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Ultrasensitive Detection of Genetically Modified Maize DNA by Capillary Gel Electrophoresis with Laser-Induced Fluorescence Using Different Fluorescent Intercalating Dyes

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In this work, four different fluorescent intercalating dyes are compared for the ultrasensitive CGE−LIF detection of DNA from transgenic maize in flours. The fluorescent intercalating dyes compared are YOPRO-1, SYBR-Green-I, Ethidium bromide (EthBr), and EnhanCE. For all the four dyes optimum concentrations are established, and efficient separations of DNA fragments ranging in size from 80 to 1000 bp are obtained. The comparative study demonstrates that SYBR-Green-I and YOPRO-1 provide better limits of detection (LODs) than EnhanCE or EthBr (i.e., LODs are, respectively, 700, 1000, 11300, and 97400 zmol, calculated for a 200-bp DNA fragment). Separations using YOPRO-1 are faster than those using SYBR-Green-I (30 min vs 47 min for the analysis of the 80−1000 bp DNA fragments). Also, separations using YOPRO-1 are more efficient than those using SYBR-Green-I (e.g., 2.4×10^6 plates/m vs 1.6×10^6 plates/m, respectively, calculated for the 200-bp fragment). Also, buffer depletion and cost per analysis are worse with SYBR-Green-I than with YOPRO-1. Therefore, YOPRO-1 was selected as the preferred intercalating dye. Using this fluorescent compound, analysis time reproducibility for the CGE−LIF separation of the DNA fragments is determined to be better than 1.7% (% RSD, $n = 10$) within the same day, and better than 1.9% (% RSD, $n = 30$) for three different days. Moreover, the fluorescence signal obtained using this dye is shown to vary linearly with the DNA concentration in the range studied, i.e., 1−500 ng/μL. It is demonstrated that by using this method 0.01% of transgenic maize can be detected in flour by direct injection of the PCR-amplified sample.

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

INTRODUCTION

Commercial use of transgenic plants and other genetically modified organisms (GMOs) has raised several ideological and ethical issues during the past few years. The debate is especially intense, for several reasons, in the case of the so-called “transgenic foods”. In the European Union, the competent authorities have dictated regulations, such as the Novel Food Regulation (258/97/CE) for labeling of GMO-containing foodstuff, and the Directive 49/2000, which restricts the need for labeling to products containing GMOs above certain percentages. Because labeling as “GMO containing” could severely affect the marketing of food products, the development of quantitative methods for transgenic DNA detection becomes a necessity to successfully control compliance of product labeling with the above-mentioned regulations. With the increasing number of GMOs that are being developed for food applications, the ability to detect several transgenes in a single reaction becomes an important additional feature for any detection method (*1*).

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GMOs could be detected by either polymerase chain reaction (PCR), for direct detection of the transgenic DNA, or immunological methods, for detection of the cognate proteins (limited to tissues in which the transgen is expressed). Because of their better reliability and sensitivity and the higher stability of DNA over proteins, PCR methods are usually preferred for GMO detection. Although the PCR technique is very sensitive, it is not quantitative in its traditional form, in which the final amplification product is detected by semiquantitative traditional electrophoretic techniques. Currently, quantification of DNA is accomplished either by real-time quantitative PCR (*2, 3*) or competitive quantitative PCR (*4, 5*).

Real-time quantitative PCR allows for the simultaneous amplification and quantification of the target DNA. In this method quantification is based on the kinetics of the amplification. However, these methods are not yet well-developed for the simultaneous detection of several transgenes.

To attain quantification, competitive PCR uses amplification targets that are similar but distinguishable from the transgene, and co-amplified in the same reaction, however, quantification is hampered by the subsequent use of conventional electro-

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phoretic techniques. The combined use of PCR and capillary gel electrophoresis (CGE) seems to be a good alternative for the detection of transgenic organisms in foods, based on DNA analysis (*6, 7*). In combination with competitive quantitative PCR, CGE analysis could allow for the accurate detection and amplification of several transgenes, as an alternative to conventional and real-time quantitative PCR. However, UV detection in CGE lacks sensitivity and generally can be applied only to samples with concentrations down to 10^{-6} M (*8*). The use of laser-induced fluorescence (LIF) in CGE improves dramatically both the limit of detection and linear dynamic range obtainable compared with that of UV detection (*9*). 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 (frequently containing fluorescein) (*10*−*12*). The second one uses intercalating dyes (for double-stranded dsDNA) added to the buffer as e.g., ethidium bromide (EtBr), thiazole orange (TO), oxazole yellow (YO), or their corresponding homodimers, that form stable fluorescent complexes when bound to dsDNA fragments (*13*−*17*). Several works have been published comparing the advantages and drawbacks of different intercalating dyes used in capillary electrophoresis (*18*−*24*). However, their application has been usually reduced to the separation of standard DNA fragments (*25*−*26*). Moreover, studies about other figures of merit different from LOD or resolution (e.g., separation reproducibility, buffer depletion, or linear detection range) are rarely found in these works (*22, 26*).

