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Detection of Genetically Modified Maize by the Polymerase Chain Reaction and Capillary Gel Electrophoresis with UV Detection and Laser-Induced Fluorescence

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In this paper, the possibilities of capillary gel electrophoresis (CGE) to detect transgenic maize in flours are shown. The method is based on the extraction and amplification by the polymerase chain reaction (PCR) of a specific DNA fragment from transgenic maize and its subsequent analysis by CGE with UV detection or laser-induced fluorescence (LIF). Some useful considerations regarding the optimization of DNA extraction and amplification conditions are given. Also, a comparison is established between the two CGE protocols for DNA detection based on ultraviolet absorption (CGE-UV) and LIF (CGE-LIF). The requirements, advantages, and limitations of both CGE methods are discussed. To our knowledge, this is the first paper on the use of CGE-LIF to detect transgenic food.

KEYWORDS: DNA; capillary gel electrophoresis; polymerase chain reaction; transgenic maize; Bt maize; GMOs; UV; LIF

1. INTRODUCTION

The application of genetic engineering for food production, including transgenic crops and genetically improved starter strains for the fermentation industries or for the production of numerous metabolites and enzymes, is a rapidly evolving field. The introduction in the market of products obtained by the use of these technologies raises social and ethical concerns, and the European Union has dictated several directives and regulations regarding their intentional liberation and commercialization. This includes the Novel Food Regulation (258/97/CE) concerning the need for labeling foods and ingredients containing or consisting in genetically modified organisms (GMOs). Some of these regulations, including the recent directive 2001/18/CE, establish that under certain percentages of approved GMOs, labeling is no longer necessary as long as their presence in the product is accidental or technically unavoidable. In this context, the development of versatile quantitative methods for GMO analysis is necessary for a successful control of the compliance of product labeling with the respective regulations.

Polymerase chain reaction (PCR)-based methods (*1*) are frequently used for GMO detection in food. Usually, amplified DNA fragments are separated by conventional electrophoretic methods (*2*, *3*). To this general strategy, improvements such as nested PCR can be added so that virtually any DNA sequence could be detected in food (*4*). However, the main deficiency of these analytical procedures for the purpose of food labeling is their semiquantitative character (*5*, *6*). This is due to the kinetics of the PCR amplification per se (*7*) and to the lack of precision in the quantification of DNA by traditional electrophoretic techniques (e.g., densitometry). In addition, many false negatives or positives are obtained by this procedure (*8*, *9*). PCR techniques, based in the use of competitive amplification targets, have been developed that allow to carry out more reliable quantitative analysis for transgenic food (*10*, *11*). However, the subsequent use of traditional electrophoretic methods still results in semiquantitative information.

In some instances, detection of GMOs in food is possible due to the presence of proteins coded by the transgenic sequences. For example, ELISA (enzyme-linked immunosorbent assay) employs antibodies that react against specific proteins, but it is expensive and has the drawback of the appearance of false negatives or positives because of cross reaction with other proteins (*12*). This method is less sensitive than PCR-based methods (*13*). Besides, proteins are thermosensitive molecules while nucleic acids are more thermostable, so that the effect of most food processing technologies on protein integrity and detectability is greater than on nucleic acids (*2*). On the other hand, the whole genome is present in every cell of the GMOs, whereas some proteins, including those coded by the transgen, may be expressed only in specific tissues. This may be the cause of false negatives in protein-based methods.

Real-time quantitative PCR has been developed by several companies (Roche, Perkin-Elmer) during the past few years as an alternative to conventional PCR for the quantification of specific nucleic acid sequences. It allows simultaneous amplification and quantification of the target DNA. However, these methods are still not well-developed for the detection of multiple PCR products (multiplex PCR). With the increasing number of GMOs that are being developed for food applications, the ability to detect several transgenic sequences in a single reaction becomes an important feature of any detection method.

