Genetically Modified Organisms in Food—Screening and Specific Detection by Polymerase Chain Reaction

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PCR methods for the detection of genetically modified organisms (GMOs) were developed that can be used for screening purposes and for specific detection of glyphosate-tolerant soybean and insect-resistant maize in food. Primers were designed to amplify parts of the 35S promoter derived from Cauliflower Mosaic Virus, the NOS terminator derived from *Agrobacterium tumefaciens* and the antibiotic marker gene NPTII (neomycin-phosphotransferase II), to allow for general screening of foods. PCR/hybridization protocols were established for the detection of glyphosate-tolerant RoundUp Ready soybean and insect-resistant Bt-maize. Besides hybridization, confirmation of the results using restriction analysis was also possible. The described methods enabled a highly sensitive and specific detection of GMOs and thus provide a useful tool for routine analysis of raw and processed food products.

Keywords: *Polymerase Chain Reaction; PCR; genetically modified organisms; food analysis*

INTRODUCTION

Three years after the introduction on the European market of the first genetically modified organism (GMO) to be processed as such in foodstuffs—the RoundUp Ready soybean of Monsanto Co.—concerns of European consumers about genetically modified organisms in food still have not vanished or even decreased. European consumers are especially skeptical about transgenes in food and demanded a strict regulation for marketing and labeling of such foods. In May 1997, the Novel Food Regulation, which demands labeling of foods that are no longer "substantially equivalent" to their conventional counterparts, came into force. Several products derived from genetically modified organisms such as insect-resistant and herbicide-tolerant maize and herbicide-tolerant oil seed rape have been notified for use in foodstuffs in Europe for the last 2 years. In September 1998, a labeling regulation came into force that regulates the labeling of products containing or consisting of GMOs. Products containing RoundUp Ready soybean (Padgette et al., 1995) or the insect-resistant "Event176" maize (Koziel et al., 1993) are also concerned, although they had been already approved for marketing before the Novel Food Regulation came into force. Detection of the difference between conventional food and food containing or consisting of GMOs, according to the Novel Food Regulation, shall be achieved by applying appropriate scientific methods. The Polymerase Chain Reaction (PCR) has been found to be appropriate for the analysis of food (Meyer et al., 1996; Allmann et al., 1993) and also seems to be the method of choice for the detection of GMOs in food (Schreiber and Bögl, 1997). However, immunological assays such as ELISAs (enzyme-linked immunosorbent assays) for the detection of the proteins expressed by these GMOs are also considered to be of use, at least for raw products.

All over Europe, efforts have been undertaken during the past few years to develop PCR methods for detection of a variety of GMOs such as the FLAVR SAVR tomato (Meyer, 1995), glyphosate-tolerant soybean (Wurz and Willmund, 1997), Bt-maize (Hupfer et al., 1998), a transgenic potato with altered starch composition (Hassan-Hauser et al., 1998), and marker genes (Pietsch et al., 1997). Furthermore, to provide the basis for meeting the requirements for labeling after the planned introduction of a GMO "threshold level" in the European Union, the first quantitative PCR methods for raw products are also being developed (Studer et al., 1998). One of the projects concerned with GMO detection is European project SMT4-CT96−2072: "Development of Methods to Identify Foods Produced by Means of Genetic Engineering". As one of the research and development partners involved in this project, we have developed methods for screening purposes as well as tests for the presence of two kinds of GMO—the Round-Up Ready soybean and insect-resistant maize of Ciba Seeds (now Novartis)—that are suitable for routine analysis. In this study we present the results of the performance testing of these protocols using defined, standardized sample material.

MATERIALS AND METHODS

Soybean Samples. RoundUp Ready (RR) soybeans and conventional soybeans that were used as positive and negative controls were kindly provided by Christian Hertel, University of Hohenheim, Stuttgart, Germany, and by Ilse Theuns, University of Gent, Belgium. Soya meal samples representing different percentages of GMO content (2%, 0.5%, 0.1%, and 0%) used for assessing sensitivity were residues of a 1998 ring trial coordinated by the Joint Research Centre (JRC) in Ispra, Italy, and were prepared at the Joint Research Centre in Geel, Belgium.