The goal of this work is to develop an ultrasensitive CGE method, using laser-induced fluorescence (LIF) to detect DNA from transgenic maize previously amplified by PCR. To do this, a comparative study of four different intercalating dyes used in CGE−LIF is accomplished. The advantages and drawbacks of each dye are discussed. Once the optimum CGE−LIF procedure is found, its figures of merit are determined. The usefulness of this method is demonstrated by detecting very low contents of transgenic maize in food.

MATERIALS AND METHODS

Chemicals. All chemicals were of analytical reagent grade and used as received. Tris[hydroxymethyl]aminomethano (TRIS), sodium dodecyl sulfate (SDS), guanidine hydrochloride, and ethylenediamine tetraacetic acid (EDTA) from Sigma (St. Louis, MO), 2-hydroxyethyl cellulose (HEC) (Mw_{av} 90000) and poly(vinyl alcohol) (PVA) (Mw_{av} 50000) from Aldrich (Milwaukee, WI), Proteinase K, RNAse A from Roche (Barcelona, Spain), chloroform from Scharlau (Barcelona, Spain), isoamyl alcohol from Merck (Darmstadt, Germany) and phenol from LabClinics (Madrid, Spain) were used. SYBR-Green-I, YOPRO-1 (both from Molecular Probes, Leiden, Holland), EthBr from Sigma-Aldrich (Madrid, Spain), and LIF EnhanCE (Beckman Instruments, Fullerton, CA, concentration not supplied) were added as intercalating dyes 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 ranging from 1 to 500 ng/μL in PCR reaction buffer (see below).

Conventional maize and BT-176 transgenic maize were 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 Gold 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).

Table 1. Sequence of the Primers Used in PCR Reactions

primer	sequence	accession number	position
cryIA(b)-V5	5′-GATCGGCAACTACACCGACCAC-3′	I41419	597–618
cryIA(b)-V6	5′-TTGGTGTAAATCTCGCGGGTCAG-3′	I41419	787–809
MSS-S	5′-TCAACATCCGTGGATTGCATC-3′	AF023159	933–954
MSS-A	5′-TTCAGGGAAATCATCAGTTAATTGC-3′	AF023159	1166–1142

DNA Extraction. To prepare samples, maize grains (transgenic and conventional) were milled to a fine powder separately using two grinders, and then mixed at several transgenic percentages (0.01, 0.5, and 1%). DNA purification was carried out by the SDS/proteinase K method modified from reference (*25*). Homogenized samples (3 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 vol phenol and subsequently with 1 vol chloroform/isoamyl alcohol (24:1). The aqueous phase was transferred to a new tube and mixed with 0.1 vol 3 M sodium acetate at pH 4.8. Then, the mix was overlaid with 2.5 vol 100% ethanol. The two phases were mixed carefully by gentle agitation with a glass bar until the DNA was spooled. DNA was immediately transferred to a new microcentrifuge tube containing 500 μL of TE buffer.

PCR Conditions. A test fragment of the modified c*ryIA(b)* gene (GenBank accession number I41419) was amplified using primers *cryIA(b)*-V5 and *cryIA(b)*-V6 (**Table 1**). Amplification of a fragment of the maize starch synthase gene *dull1* (GenBank accession number AF023159), 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 Gold reaction buffer, 2 mM $MgCl_2$, 0.25 mM dATP, 0.25 mM dCTP, 0.25 mM dGTP, 0.5 mM dUTP, 3 μM each primer, 10 μL template DNA, and 2.5 U of AmpliTaq Gold polymerase. The following thermal parameters were used for each amplification: *cryIA(b)*, first denaturation 10 min at 95 °C, 40 cycles (1 min at 95 °C, 30 s at 66 °C, 30 s at 72 °C), terminal elongation 10 min at 72 °C; starch synthase, first denaturation 10 min at 95 °C, 40 cycles (1 min at 95 °C, 30 s at 58 °C, 30 s at 72 °C), terminal elongation 10 min at 72 °C.

Before cycling, 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 to stop any residual glycosylase activity and prevent product degradation.

Capillary Electrophoresis Conditions. The analyses were carried out in a PACE-MDQ (Beckman Instruments, Fullerton, CA) equipped with an Ar+ laser working at 488 nm (excitation wavelength) and 520 nm (emission wavelength used for SYBR-Green-I, YOPRO-1, and EnhanCE), or 590 nm (emission wavelength used for EthBr). 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 1 psi for a given time (1 psi = 6894.76 Pa). The PACE-MDQ instrument was controlled by a PC running the 32 Karat Software, both from Beckman.