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Table 1. Sequence of the Primers Used in PCR Reactions

primer	sequence	accession no.	position
cryIA(b)-V3	5′-CCTGACCAAGAGCACCAACCTGG-3′	I41419	1425–1447
cryIA(b)-V4	5′-GCTCATGGTGGCGCTGAAGTTGC-3′	I41419	1668–1646
MSS-S	5′-TCAACATCCGTGGATTGCATC-3′	AF023159	933–954
MSS-A	5′-TTCAGGGAAATCATCAGTTAATTGC-3′	AF023159	1166–1142

There is, therefore, a demand for new analytical methods that will contribute new and reliable quantitative data for transgenic food characterization. In a preliminary paper, we have demonstrated the good possibilities of the combined use of PCR and capillary gel electrophoresis (CGE) to detect transgenic food via the amplification, separation, and detection of DNA (*14*). The PCR−CGE method is fast, and its sensitivity using UV detection was shown to be enough to detect 1% of transgenic maize in maize flour. However, under these conditions, the DNA signal obtained was too close to the CGE-UV detection limit. This precludes both to detect transgenic maize at percentages lower than 1% and to detect it in processed foods where the number of intact DNA fragments that can be amplified will be foreseeably lower. Therefore, it seems interesting to develop a new method that can enable higher sensitivity during the detection of DNA fragments from transgenic maize amplified by PCR.

The goal of this paper is to carry out the development of a CGE method, using laser-induced fluorescence (LIF) to detect DNA from transgenic maize previously amplified by PCR. Also, a comparative study about the use of CGE with UV detection and CGE with LIF is carried out. The advantages and drawbacks of each technique are discussed. Moreover, some interesting guidelines about optimization of DNA extraction and PCR amplification are given.

2. MATERIAL AND METHODS

2.1. Chemicals. All chemicals were of analytical reagent grade and used as received. Tris[hydroxymethyl]aminomethano (TRIS), sodium dodecyl sulfate (SDS), guanidine hydrochloride, and ethylenediaminetetraacetic acid (EDTA) from Sigma (St. Louis, MO), 2-hydroxyethyl cellulose (HEC) (M_w 90 000) and poly(vinyl alcohol) (PVA) (M_w 50 000) from Aldrich (Milwaukee, WI), proteinase K and RNAse A from Roche (Barcelona, Spain), chloroform from Scharlau (Barcelona, Spain), isoamylic alcohol and *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (CTAB) from Merck (Darmstadt, Germany), and phenol from LabClinics (Madrid, Spain) were used. LIF EnhanCE (Beckman Instruments, Fullerton, CA, concentration not supplied) was added as an intercalating dye to the CE running buffers at the different concentrations indicated. Buffers were stored at 4 °C and warmed at room temperature before use. Distilled water was deionized by using a Milli-Q system (Millipore, Bedford, MA).

The test sample was DNA 100 bp ladder from Biotools (Madrid, Spain). This sample was diluted to a final concentration of ca. 400 μg/mL in PCR reaction buffer (see below) containing Orange G as a CGE marker.

Certified reference maize powder MZ0 (conventional, i.e., containing 0% transgenic maize) and MZ0.5 (containing 2% insect-resistant Bt-176 transgenic maize) produced by the Institute of Reference Materials and Measurements were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Conventional maize and Bt-176 transgenic maize was a gift of Syngenta Seeds S. A. (Zaragoza, Spain). Oligonucleotides were synthesized at Centro de Investigaciones Biológicas (Spanish Council for Scientific Research, Madrid, Spain). AmpliTaq DNA polymerase, including reaction buffer and $MgCl_2$, was from Perkin-Elmer (Madrid, Spain). Deoxynucleotides were from Amersham Pharmacia Biotech Europe GmbH (Barcelona, Spain). Uracil DNA glycosylase and DNA glycosylase inhibitor were purchased from New England Biolabs (Beverly, MA).