Maize Samples. Maize meal from transgenic insect-resistant "Event176" maize used as a positive control in this study was kindly provided by Hermann Rüggeberg, Hanse-Analytik, Bremen, Germany. Conventional maize was purchased at a local store. Maize meal samples representing

Table 1. Oligonucleotide Primers and Probes

primer/probe	sequence	gene	position
LEC1 (forward)	5′-GTG CTA CTG ACC AGC AAG GCA AAC TCA GCG-3′	soybean lectin	70−99 of CDS[a]
LEC2 (reverse)	5′-GAG GGT TTT GGG GTG TGG CCG TTT TCG TCA AC-3′		205−233 of CDS
INVA (forward)	5′-GGC CGG ATC GTC ATG CTC TAC A-3′	maize invertase	984−1005 of CDS
INVB (reverse)	5′-TTG GCG TCC GAC TTG ACC CAC T-3′		1084−1105 of CDS
35SFZ1 (forward)	5′-CCG ACA GTG GTC CCA AAG ATG GAC-3′	CaMV 35S promotor	115−138 in: AN[b]
35SFZ2 (reverse)	5′-ATA TAG AGG AAG GGT CTT GCG AAG G-3′		252−276 in: AN
NOSFZ1 (forward)	5′-GAA TCC TGT TGC CGG TCT TGC GAT G-3′	*Agrobacterium tume-*	406−430 in: AN
NOSFZ2 (reverse)	5′-TCG CGT ATT AAA TGT ATA ATT GCG GGA CTC-3′	*faciens* NOS terminator	522−551 in: AN
NPTFZ1 (forward)	5′-ACC TGT CGG GTG CCC TGA ATG AAC TGC-3′	NPTII	155−181 of CDS
NPTFZ2 (reverse)	5′-GCC ATG ATG GAT ACT TTC TCG GCA GGA GC-3′		322−350 of CDS
CAM (forward)	5′-TCA TTT CAT TTG GAG AGG ACA CG-3′	CaMV 35S promotor	285−307 in: AN
CTP (reverse)	5′-GGA ATT GGG ATT AAG GGT TTG TAT C-3′	*Petunia hybrida* CTP	30−54 of CDS
CRYFZ1 (forward)	5′-CTG GTG GAC ATC ATC TGG GGC ATC TTC G-3′	modified *Bacillus*	178−205 of CDS
CRYFZ2 (reverse)	5′-TTG GTA CAG GTT GCT CAG GCC CTC C-3′	*thuringiensis* CryIA(b)	300−324 of CDS
CTP−S (RR soybean probe)	5′-CCT TGA GCC ATG TTG TTA ATT TGT GCC AT-3′	*Petunia hybrida* CTP	1−29 of CDS
CRY−S (Bt-maize probe)	5′-CAG TGG GAC GCC TTC CTG GTG CAG ATC-3′	modified *Bacillus thuringiensis* CryIA(b)	214−240 of CDS

[a] Coding sequence. [b] Respective accession number given in text.

Table 2. Cycling Conditions

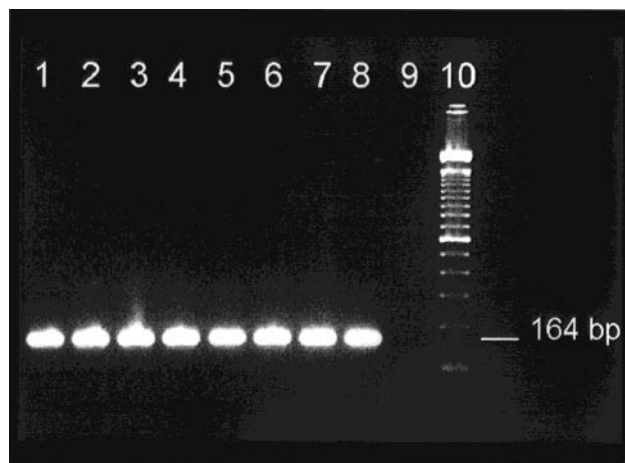| step	35SP−PCR NPTFZ-PCR LEC−PCR	NOSFZ-PCR	INV−PCR	SOJA1-PCR	CRYFZ-PCR
initial denaturation	12 min, 95 °C	12 min, 95 °C	12 min, 95 °C	12 min, 95 °C	12 min, 95 °C
denaturation	1 min, 95 °C	1 min, 95 °C	1 min, 95 °C	1 min, 95 °C	1 min, 95 °C
annealing	30 s, 72 °C	30 s, 68 °C	30 s, 66 °C	30 s, 62 °C	30 s, 70 °C
extension	30 s, 72 °C	30 s, 72 °C	30 s, 72 °C	30 s, 72 °C	30 s, 72 °C
final extension	10 min, 72 °C	10 min, 72 °C	10 min, 72 °C	10 min, 72 °C	10 min, 72 °C