Before 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. PVA brings about a neutral coating of the capillary wall that allows achieving reproducible separations reducing the electroosmotic flow (*6*). At the end of the day, the capillary was rinsed with deionized water for 5 min and stored overnight with water inside.

To calculate the LOD, a 100-bp DNA ladder (containing 3.3 pg/μL of the 200-bp fragment) was injected using N2 pressure (1 psi) for 12 s. Under these conditions, the noise signal was measured, and the quantity of the 200 bp fragment (i.e., LOD) required to obtain a signal equal to 3 times the noise signal was determined.

RESULTS AND DISCUSSION

Comparison of the Four Dyes: SYBR-Green-I, YOPRO-1, EthBr, and EnhanCE. It is known that concentration of

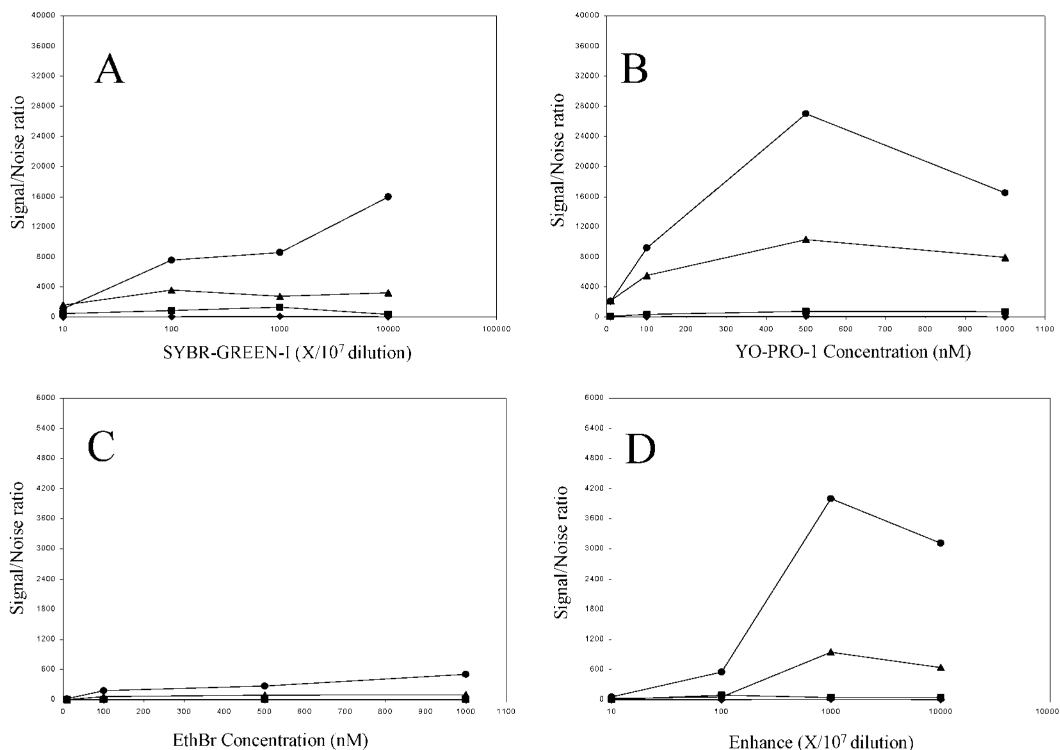

Figure 1. Fluorescence signal/noise ratio vs intercalating dye quantity determined for the 400-bp peak. In all cases a 100-bp DNA ladder was injected for 12 s at 1 psi at the following total concentrations: ● 400 ng/μL; ▲ 100 ng/μL; ■ 10 ng/μL; ◆ 1 ng/μL. Separation conditions were as follows: uncoated 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 containing different quantities of (A) SYBR-Green-I, (B) YOPRO-1, (C) EthBr, and (D) EnhanCE. LIF detection (λ_{ex} = 488 nm, λ_{em} = 520 nm for A, B, and D, and λ_{em} = 590 nm for C).

intercalating dye is important for obtaining an optimum fluorescence signal from the dye−dsDNA complexes (*23*). Therefore, a first study about this point was carried out by preparing separation buffers containing different quantities of intercalating dyes and injecting the 100-bp dsDNA ladder at total concentrations of 1, 10, 100, and 400 ng/μL. As an example, **Figure 1** shows plots of the fluorescence signal/noise ratio vs the concentration of the four dyes for the fragment of DNA of 400 bp (similar behavior was observed for the rest of the fragments of the DNA ladder, vide infra). As can be seen, the fluorescence signal/noise ratio depends on the type of dye and DNA concentration, increasing in all cases with the concentration of DNA. Also, it can be seen (**Figure 1A** and **B**) that the most favorable fluorescence signal/noise ratio is obtained by using SYBR-Green-I and YOPRO-1 (note the different *y*-scales used in **Figure 1**).