2.2. DNA Extraction. DNA purification was carried out by four alternative extraction methods.

2.2.1. CTAB Method. A 150 mg amount of homogenized samples of maize (1% transgenic and conventional) was incubated with 300 μL of 2% CTAB, 1.4 M NaCl, 20mM EDTA, 100 mM TRIS·HCl, pH 8.0, and 0.2% β-mercaptoethanol for 30 min at 60 °C and then extracted with 300 μL of chloroform/isoamyl alcohol (24:1). The nucleic acids on the aqueous phase were recovered by precipitation with 1 volume of 2-propanol, washed with 70% ethanol, and dissolved in 50 μL of TE buffer (10 mM Tris-HCl (pH 8) and 1 mM EDTA).

2.2.2. SDS/Proteinase K Method. The procedure was modified from ref *15*. Homogenized samples (1.5 g) were incubated at 37 °C overnight in 10 mL of extraction buffer (1% SDS, 100 μg/mL proteinase K, 50 mM TRIS-HCl (pH 8), and 20 mM EDTA). The suspension was centrifuged for 10 min at 5000 rpm. The supernatant was extracted with 1 volume of phenol and subsequently with 1 volume of chloroform/isoamyl alcohol (24:1). The aqueous phase was transferred to a new tube and mixed with 0.1 volume of 3 M sodium acetate at pH 4.8. Then, the mix was overlaid with 2.5 volumes of 100% ethanol. The two phases were mixed carefully by gentle agitation with a glass bar until the DNA was spooled. The DNA was immediately transferred to a new microcentrifuge tube containing 500 μL of TE buffer.

2.2.3. Potassium Acetate Method. A 1.5 g amount of homogenized samples was incubated with 1.5 mL of extraction buffer (50 mM TRIS-HCl, pH 8, 20 mM EDTA, and 20% SDS) at 65 °C for 30 min. Then, 2.25 mL of salt solution (3 M potassium acetate, pH 4.8) was added, and the mix was incubated for 1 h in ice. The solution was centrifuged for 10 min at 10 000 rpm and was filtered with a nylon mesh. Two volumes of 95% ethanol was added, and the solution was incubated at −20 °C for 1 h, then centrifuged for 10 min at 10 000 rpm, and washed with 70% ethanol. After 15 min, when the pellet was dried, 1.5 mL of TE buffer and 300 μg of RNAse A were added and incubated at 37 °C for 30 min. After the pellets were incubated, 1 volume of 2-propanol was added and mixed carefully by gentle agitation with a glass bar until the DNA was spooled. DNA was transferred to a new microcentrifuge tube containing 500 μL of TE buffer.

2.2.4. Wizard Method. DNA was extracted from 150 mg of homogenized samples using the Wizard resin and Minicolumn (Promega, Madison, WI) as follows (*16*). Every sample was mixed with 860 μL of TNE buffer (10 mM TRIS-HCl (pH 8), 150 mM NaCl, 2 mM EDTA, and 1% SDS), 40 μL of proteinase K (20 mg/mL), and 100 μL of 5 M guanidine hydrochloride. The mix was incubated at 55 °C for 3 h. After the mixture was centrifuged for 10 min at 14 000 rpm, 500 μL of supernatant was combined with 1 mL of Wizard resin, mixed by inversion and transferred to the barrel of the Minicolumn/syringe assembly. The syringe plunger was placed in the barrel and pushed carefully. The Minicolumn was centrifuged at 14 000 rpm for 1 min and washed with 2 mL of 2-propanol through the syringe again. The Minicolumn was dried for 15 min at room temperature. Then, 100 μL of TE buffer at 70 °C was added to the Minicolumn, placed in a microcentrifuge tube, and centrifuged for 2 min at 10 000 rpm.

2.3. PCR Conditions. A test fragment of the modified c*ryIA*(*b*) gene (GenBank accession number I41419) was amplified using primers *cryIA*(*b*)-V3 and *cryIA*(*b*)-V4 (**Table 1**). Amplification of a fragment of the maize starch synthase gene *dull1*, used as a control for DNA quality and amplificability, was performed with primers MSS-S and MSS-A (**Table 1**). Reaction mixtures contained 1× AmpliTaq reaction buffer, 25 mM $MgCl_2$, 2.5 mM dATP, 2.5 mM dCTP, 2.5 mM dGTP, 2.5 mM dTTP, 2.5 μM each primer, 10 μL template DNA, and 2.5 U of AmpliTaq DNA polymerase. The following thermal parameters were used for each amplification. *cryIA*(*b*): first denaturation, 12 min at 95 °C, 40 cycles (1 min at 95 °C, 30 s at 58 °C, and 30 s at 72 °C);