Figure 1. Soya, LEC−PCR: 10 µL of PCR loaded per lane. Lane 1, 2% GMO; lane 2, 0.5% GMO; lane 3, 0.1% GMO; lane 4, 0.01% GMO; lane 5, 0.001% GMO; lane 6, 0% GMO; lane 7, soybean negative control; lane 8, soybean positive control; lane 9, water control; lane 10, 100 bp ladder.
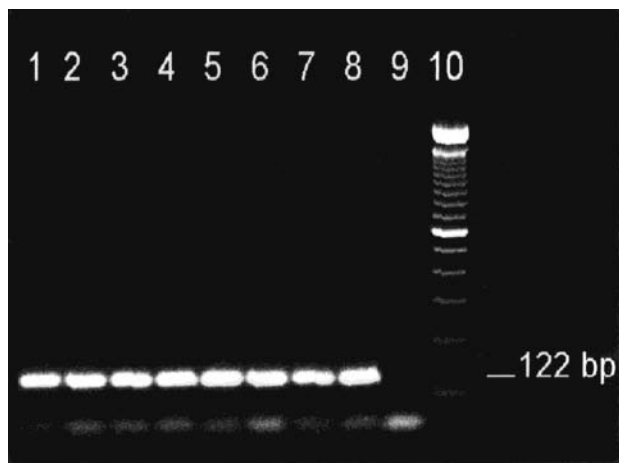

Figure 2. Maize, INV−PCR: 10 µL of PCR loaded per lane. Lane 1, 2% GMO; lane 2, 0.5% GMO; lane 3, 0.1% GMO; lane 4, 0.01% GMO; lane 5, 0.001% GMO; lane 6, 0% GMO; lane 7, maize negative control; lane 8, maize positive control; lane 9, water control; lane 10, 100 bp ladder.

different percentages of GMO content (2%, 0.5%, 0.1%, and 0%) used for assessing sensitivity were residues of the ring trial mentioned above. These soya and maize materials have lately become available as certified reference material (Fluka).

Processed Foodstuff. Samples of processed foodstuffs with unknown GMO content were either purchased at Austrian supermarkets or samples from routine analysis sent to our lab by customers. Samples with known GMO content were samples of an Austrian ringtrial conducted in May 1999, which was organized by the Austrian Federal Institute for Food Control and Research. The percentage of GMO in these samples was quantified by the Austrian Federal Institute for Food Control and Research using TaqMan technology.

NPTII Positive Control. Since no suitable transgenic reference material containing an NPTII gene was available, plasmid pMOG402, which was kindly provided by MOGEN, Inc., Leiden, The Netherlands, was used for performance testing of the NPTFZ-PCR. Dilutions representing different

copy numbers of plasmid were prepared in DNA from soybean (10 ng/µL) to simulate background DNA.

DNA Extraction. For extraction of genomic DNA from the samples, the DNeasy Plant Mini Kit (Qiagen) was used. DNA concentration was measured using a mini-fluorometer (Hoefer Scientific Instruments, model TKO-100). For PCR, DNA concentration was adjusted to 10 ng/µL with sterile distilled water. For the 0.01% and the 0.001% samples, DNA of the 0.1% sample was adequately diluted in DNA from negative controls.

Target Sequences and Primer Oligonucleotides. The DNA sequences of the 35S promoter, the NOS terminator, the NPTII gene, the soybean lectin gene, and the maize invertase gene have been published in the GenBank database (accession numbers: for 35S promoter and NOS terminator, I 08076; for NPTII gene, U 00004; for soybean lectin gene, K 00821; for maize invertase gene, U 16123).

