm as the UV detection wavelength, they successfully detected 100 bp DNA ladder and transgenic maize. RSD of migration times of the proposed method were lower than 0.92% for the same capillary in the same day, 1.46% for 4 days for the same capillary and 1.3% for 3 different capillaries. Under the optimal conditions, the corresponding LOD was 1% for transgenic maize (S/N=2.6). They also compared the CE-UV method with CE-LIF, and the results are shown in Table 2. It can be seen from this table that the sensitivity of CE-LIF was 1,000 times as high as the CE-UV method. However, the corresponding detection cost of CE-LIF was also much higher than CE-UV.

Chen *et al.* (27) reported a CE-UV method for the detection of GMOs with an enhanced sensitivity. The DNA fragments were pre-concentrated with long electrokinetic injecting time (99 sec) at a relative low voltage (−10 kV),

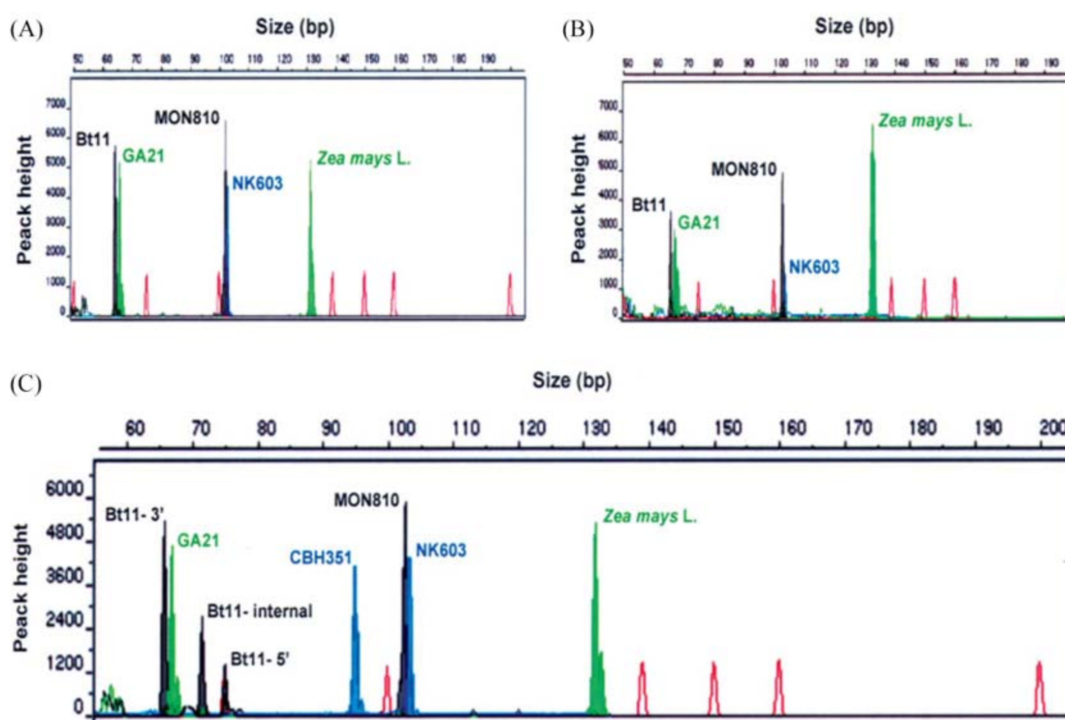

Fig. 3. Pentaplex PCR-CGE-SC assay for detection of 4 events of GM maize: Bt11, GA21, Mon810, and NK603; and maize endogenous control (*Adh1*). Analysis of maize genomic DNA corresponding to a simulated GM mixture containing 0.9% Bt11, 0.9% GA21, 0.9% Mon810, and 0.9% NK603. (A) 10 ng of maize genomic DNA. (B) 5 ng of maize genomic DNA (i.e., around 20 copies of each target). (C) CGE-SC analysis of 8 different amplicons corresponding to the following events of GM maize: Bt11 (3'), GA21, Bt11 (internal), Bt11 (5'), CBH351, Mon810, and NK603; and maize endogenous control. In red, molecular weight markers are shown. Black, HEX; green, TET; blue, FAM. (Reprinted with permission from ref. 26)

Table 2. Comparison of CGE-UV and CGE-LIF for the analysis of DNA fragments

	CGE-UV	CGE-LIF
Cost of equipment (US $)	ca. 30,000	ca. 50,000
Cost per run (US $)	0.003	0.1
Buffer durability (no. of injections)	7	4
Signal/noise ratio	11	14,000

Reprinted with permission from ref 16.

after that, a higher voltage was applied (−15 kV) for the separation of DNA fragments. Results showed that the peak height increased dramatically as a function of injection time, especially for shorter length DNA. The concentration sensitivity of DNA fragments can be improved from 20 to 100 fold relative to a normal injection (5 sec). Similar to Cifuentes' methods, they also used polyvinyl alcohol (PVA, Mw=72,000) as capillary dynamic coating reagent to eliminate the electro-osmotic flow (EOF) and DNA-wall interactions within the capillary. 0.5% poly(ethylene oxide) (PEO, Mw=4,000,000) in Tris-borate-EDTA (TBE) buffer were used as sieving matrix in this method. Under the optimal conditions, the linear coefficient of linear relation between migration time and DNA length was 0.999. The proposed method was successfully used for separation and detection of PCR amplicons from genetically modified oilseed rape.