terminal elongation, 10 min at 72 °C. Starch synthase: first denaturation, 12 min at 95 °C, 40 cycles (1 min at 95 °C, 30 s at 54 °C, and 30 s at 72 °C); terminal elongation, 10 min at 72 °C. AmpliTaq DNA polymerase was added after the first denaturation step (manual Hot-start).

For some amplification reactions, the uracil DNA-glycosylase/dUTP (uridine 5′-triphosphate) system was tried in order to avoid carry over contamination. In that case, PCR mix was prepared as above, but dUTP instead of dTTP was used. Before the tube was cycled, the tube was incubated with 1 unit of uracil DNA-glycosylase at 37 °C for 10 min. After PCR amplification, 1 unit of uracil glycosylase inhibitor was added to the reaction tube in order to stop any residual glycosylase activity and prevent product degradation.

2.4. Capillary Electrophoresis Conditions. The analyses were carried out in a P/ACE 5500 CE apparatus, equipped with an UV−vis detector working at 254 nm and in a PACE-MDQ equipped with an Ar^+ laser working at 488 (excitation wavelength) and 520 nm (emission wavelength), both instruments from Beckman Instruments (Fullerton, CA). Bare fused silica capillaries with 75 μm i.d. were purchased from Composite Metal Services (Worcester, England). Injections were made at the cathodic end using N_2 pressure of 0.5 or 1 psi for a given time (1 psi = 6894.76 Pa). The P/ACE 5500 CE instrument was controlled by a PC running the System GOLD software, and the PACE-MDQ was controlled by a PC running the 32 Karat Software, both from Beckman.

Before the first use, any uncoated capillary was preconditioned by rinsing with 0.1 M HCl for 30 min. Between injections, capillaries were rinsed using 0.1 M HCl for 4 min, 1% PVA for 2 min, and separation buffer for 4 min. At the end of the day, the capillary was rinsed with deionized water for 5 min and stored overnight with water inside.

3. RESULTS AND DISCUSSION

3.1. CGE-UV Analysis of Genetically Modified Bt Maize. Initially, the CGE-UV method for the separation of DNA fragments developed at our laboratory (*14*) was used with a slight modification. This change basically consisted of using 4.5% HEC polymer into the separation buffer instead of 4% HEC as originally described. This modification attempted to provide the complete separation of the DNA fragments from the 100 bp ladder, since under the original conditions there was comigration of two of these fragments (i.e., 700 and 800 bp). By using these new conditions (i.e., 20 mM Tris, 10 mM ortophosphoric acid, 2 mM EDTA, and 4.5% HEC at pH 7.3 together with a bare fused silica capillary dynamically coated as described under the Materials and Methods, the complete separation of all of the DNA fragments could be achieved in less than 24 min, as can be seen in **Figure 1**.

The analysis time reproducibility of this CGE method using dynamically coated fused silica capillaries was tested for the same day, four different days, and three capillaries. Results for two arbitrarily chosen DNA fragments (i.e., 100 and 500 bp) are given in **Table 2**. As can be seen, the procedure provides a good reproducibility with %RSD values lower than 1.46% (obtained in the worst case for 4 days and $n = 40$ injections).

Next, a comparison was established among four different protocols to extract DNA from maize flour. Namely, the methods tested were the CTAB method, SDS/proteinase K method, potassium acetate method, and Wizard method (they are described under Materials and Methods). It could be seen that the best conditions for extracting DNA with a high yield were provided by the SDS/proteinase K method and CTAB method, followed by the potassium acetate method. The lowest yield was obtained using the Wizard method. Amplification reactions with increasing quantities of target DNA were performed for all DNA preparations. A decrease on the yield of amplified DNA or even a complete inhibition of the amplification reaction was observed for DNA extracted by the CTAB or the potassium acetate methods (data not shown), probably due to the copurification of inhibitory substances. Therefore, the SDS/proteinase K method was chosen as the most convenient.