From **Figure 1** it can also be deduced that, except for the 400 ng/μL DNA sample, there is an optimum concentration of each dye for which the fluorescence signal/noise ratio reaches a maximum or a plateau value independently of the DNA concentration (e.g., 500 nM for YOPRO-1 and EthBr, a dilution of 1000/10^7 for EnhanCE or SYBR-Green-I). This happens for all dyes and DNA concentrations except for the sample containing 400 ng/μL of DNA injected in a buffer containing SYBR-Green-I (**Figure 1A**) or, in a less extent, containing EthBr (**Figure 1C**). In these two cases an unexpected increase of the fluorescence signal/noise ratio is obtained. In a previous work, the linearity in fluorescence intensity versus DNA concentration was demonstrated for SYBR-Green-I (*21*). However, in that study only concentrations of DNA up to 30 ng/μL were used. Therefore, some cautions have to be taken when using SYBR-Green-I and high DNA concentrations. Logically, this point is not a big concern because the most interesting applications relate to the lowest DNA concentrations. Thus, for DNA concentrations of 10 ng/μL and 1 ng/μL, the fluorescence signal/noise ratio increases up to a concentration of dye of 500 nM for YOPRO-1 and a dilution of 1000/10^7 for SYBR-Green-I, and then it levels off at higher dye concentrations. In addition, it could be seen that these two dyes (SYBR-Green-I and YOPRO-1) provide fluorescence signal/noise ratios much better than those of EthBr (DNA detection at these low concentrations is not achieved with this dye) and EnhanCE. Also, at these low DNA concentrations, SYBR-Green-I provides slightly better fluorescence signal/noise ratios than YOPRO-1.

These results on the effect of the concentration of both dye and DNA on the fluorescence signal/noise ratio allowed us to optimize of the separation conditions of the 80−1000 bp DNA fragments for the four dyes. As shown in **Figure 2**, it was possible to obtain a good separation of the 11 DNA fragments using the 4 intercalating dyes without detrimental effect of the intercalating dye on the electrophoretic performance (*26*). Under these optimal conditions a complete study of different figures of merit (namely LOD, analysis speed, buffer depletion, efficiency, and cost) of the four dyes was carried out, and the results obtained are shown in **Table 2**. Thus, the LOD using SYBR-Green-I was slightly better than that obtained with YOPRO-1 (700 vs 1000 zmol, respectively, **Table 2**) and much lower than those attained using EnhanCE (11300 zmol) and EthBr (97400 zmol). However, cost and analysis speed are better using YOPRO-1 than using SYBR-Green-I (**Table 2**). Moreover, the buffer depletion induced by the high electric field (*6*) and the separation efficiency are also favorable to YOPRO-1 (**Table 2**). Despite the slightly better LOD obtained with SYBR-Green-I, the rest of the figures of merit clearly indicate the convenience of using YOPRO-1. This dye was, therefore, selected for the next experiments.