Dinelli *et al.* (28) established a method for quantitative detection of transgenic soy and maize with QC-PCR coupled with CE-UV detection. A specific competitor DNA for transgenic event was developed for roundup ready maize. A commercial available competitor was used for roundup ready soy. These internal standards were calibrated by coamplifying with mixtures contain roundup ready soybean and maize DNAs. The amplicons were separated and detected by CGE-UV. They also compared the detection of QC-PCR products by slab gel and CGE-UV detection. They found that CGE provided both better precise and sensitivity.

Application of CE-CL Chemiluminescence (CL) is the generation of electromagnetic radiation as light by the release of energy from a chemical reaction. The attractiveness of CL as an analytical tool is the simplicity and high sensitivity of detection. The application of CE-CL for the detection of GMOs was first reported by our group (29). An ultra sensitive CE-CL detection system was developed for the detection of PCR products (Fig. 4). This system was based on the detection the CL of acridinium ester (AE). AE was easily hydrolyzed to give their pseudo-base form at pH>3 and the corresponding pseudo-base form of AE would not produce CL emission. Therefore, an additional acidification procedure had been added at the end of the separation capillary with a double-on-column CE-CL detection system to turn none CL acridinium pseudo-bases into acridinium esters. A reliable procedure based on this

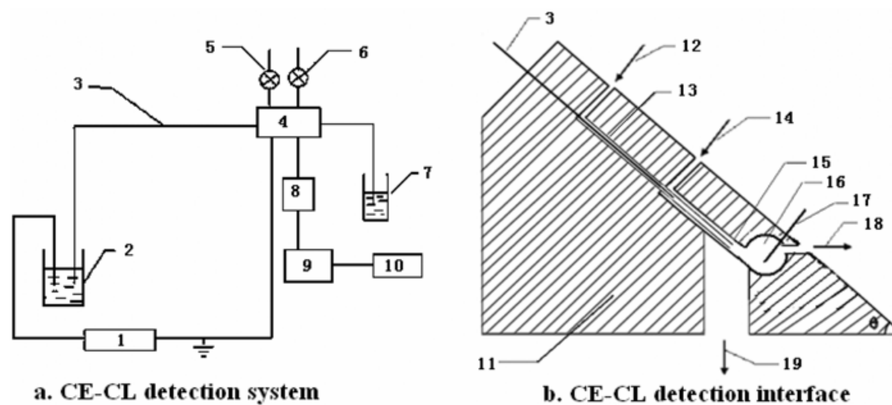

Fig. 4. Schematic image of the proposed CE-CL detection system. 1, high voltage supply; 2, electrophoretic buffer reservoir; 3, separation capillary; 4, CE-CL detection interface; 5, 6, micro-infusion pump; 7, waste reservoir; 8, PMT detector; 9, Ultra Weak CL analyzer; 10, computer; 11, plexiglass profile; 12, acidification solution entrance; 13, acidification capillary; 14, trigger solution entrance; 15, CL reaction capillary; 16, bubble formation pond; 17, grounding electrode; 18, waste exit; 19, CL detection window. (Reprinted with permission from ref. 29)