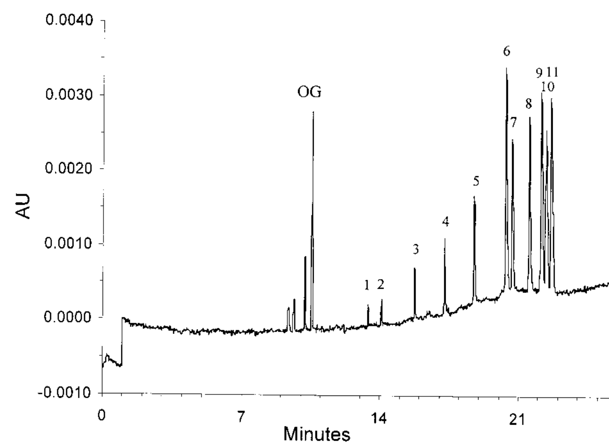

Figure 1. Separation of a 100 bp DNA ladder with CGE-UV using an uncoated fused silica capillary with 47 cm of total length, 40 cm of effective length, and 75 μm i.d. Separation voltage: −14 kV. Running buffer: 20 mM Tris, 10 mM ortophosphoric acid, 2 mM EDTA, and 4.5% HEC at pH 7.3. Injection for 50 s using N_2 pressure (0.5 psi) of (1) 80, (2) 100, (3) 200, (4) 300, (5) 400, (6) 500, (7) 600, (8) 700, (9) 800, (10) 900, and (11) 1000 bp. Detection at 254 nm.

Table 2. Reproducibility of Migration Times of the DNA Fragments of 100 and 500 bp Using Uncoated Capillaries for the Same day, Four Different days, and Three Different Capillaries[a]

	100 bp, t_{av} (min)	% RSD	500 bp, t_{av} (min)	% RSD
same day ($n = 10$)[b]	14.22	0.54	20.48	0.92
4 days ($n = 40$)[b]	14.12	1.04	20.40	1.46
3 capillaries ($n = 15$)	14.28	1.14	20.70	1.3

[a] All of the conditions as in **Figure 1**. [b] Same capillary.

DNA samples extracted using the SDS/proteinase K method from maize flour containing transgenic maize or not were next amplified by PCR and injected into the CGE-UV equipment. The samples were flour from conventional maize (0% transgenic), flour containing 1% of transgenic maize, and a blank. CGE-UV electrophoregrams of these samples are given in **Figure 2**. The quality and amplificability of the extracted DNA were tested by amplifying the maize *dull1* gene (**Figure 2A**). Amplification of a fragment of the modified *cryIA*(*b*) allowed detection of 1% of transgenic maize in the corresponding sample (**Figure 2B**). The peak observed for the electroforegram in **Figure 2B** is specific for *cryIA*(*b*), because no signal is observed for conventional maize in the same conditions (**Figure 2C**). **Figure 2D** (blank) is the result for an amplification reaction including all of the PCR reactives except for the target DNA and subsequent analysis by CGE-UV, showing that no interference is expected from this step.

Although the sensitivity of the PCR−CGE-UV procedure is enough to detect 1% of transgenic maize in food samples (**Figure 2B**), it would be convenient to have higher sensitivity since the peak obtained is too close to the detection limit (the signal-to-noise ratio is equal to 2.6 for that peak). A new procedure for detecting DNA fragments from PCR using CGE together with LIF as detection was then developed.