For detection of RR soya, a primer pair was designed that amplifies a fragment covering the junction between the 35S
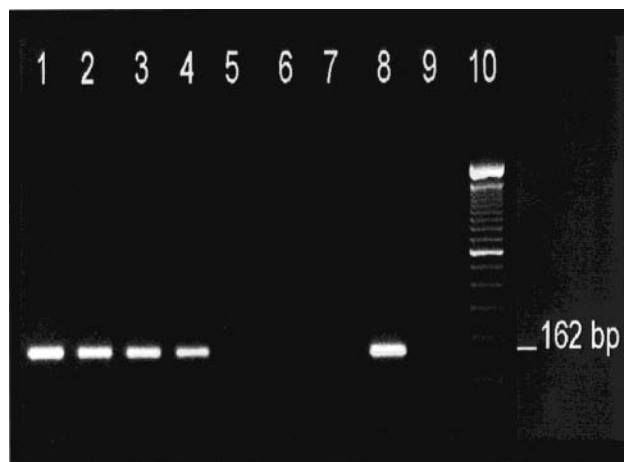
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Figure 3. Soya, 35SP−PCR: 10 µL of PCR loaded per lane. Lane 1, 2% GMO; lane 2, 0.5% GMO; lane 3, 0.1% GMO; lane 4, 0.01% GMO; lane 5, 0.001% GMO; lane 6, 0% GMO; lane 7, soybean negative control; lane 8, soybean positive control; lane 9, water control; lane 10, 100 bp ladder.
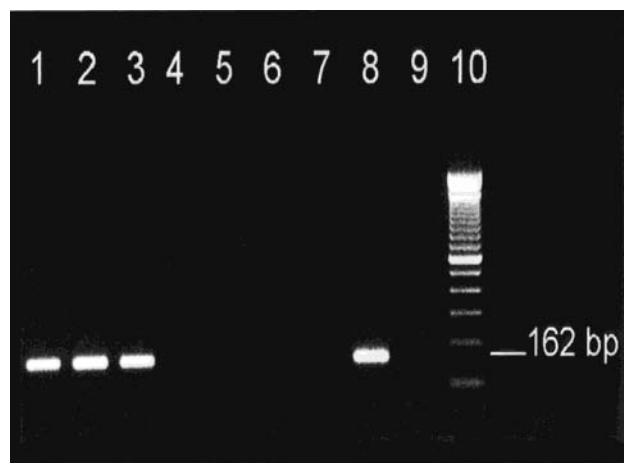

Figure 5. EcoRV restriction digestion of 35SP−PCR fragments. Total digestion reaction loaded. Lanes 1−3, positive maize samples; lane 4, maize positive control; lane 5, Molecular Weight Marker V; lanes 6−9, positive soya samples; lane 10, soybean positive control.

Figure 4. Maize, 35SP−PCR: 10 µL of PCR loaded per lane. Lane 1, 2% GMO; lane 2, 0.5% GMO; lane 3, 0.1% GMO; lane 4, 0.01% GMO; lane 5, 0.001% GMO; lane 6, 0% GMO; lane 7, maize negative control; lane 8, maize positive control; lane 9, water control; lane 10, 100 bp ladder.

Figure 6. Soya, NOSFZ-PCR: 10 µL of PCR loaded per lane. Lane 1, 2% GMO; lane 2, 0.5% GMO; lane 3, 0.1% GMO; lane 4, 0.01% GMO; lane 5, 0.001% GMO; lane 6, 0% GMO; lane 7, soybean negative control; lane 8, soybean positive control; lane 9, water control; lane 10, 100 bp ladder.

promoter and the chloroplast transit peptide (CTP) sequence derived from the *Petunia hybrida* 5-enol-pyruvylshikimate-3-phosphate-synthase (EPSPS) gene. This leader sequence has been fused to the *Agrobacterium sp.* CP4 EPSPS gene in the RR soybean (Padgette et al., 1995). The complete sequence of the *P. hybrida* EPSPS gene has been published in the GenBank database (accession number, M 21084) and in the literature (Gasser et al., 1988).

The modified sequence of the Bt toxin gene (CryIA(b) gene) originally derived from *Bacillus thuringiensis* var. Kurstaki HD1) present in transgenic "Event 176" maize was kindly provided by Christine Hupfer, Technical University of Munich, Freising-Weihenstephan, Germany. Primer pairs and probes were designed using the PC/GENE software of IntelliGenetics, Inc., and synthesized by Vienna Biocenter, Vienna, Austria. Sequences of primers are listed in Table 1.