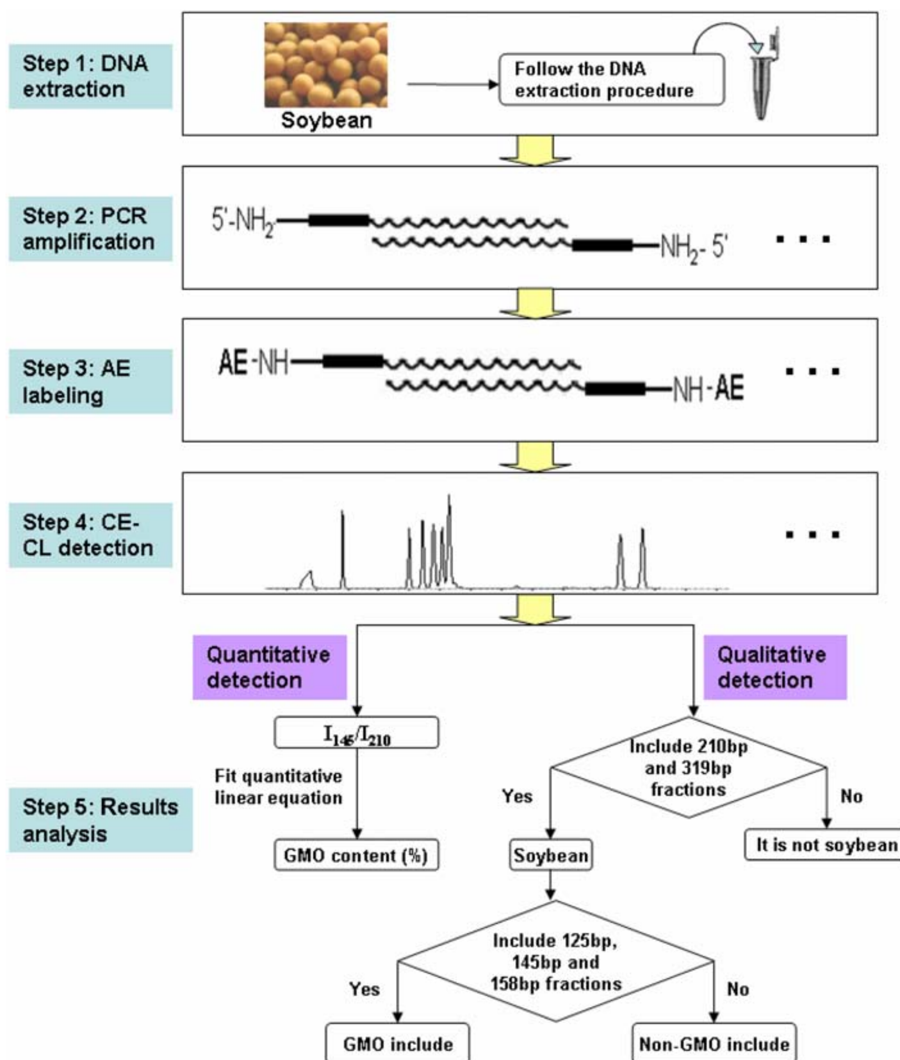

Fig. 5. Schematic diagram of the proposed approach. (Reprinted with permission from ref. 29)

system had been demonstrated for simultaneous qualitative and quantitative analysis of RRS. The schematic diagram of the proposed approach is shown in Fig. 5. The promoter, terminator, function, and 2 reference genes of RRS were amplified with multiplex PCR (MPCR) simultaneously. After that, the PCR amplicons were labeled with acridinium

ester at the 5'-terminal through an amino modification and then analyzed by the proposed CE-CL system. Reproducibility of analysis times and peak heights for the CE-CL analysis were determined to be better than 0.91 and 3.07% (RSD, n=15) respectively, for 3 consecutive days. Results showed that this method could accurately qualitative and quantitative detect RRS standards and the simulative samples with only 16 PCR cycles. The evaluation in terms of quantitative analysis provided by this approach was confirmed by comparing the assay results with those of the standard RT-QPCR using SYBR® Green I dyes. The results showed a good coherence between the 2 methods. The use of a more convenient CE-ECL method for the detection of GMOs is now being carried out at our laboratory.

CE-based Methods for the Detection of Proteins and Metabolites of GMOs

Most CE-based methods for GMOs detection are to detect the exogenous DNA amplified by PCR. More recently, Cifuentes' group has done some works to detect the protein profiles and metabolites of GMOs with CE, and they were trying to distinguish transgenic and nontransgenic species from these detection (16,17).

Detection of protein profile CE-UV was used for detection of protein profiles for both transgenic and nontransgenic soybean (30). Before CE injection, the soy samples were treated as follow: All beans were ground with an automatic miller, then appropriate amount of the ground sample was dissolved in ACN/water (25:75 v/v) containing 0.3%(v/v) acetic acid, the mixtures were manually shaken and centrifuged (3,362×g for 10 min, 25°C). Finally, the supernatant was used for CE injection. Figure 6 shows the electrophoretic profiles of transgenic and nontransgenic soybean under 2 different UV detection wavelengths (254 and 280 nm). From these electrophoretic profiles it can be seen that the peak area of transgenic and nontransgenic soybean were much different at the peak 2, 4, and 5. Therefore, discriminant analysis was setup using these protein peak areas as variable to discriminate transgenic and nontransgenic soybean. Thirty-four new samples analyzed by the CE method were used to prove the validity of the discriminant model. A 93% of the cases were correctly classified. In a following work (31), they demonstrated the other method for protein detection using CE-MS. Four protein fragments, 15-kDa-β-zein, 16-kDa-γ-zein, 19 and 22-kDa-α-zeins were identified, among which 2 of them (19 and 22-kDa-α-zeins) were identified for the first time. Although the proposed procedures were able to differentiate different varieties of maizes, they got a tentative conclusion that no significant differences were observed between the transgenic and its parental non-transgenic line.