3.2. CGE-LIF Optimization and Comparison with CGE-UV. The better sensitivity of LIF as compared with UV detection

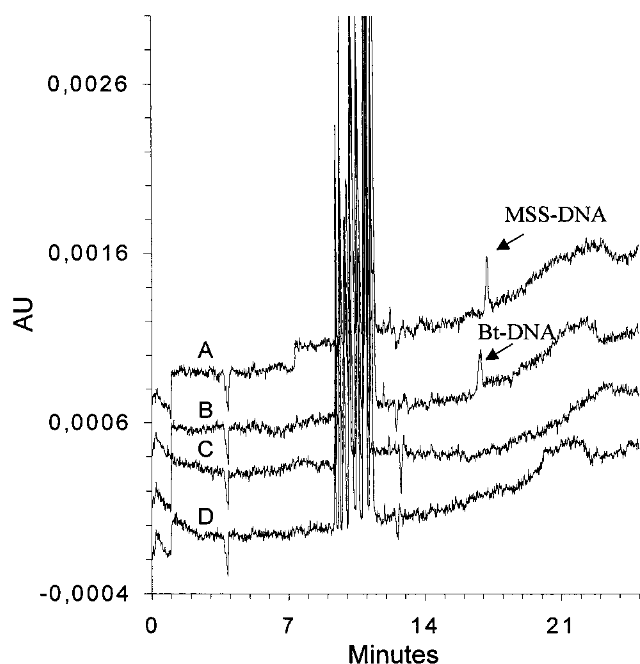

Figure 2. CGE-UV electropherograms obtained for the PCR amplification reactions using (A) transgenic maize DNA and the primer pair MSS-S/MSS-A (see **Table 1**); (B) transgenic maize DNA and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4; (C) conventional maize DNA and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4; and (D) control amplification reaction without template DNA (i.e., blank) and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4. Samples injected for 70 s using N_2 pressure (0.5 psi). Other conditions as in **Figure 1**.

Table 3. Signal/Noise Ratio of Three dsDNA Fragments (80, 300, and 1000 bp) Depending on the Volume of EnhanCE Added Per Milliliter of Running Buffer

nL of EnhanCE	80 pb	300 pb	1000 pb
62.5[a]	2176	4950	45 500
125	6494	14 373	96 212
250	5265	13 647	61 323
500	3540	9060	73 650

[a] Vials containing 2 mL of running buffer were used.

is well-known (*17*); therefore, CGE-LIF should allow an easier quantitative analysis of DNA fragments below the mentioned threshold of 1%. Basically, there are two procedures to supply fluorescence to DNA fragments when excited with an Ar^+ laser (usually λ_{ex}= 488 nm). The first one is based on covalently binding the DNA molecules with a derivatizing agent (containing frequently fluorescein) (*18*). The second one uses intercalating dyes (for double-stranded dsDNA) added to the buffer, such as, e.g., ethidium bromide, thiazole orange, oxazole yellow, or their corresponding homodimers, which form stable fluorescent complexes when binding to dsDNA fragments (*19*). In our case, the LIF EnhanCE was used as the intercalating dye for the dsDNA fragments.

It is known that the concentration of the intercalating dye is important for obtaining an optimum fluorescence signal from the dye−dsDNA complexes (*20*). Therefore, a study about this point was carried out, preparing separation buffers containing different quantities of intercalating dye and injecting the 100 bp dsDNA ladder. The results are given in **Table 3**. As can be seen, there is an optimum value for which the signal-to-noise ratio is maximum, and it corresponds to a volume of 125 nL of dye per mL of running buffer. However, it could be seen that