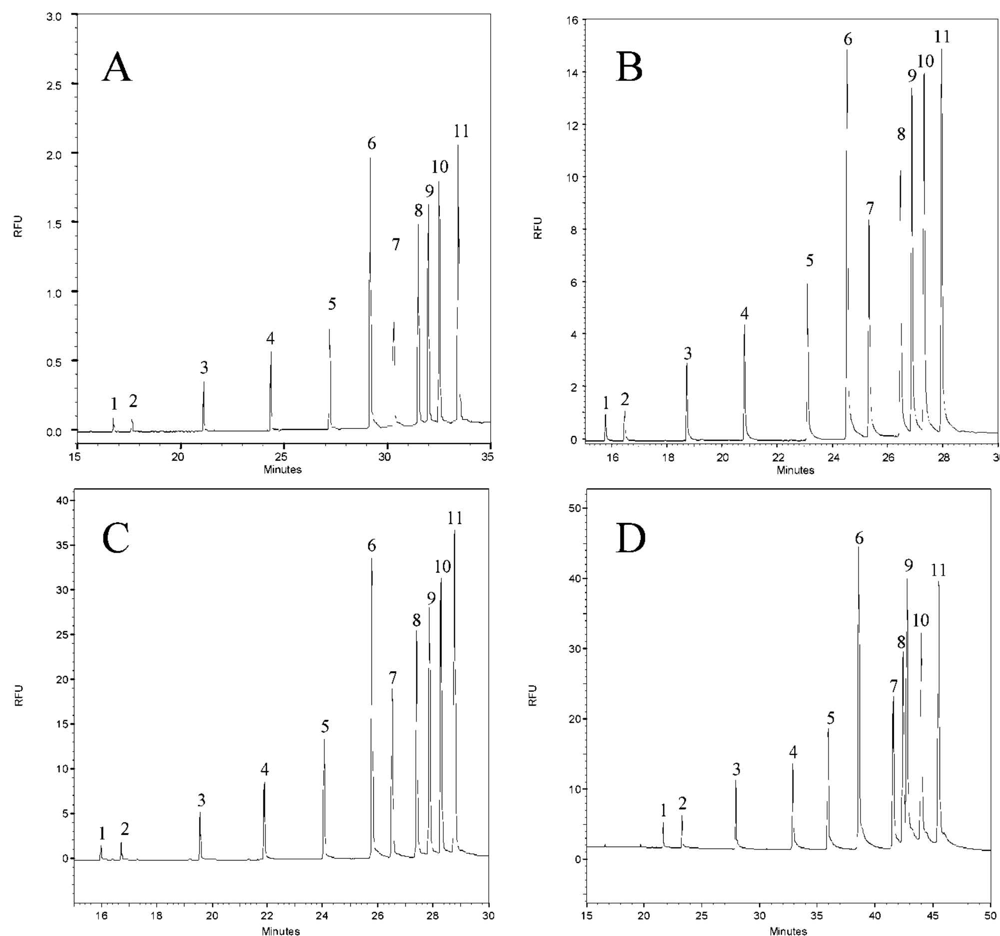

Figure 2. Separation of a 100-bp DNA ladder with CGE–LIF using an uncoated fused silica capillary with 50 cm of total length, 40 cm of effective length, and 75 μm i.d; separation voltage of –15 kV; running buffer of 20 mM Tris, 10 mM ortophosphoric acid, 2 mM EDTA, and 4.5% HEC at pH 7.3. Injection for 12 s using N_2 pressure (1 psi) of (1) 80 bp, (2) 100 bp, (3) 200 bp, (4) 300 bp, (5) 400 bp, (6) 500 bp, (7) 600 bp, (8) 700 bp, (9) 800 bp, (10) 900 bp, and (11) 1000 bp. (A) EthBr and 100 ng/μL of DNA injected; (B) EnhanCE and 100 ng/μL injected; (C) YOPRO-1 and 10 ng/μL of DNA injected; and (D) SYBR-Green-I and 10 ng/μL of DNA injected. LIF detection (λ_{ex} = 488 nm, λ_{em} = 590 nm for A; and λ_{em} = 520 nm for B, C, and D).

Table 2. Characteristics at the Optimum Concentrations of the Four Fluorescent Intercalating Dyes Used in This Work (All Conditions Same as Those in **Figure 2**)

	LOD[a]	analysis time[b]	buffer renewal[c]	efficiency[d]	cost[e]
SYBR-Green-I	700	47	after 3 runs	1600000	0.25
YOPRO-1	1000	30	after 5 runs	2400000	0.04
EnhanCE	11300	29	after 4 runs	2400000	0.29
EthBr	97400	34	after 5 runs	2700000	0.003

[a] Limit of detection in zmol for the 200 bp fragment. [b] Analysis time in min for separation of the DNA fragments from 80 to 1000 bp. [c] Calculated for 2 mL of running buffer. [d] Number of theroretical plates per meter of column for the 200 bp peak of **Figure 2**. [e] Cost per run in US dollars.

Table 3. Reproducibility of Migration Times of the DNA Fragments of 100, 300, and 500 bp Using Uncoated Capillaries for the Same Day and Three Different Days (All Conditions are the Same as Those in **Figure 2C**)

	100 bp		300 bp		500 bp	
	t_{av} (min)	%RSD	t_{av} (min)	%RSD	t_{av} (min)	%RSD
same day (n=10)	16.8	1.2	22.0	1.7	25.8	1.5
three days (n=30)	16.9	1.4	22.1	1.9	25.9	1.7

Reproducibility of this CGE-LIF procedure using YOPRO-1, an uncoated capillary, and commercially available polymers in the running buffer (*6, 7*) was good. In **Table 3**, the %RSD values for the same day and three different days obtained for the DNA fragments of 100, 300, and 500 bp peaks are given. As can be seen, high reproducibility was obtained in all cases, with %RSD values up to 1.7 within the same day and 1.9 for three different days (i.e., the worst case). Moreover, the efficiency achieved was up to 3.2×10^6 plates/m calculated for the DNA fragment of 700 bp in **Figure 2C** (peak 8). Also, it could be seen that the fluorescence intensity of DNA fragments complexed with YOPRO-1 increased linearly as a function of DNA concentration over nearly 3 orders of magnitude (i.e., from 1 to 500 ng/μL of total DNA). For example, for the 200 bp peak the linear dependence was given by the equation (obtained after least-squares fitting) $y = 4.14 + 0.40x$, where x is the peak height and y is the fluorescence signal, with a regression factor $r = 0.995$ ($n = 6$).