Polymerase Chain Reaction. Amplification reactions were carried out in a 25 µL total volume on a PTC-100 thermocycler (MJ Research, Inc., Watertown, MA). End concentrations of PCR components were as follows: PCR buffer (Perkin-Elmer), 1×; $MgCl_2$, 2.5 mM; dNTPs, 0.2 mM each; primers, 1 µM each; Amplitaq Gold Polymerase (Perkin-Elmer), 2.5 U/reaction. PCRs were performed using 5 µL (50 ng) of DNA.
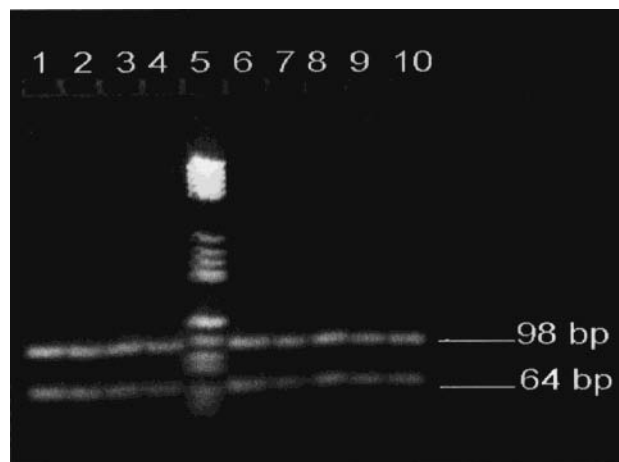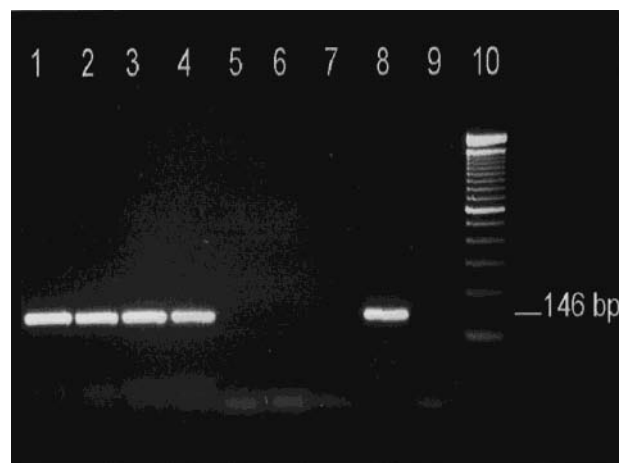
PCR Conditions. Cycling conditions for the different PCR reactions are listed in Table 2. The cycling number was 50 in all cases.

Agarose Gel Electrophoresis. Agarose gel electrophoresis was carried out according to Sambrook et al. (1989) using TBE buffer. After completion of PCR, 10 µL of each sample was loaded on a 2% agarose gel. A 100 bp ladder (Gibco BRL) was used for size control of amplified fragments. The complete restriction analysis reactions (20 µL each) were loaded on 4% agarose gels. Molecular Weight Marker V (Boehringer Mannheim) was used for size control of restriction fragments.

Southern Blot and Hybridization. DNA from agarose gels was blotted onto positively charged nylon membranes (Boehringer Mannheim) according to standard methods (Sambrook et al., 1989). Digoxigenin-labeled oligonucleotide probes were used for hybridization. The sequences of the CTP−S probe for RR soya and the CRY−S probe for Bt-maize are shown in Table 1. Hybridizations were carried out in sealed plastic bags in a shaking water bath as follows: prehybridization in 5×SSC containing 1 g/L N−lauroylsarcosine, 0.2 g/L SDS, 10 g/L Blocking Reagent (Boehringer Mannheim) for 1 h at 50 (RR soybean) or 55 °C (Bt-maize), hybridization in the same solution containing 200 ng/mL probe at the same respective temperature for 3 h, washing with 2×SSC, 0.1% SDS for 5 min at ambient temperature (2 times), then with

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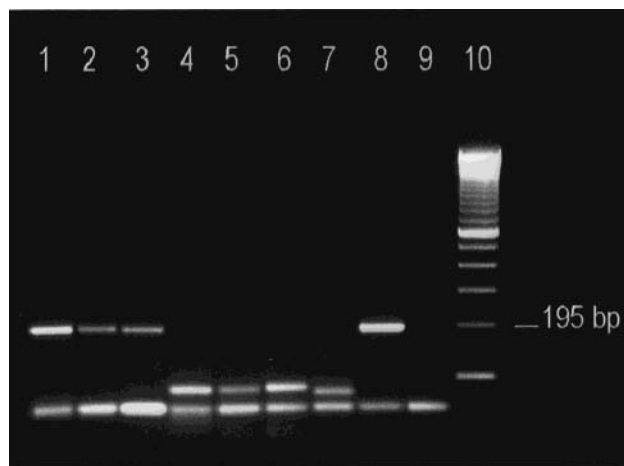*J. Agric. Food Chem.,* Vol. 47, No. 12, 1999 **5041**