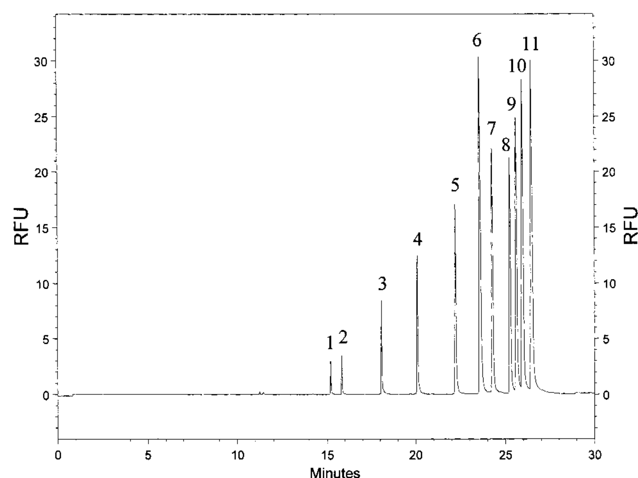

Figure 3. Separation of a 100 bp DNA ladder with CGE-LIF using an uncoated fused silica capillary with 50 cm of total length, 40 cm of effective length, and 75 μm i.d. Separation voltage: −15 kV. Running buffer: 20 mM Tris, 10 mM ortophosphoric acid, 2 mM EDTA, and 4.5% HEC at pH 7.3. Injection for 25 s using N_2 pressure (1 psi) of (1) 80, (2) 100, (3) 200, (4) 300, (5) 400, (6) 500, (7) 600, (8) 700, (9) 800, (10) 900, and (11) 1000 bp. LIF detection (λ_{ex} = 488 nm, λ_{em} = 520 nm).

Table 4. Comparison of CGE-UV and CGE-LIF for the Analysis of DNA Fragments[a]

	CGE-UV	CGE-LIF
cost of equipment (US $)	ca. 30000	ca. 50000
cost per run (US $)	0.003	0.1
buffer durability (no. of injections)[b]	7	4
signal/noise ratio[c]	11	14000

[a] All of the conditions as in **Figures 1** (CGE-UV) and **3** (CGE-LIF). [b] Calculated for 2 mL of running buffer. [c] Calculated for the dsDNA fragment of 300 bp in **Figures 1** and **3**.

by using 125 nL of dye the durability of the buffer was only 2 injections, after which a lower signal was observed, probably due to depletion of the dye induced by the high electric field applied. Using 250 nL, the same buffer could be used for 4 injections without a noticeable variation of separation. Therefore, the latter ratio (i.e., 250 nL dye/mL of buffer) was chosen as more adequate since less buffer is wasted for the same consumption of dye.

Figure 3 shows the separation of a 100 bp dsDNA ladder carried out using CGE-LIF under these optimum conditions. As can be seen, a good separation of the DNA fragments is also obtained with resolution slightly better than for CGE-UV (see **Figure 1**) at the expense of larger analysis times. This effect has already been observed and attributed to the effect of the intercalating dye onto the electrophoretic mobility of DNA fragments (*21*). Other interesting conclusions that can be extracted from the comparison of these two methodologies, CGE-UV vs CGE-LIF, are summarized in **Table 4**. Thus, it is clear that the much higher DNA signal obtained with CGE-LIF as compared to CGE-UV (14 000 vs 11 in our case) must compensate for (i) the higher price of the CGE-LIF equipment, (ii) its superior cost per analysis, and (iii) the lower durability of LIF buffers containing intercalating dyes.

3.3. CGE-LIF Analysis of Genetically Modified Bt Maize. The same samples analyzed by CGE-UV of **Figure 2** were analyzed by CGE-LIF using the optimized conditions mentioned above. The results of the control, transgenic maize, conventional maize, and blank are given in **Figures 4A−D**, respectively. As

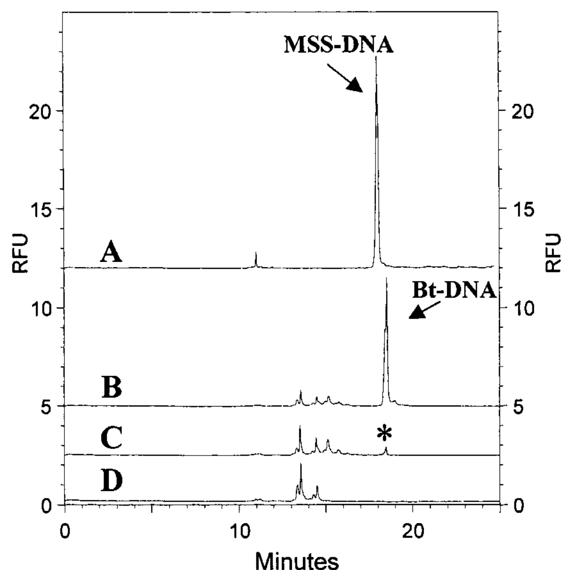

Figure 4. CGE-LIF electrophoregrams obtained for the PCR amplification reactions using (A) transgenic maize DNA and the primer pair MSS-S/MSS-A (see **Table 1**); (B) transgenic maize DNA and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4; (C) conventional maize DNA and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4; and (D) control amplification reaction without template DNA (i.e., blank) and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4. Samples injected for 38 s using N_2 pressure (1 psi). Other conditions as in **Figure 3**.