These values demonstrate that the method proposed is sensitive, reproducible, and efficient, and can be used with confidence for the quantitative analysis of GMOs in foods. To demonstrate that, detection of transgenic maize addition in conventional maize powder was carried out via PCR amplification of a DNA fragment corresponding to the *cryIA(b)* gene and subsequent analysis by this CGE–LIF method.

Detection of Different Percentages of Genetically Modified Maize by CGE–LIF. To check the quality of the DNA extracted from transgenic maize and its suitability for PCR amplification, a starch synthase gene fragment of 234 bp was

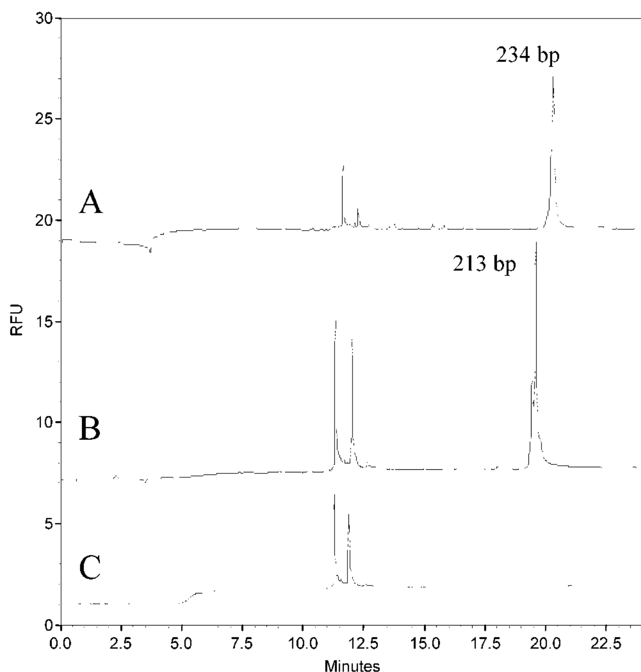

Figure 3. CGE–LIF electropherograms obtained for the PCR amplification reactions using the following: (A) 0.01% transgenic maize DNA and the primer pair *MSS*-S/*MSS*-A (**Table 1**); (B) 0.01% transgenic maize DNA and the primer pair *cryIA(b)*-V5/*cryIA(b)*-V6; (C) conventional maize DNA and the primer pair *cryIA(b)*-V5/*cryIA(b)*-V6. Samples were injected for 12 s using N_2 pressure (1 psi). Other conditions were as in **Figure 2C**.

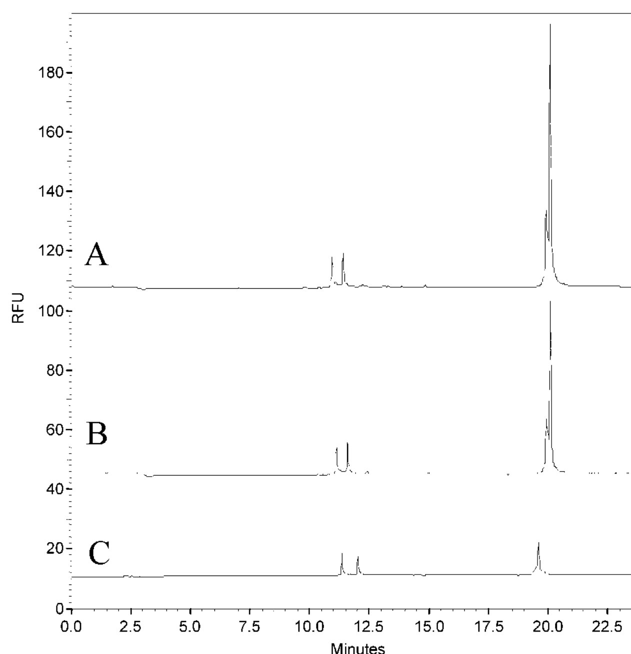

Figure 4. CGE–LIF electropherograms obtained for PCR amplification reactions using the primer pair *cryIA(b)*-V5/*cryIA(b)*-V6 (**Table 1**) and DNA from flours containing the following: (A) 1% of transgenic maize; (B) 0.5% of transgenic maize; and (C) 0.01% of transgenic maize. Samples were injected for 12 s using N_2 pressure (1 psi). Other conditions were as in **Figure 2C**.