Figure 7. Plasmid pMOG402 in soybean DNA, NPTFZ-PCR: 10 μL of PCR loaded per lane. Lane 1, 500 fg plasmid; lane 2, 50 fg plasmid; lane 3, 5 fg plasmid; lane 4, 500 ag plasmid; lane 5, 50 ag plasmid; lane 6, 5 ag plasmid; lane 7, soybean DNA; lane 8, positive control (50 pg plasmid); lane 9, water control; lane 10, 100 bp ladder.

Figure 9. Southern blot and hybridization of SOJA1-PCR products. Lanes 1–10 correspond to lanes 1–10 in Figure 8.

Figure 8. Soya, SOJA1-PCR: 10 μL of PCR loaded per lane. Lane 1, 2% GMO; lane 2, 0.5% GMO; lane 3, 0.1% GMO; lane 4, 0.01% GMO; lane 5, 0.001% GMO; lane 6, 0% GMO; lane 7, soybean negative control; lane 8, soybean positive control; lanes 9 and 10, water controls; lanes 11 and 12, 100 bp ladder.

0.1×SSC, 0.1% SDS for 20 min at the respective hybridization temperature (2 times). Immunological detection of probe bound to target was performed according to the instructions of the DIG DNA Labeling and Detection Kit of Boehringer Mannheim.

Restriction Analysis. To 15 μL of PCR fragment, 2 μL of the respective restriction buffer, 2 μL of sterile water, and 1 μL (10 U) of the respective restriction enzyme was added. The reaction was incubated at 37 °C for at least 3 h.

RESULTS AND DISCUSSION

DNA Extraction. Using the DNeasy Plant Mini Kit, high-quality DNA could be extracted from the samples (not shown). High purity of the extracted DNA is an important prerequisite for further analysis, and commercially available kits seem to be best suited to meet this demand (Zimmermann et al., 1998). Standard methods are also applicable in many cases and are much cheaper. One of the disadvantages of commercial kits besides their high cost is that they cannot be used with samples that contain very small amounts of DNA, since scaling up is not possible in most cases. Raw products
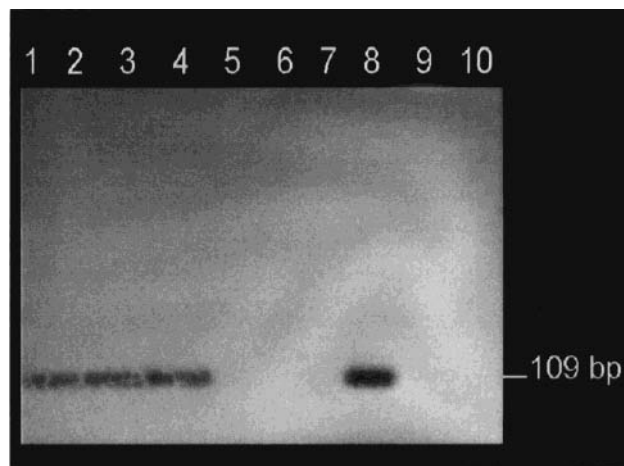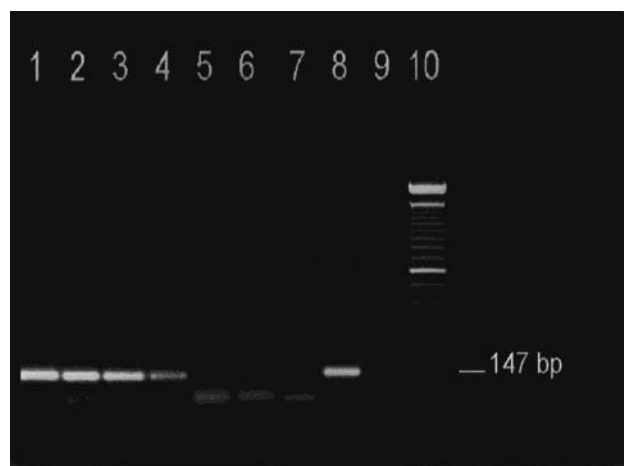