Detection of metabolites Capillary electrophoresis-time-of-flight mass spectrometry (CE-TOF-MS) was used to identify and quantify the main metabolites found in both transgenic soybean and its corresponding nontransgenic parental line grown under identical conditions (32). The proposed method included 4 steps: optimization of metabolites extraction, separation by CE, on-line electrospray-

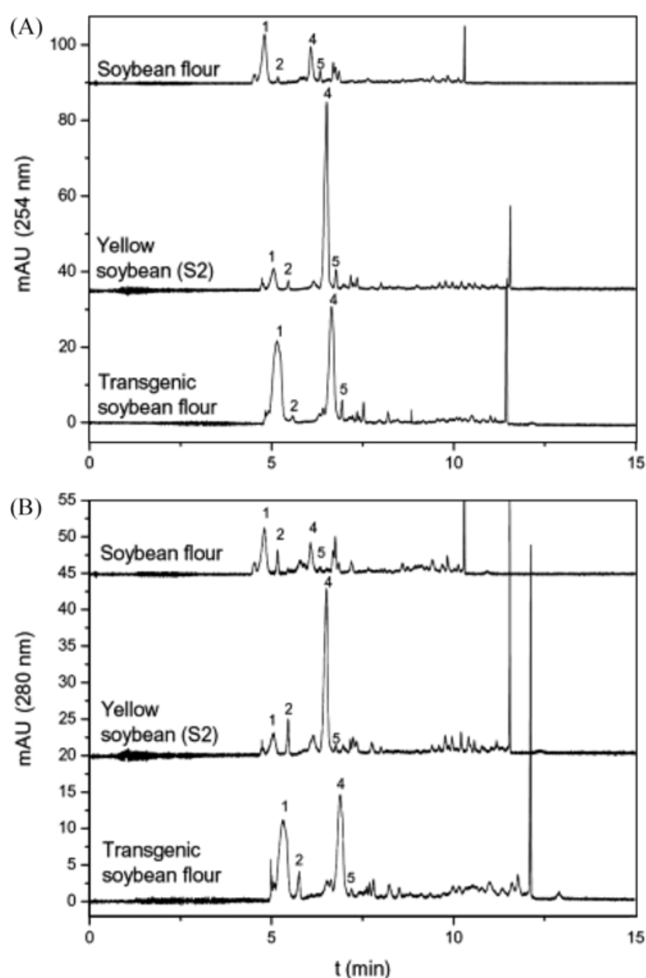

Fig. 6. Electropherograms corresponding to the injection of the protein extracts obtained from commercial soybean flour (ca. 40 mg/mL), yellow soybean (ca. 60 mg/mL), and transgenic soybean flour (ca. 60 mg/mL) using a solution of water/ACN (75:25 v/v) with 0.3%(v/v) acetic acid. (Reprinted with permission from ref. 30)

TOF-MS analysis, and data evaluation. The extraction procedures and the background electrolytes were optimized. The optimal extraction protocol was as follows: soybean seeds were finely grounded at 5°C using a mill and maintained in the fridge at 4°C. One g of the milled samples was mixed with 15 mL of the different solvents and extracted during 30 min in the ultrasonic bath. The extracts were then centrifuged at 5,750×g for 15 min at 5°C. The supernatants were separated in 3 aliquots of 4 mL and evaporated in a concentrator. The residue was finally dissolved in 500 μL of the solvent. Then the above solutions were separated and detected with CE-TOF-MS. Results showed that both transgenic and nontransgenic soy extraction solutions had the same metabolites. However, the concentrations of some metabolites were much different. As it is shown in Fig. 7, the concentration of 4-hydroxylthreonine (the peak which was labeled with '*' in the electropherogram) was high in nontransgenic soybean, however, it almost could not be found in the electropherogram obtained with transgenic soybean.