selected as marker and amplified using the primers indicated in **Table 1**. **Figure 3A** shows the CGE−LIF electropherogram obtained for the injection of the PCR amplification reaction of this starch synthase gene fragment amplified from transgenic maize DNA. The good signal obtained for this amplicon supports the suitability of the extracted DNA for PCR−CGE−LIF analysis. Similar results were obtained after injecting the PCR amplification reaction of the starch synthase gene fragment amplified from conventional maize DNA (not shown). A transgenic maize specific *cryIA(b)* 213 bp fragment was amplified from both 0.01% transgenic and conventional maize DNA using the primers *cryIA(b)*-V5/*cryIA(b)*-V6 of **Table 1**. These two amplified DNA samples (i.e., from transgenic and conventional maize) were injected, and the electropherograms of **Figure 3B** and **C** were obtained. As can be seen, the PCR−CGE−LIF method allows the detection of maize containing transgenic organisms (**Figure 3B**) and its differentiation from conventional maize (**Figure 3C**). No peak could be observed for conventional maize DNA (**Figure 3C**) in the region where the amplicon used for transgenic DNA detection should come out (about 20 min in **Figure 3B**).

Moreover, by using this PCR−CGE−LIF procedure, samples containing transgenic maize at concentrations of 1, 0.5, and 0.01% could be easily detected as can be seen in the electropherograms of **Figure 4A**, **B**, and **C**, respectively. Therefore, this method allows for the specific detection of transgenic maize in conventional maize powder below the 1% threshold imposed by the European regulation (49/2000). Moreover, it is interesting to mention that the fluorescence signal/noise ratio obtained for the sample containing 0.01% of transgenic maize was ca. 600, this would theoretically allow us to detect percentages of transgenic maize as low as 0.00005% (for a signal/noise ratio equal to 3 and considering similar degree of amplification from the PCR reaction). However, in this case the high amount of DNA sample required to include at least one molecule of transgene would probably avoid the amplification.

In future works, the development of competitive PCR reactions to accurately estimate the percentage of transgenic maize in commercial samples will be addressed by PCR−CGE−LIF. The use of multiplex PCR reactions and CGE−LIF to simultaneously detect and quantify several transgenic sequences in a given sample will then be studied.

ABBREVIATIONS USED

CGE, capillary gel electrophoresis; LIF, laser-induced fluorescence; PCR, polymerase chain reaction; EthBr, ethidium bromide; LOD, limit of detection; HEC, hydroxyethylcellulose; TRIS, tris[hydroxymethyl]aminomethano; GMO, genetically modified organism; ELISA, enzyme-linked immunosorbent assay, EDTA, ethylenediamine tetraacetic acid; PVA, poly(vinyl alcohol); SDS, sodium dodecyl sulfate.

LITERATURE CITED

(1) McLaren, J. S. Agricultural biotechnology: myth and measurement. *Mol. Diagn.* **2000**, *5*, 257−265.
(2) Higuchi, R.; Dollinger, G.; Walsh, P. S.; Griffith, R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology* **1992**, *10*, 413−417.
(3) Higuchi, R.; Fockler, C.; Dollinger, G.; Watson, R. Dinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Biotechnology* **1993**, *11*, 1026−1030.
(4) Hardegger, M.; Brodmann, P.; Herrmann, A. Quantitative detection of the 35S promoter and the NOS terminator using quantitative competitive PCR. *Eur. Food Res. Technol.* **1999**, *209*, 83−87.
(5) Studer, E.; Rhyner, C.; Luethy, J.; Huebner, P. Quantitative competitive PCR for the detection of genetically modified soybean and maize. *Z. Lebensm.-Unters. Forsch. A*, **1998**, *207*, 207−213.

(6) García-Cañas, V.; González, R.; Cifuentes, A. Detection of genetically modified maize by PCR and capillary gel electrophoresis with UV detection and laser induced fluorescence. *J. Agric. Food Chem.* **2002**, *50*, 1016−1021.

(7) García-Cañas, V.; González, R.; Cifuentes, A. Detection of genetically modified maize by PCR and capillary gel electrophoresis (CGE) using uncoated columns. MCFA Annals, Vol II, 2002 (in press).

(8) Nouadje, G.; Amsellem, J.; Couderc, B.; Verdeguer, P.; Couderc, F. Capillary electrophoresis with laser-induced fluorescence detection: optical designs and applications. *Prog. HPLC−HPCE* **1997**, *5*, 49−72.

(9) Dovichi, N. J. Capillary electrophoresis for DNA sequencing. In *Analysis of Nucleic Acids by Capillary Electrophoresis*; Heller, C., Ed.; Vieweg & Sohn: Wiesbaden, Germany, 1997.