Figure 10. Maize, CRYFZ-PCR: 10 μL of PCR loaded per lane. Lane 1, 2% GMO; lane 2, 0.5% GMO; lane 3, 0.1% GMO; lane 4, 0.01% GMO; lane 5, 0.001% GMO; lane 6, 0% GMO; lane 7, maize negative control; lane 8, maize positive control; lane 9, water control; lane 10, 100 bp ladder.

and products that are not too highly processed can, however, be extracted very conveniently with this method. Nevertheless, difficulties in finding the optimal extraction method for a given food matrix still pose a problem for routine testing.

Amplifiability of DNA. In Figures 1 and 2, PCR results of the LEC–PCR for soybean and the INV–PCR for maize are shown. Both PCR protocols are suitable for controlling the amplifiability of DNA derived from soybean and maize samples. Primers had been checked for homologies with other sequences published in the GeneBank database using a BLAST search to rule out as far as possible the eventuality of the formation of PCR products with samples from plants other than soybean or maize, and no substantial similarities to other sequences could be detected. However, it has to be kept in mind that, nevertheless, there might be sequence homologies with genes of various plant species. This fact can pose problems when quantitative PCR protocols are applied to complex food matrices and when the percentage of GMO, for instance transgenic maize, in a sample must be calculated after quantification of maize DNA present in total sample DNA. Thus, the availability of a sequence that is unique for a specified plant will be a prerequisite for reliable quantitative protocols for complex food matrices. No problems for

Table 3. PCR, Hybridization, and Restriction Analysis

PCR	fragment length	sensitivity	hybridization	restriction enzyme	restriction fragment lengths
LEC	164 bp				
INV	122 bp				
35SP	162 bp	0.1−0.01% GMO	no	*Eco*RV	98 bp + 64 bp
NOSFZ	146 bp	0.01% GMO	no	*Afl*III	74 bp + 72 bp
NPTFZ	195 bp	500 copies (plasmid)	no	*Pvu*II	114 bp + 81 bp
SOJA1	109 bp	0.01% GMO	yes	*Bgl*II	66 bp + 43 bp
CRYFZ	147 bp	0.01% GMO	yes	*Pvu*II	78 bp + 69 bp

Table 4. GMO Detection in Processed Food Samples

sample	amount extracted	extraction method	DNA concentration (ng/μL)	amount of DNA used for PCR (ng)
hazelnut-nougat-cream	100 mg	Qiagen	< 10	< 50
soya cubes	100 mg	Qiagen	< 10	< 50
soya protein	35 mg	Qiagen	< 10	< 50
specialty food for tube feeding	100 mg	Qiagen	< 10	< 50
paste food containing soya meal	2 g	CTAB	30	30
soya drink	5 mL	CTAB	< 10	< 50
strawberry roll	1 g	CTAB	20	100
spring roll	1 g	CTAB	100	500
chicken sticks	1 g	CTAB	130	650
toast ham	2 g	CTAB	300	300
wholemeal soya spaghetti	2 g	CTAB	1600	250
instant diet food (banana), approx. 10% GMO	100 mg	CTAB	20	100
instant diet food (chocolate), approx. 15% GMO	100 mg	CTAB + purification	20	100
soya meal, < 0.05% GMO	100 mg	CTAB	50	250
pretzel containing soya meal, > 10% GMO	1 g	CTAB	< 10	< 50
corn chips[a]	1 g	CTAB	10	10
cheese chips[a]	1 g	CTAB	10	10

[a] Samples positive for Bt-maize. All other samples positive for presence of RR soya.

quantification occur when the sample consists entirely of the plant species under investigation or when, like in this study, only the amplifiability of DNA has to be controlled prior to GMO detection.

PCR, Hybridization, and Restriction Analysis. A short summary of the results is shown in Table 3. In Figures 5 and 9, results of a restriction analysis and a hybridization, respectively, are shown as an example.