In a latest work (33), they used the similar approach as

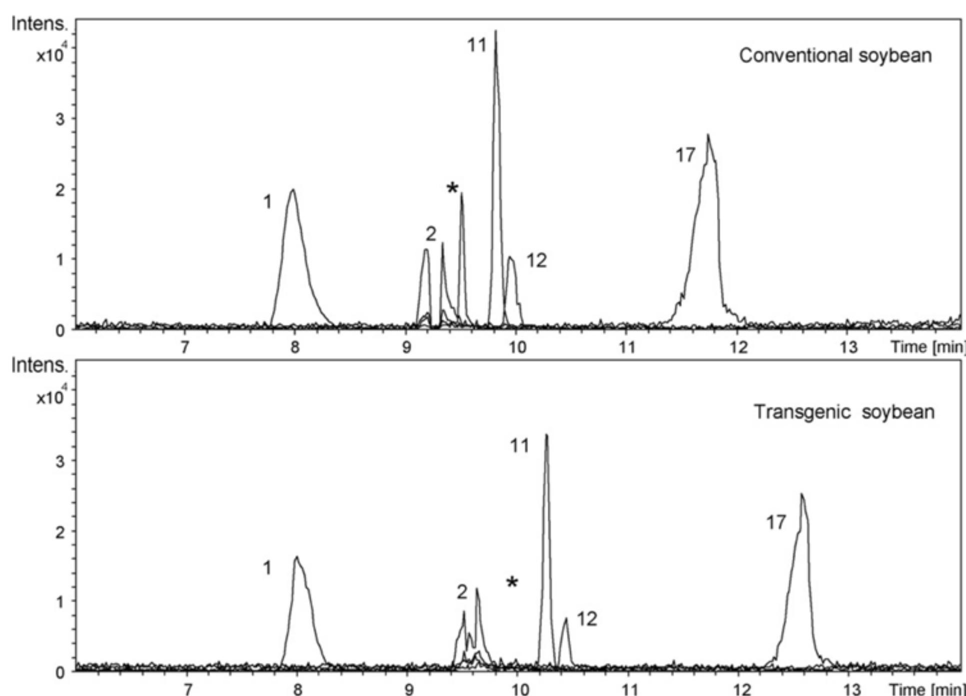

Fig. 7. Comparison of the CE-TOF-MS extracted ions electropherograms of some metabolites found in conventional and transgenic soybean. (*) Compound that changes; 1, 2, 11, 12, 17 unmodified compounds. (Reprinted with permission from ref. 32)

above to identify and quantify the main metabolites in 3 lines of genetically modified (GM) maize and their corresponding nontransgenic parental lines grown under identical conditions. Significant differences were systematically observed between the detected amounts of some metabolites in conventional varieties compared with their corresponding transgenic lines.

Applications of MCE for the Detection of GMOs

Microchip capillary electrophoresis (MCE) is an emerging separation technique that has attracted wide attention and gained considerable popularity. Because of miniaturization of the separation format, MCE typically offers shorter analysis time and lower reagent consumption with potential development of portable analytical instrumentation. Hitherto, MCE technology has been widely used in chemical and biochemical analysis, such as proteins and peptides analysis, DNA separation including fragment sizing, genotyping, mutation detection, and sequencing, and also the analysis of low-molecular-weight compounds, e.g., explosive residues and warfare agents, pharmaceuticals and drugs of abuse, and various small molecules in body fluids. MCE is also one of the earliest CE-based methods used for GMOs detection.

In 2001, Birch *et al.* (34) evaluated the usefulness of a commercial available MCE LabChip instrument (the Bioanalyser 2100; Agilent Technologies) for GMOs analysis in food. Both the lectin and CaMV35S from genetically modified soya were amplified with PCR first. Then they compared a traditional AGE method with the LabChip system to assess their relative performance in the quantitative analysis of these PCR amplicons. Results demonstrated that the LabChip system offered improvements in quantitation accuracy, objectivity and ease of use. In 2003, Burns *et al.* (35) successfully detected transgenic soy using the same instrument and similar method above. They found that the quantification results of the proposed method giving comparable results to a real-time PCR technique. Since MCE presented associated cost saving implications, it seems that MCE was a good alternative for genetic modification detection and quantification to that of real time PCR.

Obeid *et al.* (36) developed an in-house glass CE chip with LIF detection for the rapid DNA-based analysis of GMOs. The schematic diagram of the in-housed developed CE chip and the holding apparatus are shown in Fig. 8B. It consisted of 3 components: CE chip, laser induced fluorescence detection system, and the holding apparatus. The CE chip (Fig. 8A) was composed of 2 glass plates (each 25×76 mm) thermally bonded together to form a closed structure. The widths of the injection and separation channels were 30 and 70 μm, respectively. The glass slide was etched to a depth of 30 μm for both the injection and separation channel. The effective separation length is 4.5 cm. The cost of the chip was less than $ 1 and required 2 days for preparation. The separation and detection of PCR amplified NOS, 35S, and lectin sequences was completed in less than 60 sec. The corresponding LODs were 0.1% GMOs content after 35 and 40 amplification cycles for 35S and NOS, respectively.

Kim *et al*. (37) reported a MCE-based method for ultra-fast analysis of genetically modified organisms in soybeans. 0.5% PVP (Mr 1,000,000) was used as dynamic capillary coating matrix and the sieving matrix was 0.3% PEO (Mr 8,000,000). Under the optimal conditions, 2 PCR amplicons for transgenic soybeans were analyzed within only 11 sec with the microchip (Fig. 9). The main