(10) Vincent, U.; Patra, G.; Therasse, J.; Gareil, P. Quantitation of polymerase chain reaction-amplified DNA fragments by capillary electrophoresis and laser-induced fluorescence detection. *Electrophoresis* **1996**, *17*, 512−517.

(11) Wang, Y.; Wallin, J. M.; Ju, J.; Sensabaugh, G. F.; Mathies, R. A. High-resolution capillary array electrophoretic sizing of multiplexed short tandem repeat loci using energy-transfer fluorescent primers. *Electrophoresis* **1996**, *17*, 1485−1490.

(12) Deforce, D.; Millecamps, R.; Van Hoofstat, D.; Van den Eeckhout, E. G. Comparison of salb gel electrophoresis and capillary electrophoresis for the detection of the fluorescently labeled polymerase chain reaction products of short tandem repeat fragments. *J. Chromatogr. A* **1998**, *806*, 149−155.

(13) Kim, Y.; Morris, M. D. Separation of nucleic acids by capillary electrophoresis in cellulose solutions with mono- and bis-intercalating dyes. *Anal. Chem.* **1994**, *66*, 1168−1174.

(14) Guttman, A.; Cooke, N. Capillary gel affinity electrophoresis of DNA fragments. *Anal. Chem.* **1991**, *63*, 2038−2042.

(15) Schartz, H. E.; Ulfelder, K. J. Capillary electrophoresis with laser-induced fluorescence detection of PCR fragments using thiazole orange. *Anal. Chem.* **1992**, *64*, 1737−1740.

(16) Marino, M. A.; Weaver, K. R.; Tully, L. A.; Girard, J. E.; Belgrader, P. Characterization of mitochondrial DNA using low-stringency single specific primer amplification analyzed by laser induced fluorescence-capillary electrophoresis. *Electrophoresis* **1996**, *17*, 1499−1504.

(17) Siles, B. A.; O'Neil K. A.; Fox, M. A., Anderson, D. E.; Kuntz, A. F.; Ranganath, S. C.; Morris, A. C. Genetic fingerprinting of grape plant (*Vitis vinifera*) using random amplified polymorphic DNA (RAPD) analysis and dynamic size-sieving capillary electrophoresis. *J. Agric. Food Chem.* **2000**, *48*, 5903−5912.

(18) Brian, K.; Sepaniak, M. J. Evaluation of On-column labeling with intercalating dyes for fluorescence detection of DNA fragments separated by capillary electrophoresis. *J. Microcolumn Sep.* **1993**, *5*, 275−282.

(19) Zhu, H.; Clark, S. M.; Benson, S. C.; Rye, H. S.; Glazer, A. N.; Mathies, R. A. High-Sensitivity capillary electrophoresis of double-stranded DNA fragments using monomeric and dimeric fluorescent intercalating dyes. *Anal. Chem.* **1994**, *66*, 1941−1948.

(20) Figeys, D.; Arriaga, E.; Renborg, A.; Dovichi, N. J. Use of the fluorescent intercalating dyes POPO-3, YOYO-3 and YOYO-1 for ultrasensitive detection of double-stranded DNA separated by capillary electrophoresis with hydroxypropylmethyl cellulose and non-cross-linked polyacrylamide. *J. Chromatogr. A* **1994**, *669*, 205−216.

(21) Kim, Y.; Morris, M. D. Separation of nucleic acids by capillary electrophoresis in cellulose solutions with mono- and bis-intercalating dyes. *Anal. Chem.* **1994**, *66*, 1168−1174.

(22) Skeidsvoll, J.; Ueland, P. M. Analysis of double-stranded DNA by capillary electrophoresis with laser-induced fluorescence detection using the monomeric dye SYBR Green I. *Anal. Biochem.* **1995**, *231*, 359−365.

(23) Marino, M. A.; Devaney, J. M.; Davis, P. A.; Girard, J. E. Optimization of intercalation dye concentration for short tandem repeat allele genotyping using capillary electrophoresis with laser-induced fluorescence detection. *J. Chromatogr. B* **1999**, *732*, 365−374.

(24) Zabzdyr, J. L.; Lillard, S. UV- and visible-excited fluorescence of nucleic acids separated by capillary electrophoresis. *J. Chromatogr. A* **2001**, *911*, 269−276.

(25) Sambroock, J.; Fristsch, E. F.; Maniatis, T. *Molecular Clonning-A Laboratory Manual*. Cold Spring Harbor Laboratoy Press: Plainview, NY, 1989, p 6.15.

(26) Clark, B. K.; Sepaniak, M. J. Evaluation of on-column labeling with intercalating dyes for fluorescence detection of DNA fragments separated by capillary electrophoresis. *J. Microcolumn Sep.* **1993**, *5*, 275−282.

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