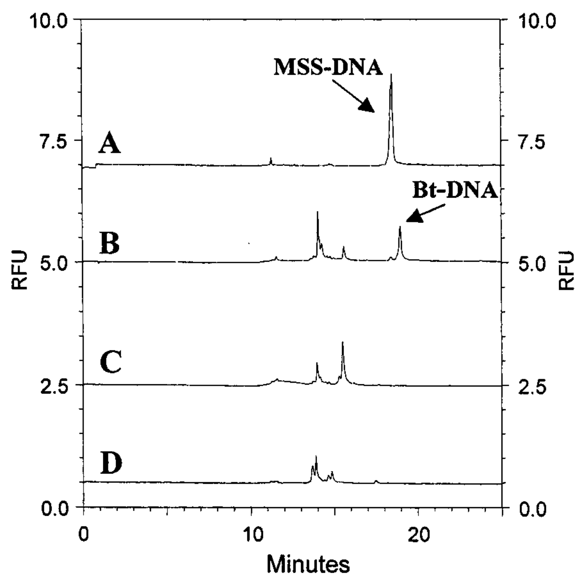

Figure 5. CGE-LIF electrophoregrams obtained for PCR amplification reactions using the uracil DNA-glycosylase/dUTP system using (A) transgenic maize DNA and the primer pair MSS-S/MSS-A (see **Table 1**); (B) transgenic maize DNA and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4; (C) conventional maize DNA and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4; and (D) control amplification reaction without template DNA (i.e., blank) and the primer pair *cryIA*(*b*)-V3/*cryIA*(*b*)-V4. Samples injected for 38 s using N_2 pressure (1 psi). Other conditions as in **Figure 4**.

can be seen, as a result of the much higher sensitivity obtained with the LIF detector (e.g., the signal-to-noise ratio for the Bt-DNA peak is 2.6 in **Figure 2B** and 2633 in **Figure 4B**), a contamination in the conventional maize that was not detected by using CGE-UV (**Figure 2C**) can now be seen by CGE-LIF (**Figure 4C**, peak marked with an asterisk). This could be erroneously assigned as maize flour containing Bt maize, i.e., a false positive. However, the existence of DNA from the *cryIA*(*b*) gene in the sample of **Figure 4C** (conventional maize) is due, as next shown, to carry over contamination. This point is demonstrated through the use of uracil DNA-glycosylase together with dUTP instead of dTTP as described under the Materials and Methods. By using this procedure, any carry over contamination due to DNA (transgenic or conventional) amplified from previous samples will be removed from the system. The results from the use of this uracil DNA-glycosylase/dUTP system are given in **Figure 5**. As can be seen, no contamination is now observed for the conventional maize (**Figure 5C**), although this is to the expense of slightly lower signals due to a lower yield of the PCR amplification under these conditions.

By using this combination of CGE-LIF with the uracil DNA-gluycosylase/dUTP system, it is now possible to address the development of, first, competitive PCR reactions to accurately estimate the percentage of transgenic maize in commercial samples. Second, the use of multiplex PCR reactions and CGE-LIF to simultaneously detect and quantify several transgenic sequences in a given sample will be investigated. These works are now being carried out at our laboratory.

ABBREVIATIONS USED

CGE, capillary gel electrophoresis; LIF, laser-induced fluorescence; PCR, polymerase chain reaction; HEC, hydroxyethyl cellulose; TRIS, tris[hydroxymethyl]aminomethano; GMO, genetically modified organism; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediaminetetraacetic acid; CTAB, *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide; PVA, poly(vinyl alcohol); SDS, sodium dodecyl sulfate.

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