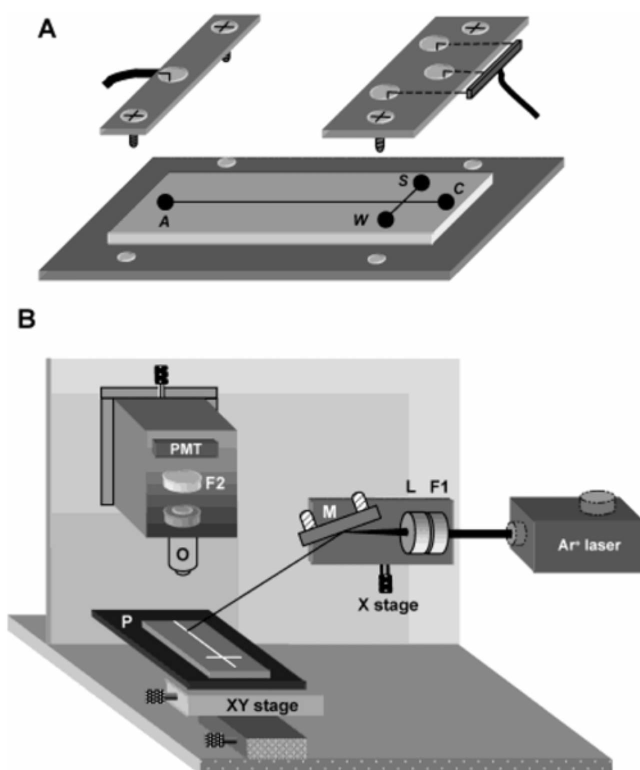

Fig. 8. (A) Schematic diagram of the in-housed developed CE chip and the holding apparatus. S, sample reservoir; W, waste reservoir; C, CE cathode reservoir. A, CE anode reservoir. The holding apparatus is designed to securely hold the chip, to allow for quick electrical connections, and provide leak-proof reservoirs for the application of buffers and samples. The time required to exchange chips is less than 2 min. **(B) Schematic representation of the in-house developed LIF detection system.** The system comprises the following components: Ar^+ laser, translation stages (X and XY); F1, 488 nm band pass excitation filter; L, 10 cm focal length lens; M, adjustable mirror; PMT, R928 Hamamatsu PMT; F2, 520 nm band pass emission filter; O, 106 objective lens; and P, Plexiglas platform to support the CE chip. The detection system allows for rapid mounting of the chip and offers flexibility for any modification. (Reprinted with permission from ref. 36)

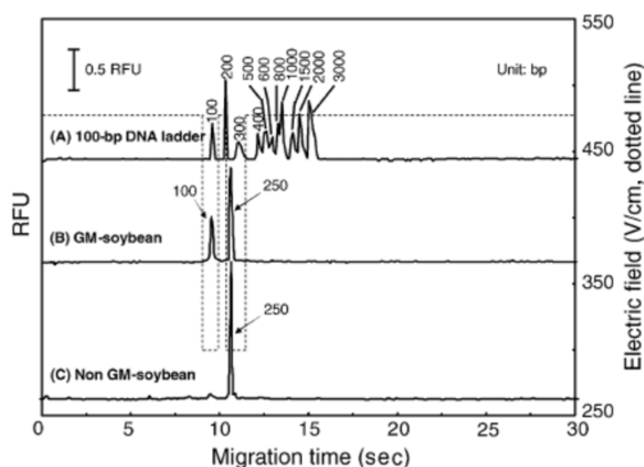

Fig. 9. MCGE separation of PCR products, 100- and 250-bpDNAfragments by programmed field strength gradient. 100-bp DNA ladder; running buffer, 1× TBE buffer (pH 8.3) with 0.5 μg/mL EtBr; coating matrix, 0.5% PVP (Mr 1,000,000); sieving matrix, 0.3% PEO (Mr 8,000,000); injection 60 sec at 0.48 kV; applied separation voltage, 470.6 V/cm for 9 sec, 294.1 V/cm for 1 sec, 470.6 kV for 0.5 sec, 294.1 V/cm for 1.5 sec, and 470.6 V/cm for 20 sec. (Reprinted with permission from ref. 37)

contribution of this method was the use of a programmed field strength gradients (PFSG) during the MCE separation. The PFSG separation was optimized with the separation of 100 to 300 bp DNA fragments. The shortest time was used in terms of all target DNA fragments with resolutions >1.5.

Conclusions and Prospects

CE and MCE have been presented for GMOs detection in recent years, and the applications of these techniques have shown some unique advantages. In comparison with AGE, the CE technology has higher resolution efficiency and lesser time consumption. The current trend is towards the replacement of AGE by CE in routine GMOs analysis. Although MCE is still in the early stages of development, the technique has already shown applicability in GMOs analysis. The possible prospects of these techniques for GMOs detection can be included as follow:

1) Development of sensitive, precise, and accurate quantitative methods

Most of the current CE-based methods focus on screening and identifying of GMOs. However, to satisfying the needs of labeling policy, a quantitative method covering the threshold is necessary. The development and application of high sensitive detector is a significant previous question for quantitative detection of GMOs due to the nanolitre-scale sample injection of CE. Moreover, in order to obtain competitive applications for real samples detection, it is vital to improve the precision and accuracy of the CE-based methods, especially to improve reproducibilities among laboratories.

2) Development of rapid, automated, and high-throughput analytical systems

Currently, hundreds of transgenic species have been approval for commercialization. With the increasing development of genetic technology, more variety of transgenic products would be produced. In order to satisfy the rapid detection of a great deal of samples, it is essential to develop rapid, automated, and high-throughput analytical systems. Miniature and highly integrated CE chip would find to be useful for this purpose.

3) On line coupling of CE to PCR

Current CE-based methods for the detection of exogenous DNA in GMOs are divided into 2 steps: PCR detection and CE separation. To online couple the 2 apparatus, so that to realize real-time and automatic detection of PCR amplicons would be the main problem to be overcome.

4) Construction of CE fingerprints for diverse GMOs

It should be some trace differences between the transgenic and its parental non-transgenic line for its genome, proteome, or metabolites. CE, especially multiplex dimensional CE have extremely high resolution, therefore, it may have the ability to distinguish these trace differences between transgenic and its parental non-transgenic line.

Acknowledgments

This project was supported by the National Nature Sciences Foundation of China (20735002, 20877019), the Key Program of Science and Technology Department of Fujian Province, China (2007Y0026).

References

1. Paparini A, Romano-Spica V. Public health issues related with the consumption of food obtained from genetically modified organisms. Biotechnol. Annu. Rev. 10: 85-122 (2004)
2. Anklam E, Gadani F, Heinze P, Pijnenburg H, Van Den Eede G. Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. Eur. Food. Res. Technol. 214: 3-26 (2002)
3. Deisingh AK, Badrie N. Detection approaches for genetically modified organisms in foods. Food Res. Int. 38: 639-649 (2005)
4. Michelini E, Simoni P, Cevenini L, Mezzanotte L, Roda A. New trends in bioanalytical tools for the detection of genetically modified organisms: An update. Anal. Bioanal. Chem. 392: 355-367 (2008)
5. Ahmed FE. Detection of genetically modified organisms in foods. Trends Biotechnol. 20: 215-223 (2002)
6. Grothaus GD, Bandla M, Currier T, Giroux R, Jenkins GR, Lipp M, Shan G, Stave JW, Pantella V. Immunoassay as an analytical tool in agricultural biotechnology. J. AOAC Int. 89: 913-928 (2006)
7. Brett GM, Chambers SJ, Huang L, Morgan MRA. Design and development of immunoassays for detection of proteins. Food Control 10: 401-406 (1999)
8. Lipton CR, Dautlick JX, Grothaus GD, Hunst PL, Magin KM, Mihaliak CA, Rubio FM, Stave JW. Guidelines for the validation and use of immunoassays for determining of introduced proteins in biotechnology enhanced crops and derived food ingredients. Food Agric. Immunol. 12: 153-164 (2000)
9. Fagan J, Schoel B, Haegert A, Moore J, Beeby J. Performance assessment under field conditions of a rapid immunological test for transgenic soybeans. Int. J. Food Sci. Tech. 36: 357-367 (2001)
10. Liu G, Su W, Xu Q, Long M, Zhou J, Song S. Liquid-phase hybridisation based PCR-ELISA for detection of genetically modified organisms in food. Food Control 15: 303-306 (2004)
11. Hernandez M, Esteve T, Prat S, Pla M. Development of real-time PCR systems based on SYBR Green I, Amplifluore, and Taqman technologies for specific quantitative detection of the transgenic maize event GA 21. J. Cereal Sci. 39: 99-107 (2004)
12. Trifa Y, Zhang D. DNA content in embryo and endosperm of maize kernel (*Zea mays* L): Impact on GMOs quantification. J. Agr. Food Chem. 52: 1044-1048 (2004)
13. Taverniers I, van Bockstaele E, De Loose M. Cloned plasmid DNA fragments as calibrators for controlling GMOs: Different real-time duplex quantitative PCR methods. Anal. Bioanal. Chem. 378: 1198-1207 (2004)
14. Holst-Jensen A, Ronning SB, Lovseth A, Berdal KG. PCR technology for screening and quantification of genetically modified organisms (GMOs). Anal. Bioanal. Chem. 375: 985-993 (2003)
15. Elenis DS, Kalogianni DP, Glynou K, Ioannou PC, Christopoulos TK. Advances in molecular techniques for the detection and quantification of genetically modified organisms. Anal. Bioanal. Chem. 392: 347-354 (2008)
16. García-Cañas V, González R, Cifuentes A. Detection of genetically modified maize by the polymerase chain reaction and capillary gel electrophoresis with UV detection and laser-induced fluorescence. J. Agr. Food Chem. 50: 1016-1021 (2002)
17. García-Cañas V, González R, Cifuentes A. Highly reproducible capillary gel electrophoresis (CGE) of DNA fragments using uncoated columns. Detection of genetically modified maize by PCR-CGE. J. Sep. Sci. 25: 577-583 (2002)
18. García-Cañas V, González R, Cifuentes A. Ultrasensitive detection of genetically modified maize DNA by capillary gel electrophoresis with laser-induced fluorescence using different fluorescent intercalating dyes. J. Agr. Food Chem. 50: 4497-4502 (2002)
19. García-Cañas V, Cifuentes A, González R. Quantitation of transgenic Bt Event-176 maize using double quantitative competitive polymerase chain reaction and capillary gel electrophorsesis laser-induced fluorescence. Anal. Chem. 76: 2306-2313 (2004)
20. García-Cañas V, González R, Cifuentes A. Sensitive and simultaneous analysis of five transgenic maizes using multiplex polymerase chain reaction, capillary gel electrophoresis, and laser-induced fluorescence. Electrophoresis 25: 2219-2226 (2004)
21. Sánchez L, González R, Crego AL, Cifuentes A. A simple capillary gel electrophoresis approach for efficient and reproducible DNA separations. Analysis of genetically modified soy and maize. J. Sep. Sci. 30: 579-585 (2007)
22. Zhou Y, Li Y, Su N, Pei X, Yong L. Rapid analysis of genetically modified soybean by a duplex PCR-capillary electrophoresis system with laser-induced fluorescence detection. Sichuan Daxue Xuebao 36: 119-123 (2005)
23. Heide B, Dromtorp SM, Rudi K, Heir E, Holck AL. Determination of eight genetically modified maize events by quantitative, multiplex PCR, and fluorescence capillary gel electrophoresis. Eur. Food Res. Technol. 227: 1437-2377 (2008)
24. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 30: e57 (2002)
25. Moreano F, Ehlert A, Busch U, Enge KH. Ligation-dependent probe amplification for the simultaneous event-specific detection and relative quantification of DNA from two genetically modified organisms. Eur. Food Res. Technol. 222: 479-485 (2006)
26. Nadal A, Coll A, La Paz JL, Esteve T, Pla M. A new PCR-CGE (size and color) method for simultaneous detection of genetically modified maize events. Electrophoresis 27: 3879-3888 (2006)
27. Chen H, Wu YH, Song DY, Zhang W, Dong XY, Li PW, Lu CM. On-line pre-concentration and UV determination of DNA fragments by dynamic coating capillary electrophoresis and its application to detection of genetically modified oilseed rape based on PCR. Microchem. J. 86: 17-22 (2007)
28. Dinelli G, Bonetti A, Marotti I, Minelli M, Navarrete-Casas M, Segura-Carretero A, Fernández-Gutiérrez A. Quantitative-competitive polymerase chain reaction coupled with slab gel and capillary electrophoresis for the detection of roundup ready soybean and maize. Electrophoresis 27: 4029-4038 (2006)
29. Guo L, Qiu B, Chi Y, Chen G. Using of multiple PCR and capillary electrophoresis with chemiluminescent detection for simultaneous qualitative and quantitative assay of genetically modified organism. Electrophoresis 29: 3801-3809 (2008)
30. García-Ruiz C, García MC, Cifuentes A, Marina ML. Characterization and differentiation of diverse transgenic and nontransgenic soybean varieties from CE protein profiles. Electrophoresis 28: 2314-2323 (2007)
31. Erny GL, Marina ML, Cifuentes A. CE-MS of zein proteins from conventional and transgenic maize. Electrophoresis 28: 4192-4201 (2007)
32. García-Villalba R, León C, Dinelli G, Segura A, Fernández A, Garcia-Cañas V, Cifuentes A. Comparative metabolomic study of transgenic versus conventional soybean using capillary electrophoresis-time-of-flight mass spectrometry. J. Chromatogr. A 1195: 164-173 (2008)
33. Levandi T, Leon C, Kaljurand M, Garcia-Cañas V, Cifuentes A. Capillary electrophoresis time-of-flight mass spectrometry for comparative metabolomics of transgenic versus conventional maize. Anal Chem. 80: 6329-6335 (2008)
34. Birch L, Archard CL, Parkes HC, McDowell DG. Evaluation of LabChip technology for GMOs analysis in food. Food Control 12: 535-540 (2001)
35. Burns M, Shanahan D, Valdivia H, Harris N. Quantitative event-specific multiplex PCR detection of Roundup Ready soya using LabChip technology. Eur. Food Res. Technol. 216: 428-433 (2003)
36. Obeid PJ, Christopoulos TK, Ioannou PC. Rapid analysis of genetically modified organisms by in-house developed capillary electrophoresis chip and laser-induced fluorescence system. Electrophoresis 25: 922-930 (2004)
37. Kim YJ, Chae JS, Chang JK, Kang SH. Microchip capillary gel electrophoresis using programmed field strength gradients for the ultra-fast analysis of genetically modified organisms in soybeans. J. Chromatogr. A 1083: 179-184 (2005)