35SP−PCR. Figures 3 and 4 show the results of the 35SP−PCR using soya and maize meal samples representing different percentages of GMO. For soybean, the limit of detection was 0.01% GMO and for maize 0.1% GMO under the conditions described above. Taking into account the genome size (1 C) of soya, which has been reported to be approximately 1.134 pg (Greilhuber and Obermayer, 1997), this means that about 4 copies of the 35S promoter could be detected in the soya samples. No unspecific fragments were amplified under the chosen conditions. The 35SP−PCR led to amplification of the predicted 162 bp fragment that was cleaved by EcoRV, resulting in two fragments of 98 and 64 bp length (Figure 5).

NOSFZ-PCR. This PCR resulted in the formation of a 146 bp fragment (Figure 6). The identity of the amplicon could be confirmed by restriction digestion with AflIII, yielding two fragments of 72 and 74 bp (not shown). 0.01% GMO could be detected.

NPTFZ-PCR. Results of the NPTFZ-PCR are shown in Figure 7. Under the chosen conditions, 500 copies of the plasmid could be detected in a background of 50 ng soybean DNA, a result that was not as sensitive as the results of the other PCRs. Due to this lower sensitivity, the specific band is weaker and more unincorporated primer is visible. Furthermore, a low molecular weight unspecific band appears as the concentration of specific target decreases. However, a definitive statement concerning the sensitivity and specificity of this PCR can

be made only after analysis of suitable transgenic reference material in a more realistic setting. The expected length of the PCR product is 195 bp. The fragment was cleaved by restriction enzyme *Pvu*II, yielding two fragments of 114 and 81 bp length (not shown).

SOJA1-PCR for RoundUp Ready Soybean. In Figure 8, results of the SOJA1-PCR are presented. The identity of the expected 109 bp PCR fragment could be confirmed by hybridization with the specific CTP−S probe (Figure 9). Again, 0.01% GMO could be detected. Restriction digestion of the amplicon with *Bgl*II resulted in two fragments of 66 and 43 bp length (not shown).

CRYFZ-PCR for Modified Bt Toxin Gene. GMO (0.01%) was detected with the CRYFZ-PCR, as shown in Figure 10. Again, this was very close to the theoretical detection limit considering that the maize genome has been reported to have an approximate size (1 C) of 2.685 pg (Michaelson et al., 1991), and a few copies of the CryIA(b) gene are present in "Event176" maize (Koziel et al., 1993). As in Figure 7, some low molecular weight unspecific bands can be seen, most probably representing primer artifacts preferentially forming as the copy number of target decreases. Confirmation of the result by hybridization was possible using probe CRY−S (not shown). Restriction digestion of the 147 bp fragment with restriction enzyme *Pvu*II yielded two fragments of 78 and 69 bp length, respectively (not shown). It has to be noted that this PCR will not only detect "Event176" maize, but also other transformation events containing the same modified Bt toxin sequence.

With the described PCR/hybridization protocols for RoundUp Ready soybean and for Bt-maize, the respective GMOs could also be identified in a variety of processed foods in our laboratory. Table 4 shows some

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examples. DNA was extracted using a modified CTAB (*N*-cetyl-*N,N,N*-trimethylammonium bromide) protocol, and in one case the DNA had to be further purified for successful amplification (Vollenhofer et al., manuscript in preparation).

Although more and more sophisticated methods for DNA extraction from highly processed products are being developed, identification of GMOs in processed food remains to be very challenging due to low DNA content, highly degraded DNA, and the presence of inhibitors of PCR. Thus, the DNA extraction method and PCR detection method are equally important for successful analysis.

CONCLUSION

The sensitivity of most of the PCR protocols described here was very high, allowing the detection of very low copy numbers. Due to their high annealing temperatures, the primer pairs used in this study also showed a high specificity in the PCR reactions, which facilitates confirmation by restriction analysis, where unspecific fragments are particularly disturbing. Furthermore, we have tried to select primer pairs that would allow restriction enzyme digestion of the PCR product using one of the cheaper restriction enzymes in order to reduce the costs of the analysis. None of the amplified fragments is longer than 200 bp, so analysis of degraded DNA from highly processed products should not pose a serious problem. The PCR, hybridization, and restriction digestion protocols presented in this study should provide a very useful tool for routine GMO detection in food.

ACKNOWLEDGMENT

LITERATURE CITED

Allmann, M.; Candrian, U.; Höfelein, C.; Lüthy, J. Polymerase Chain Reaction (PCR): a possible alternative to immunochemical methods ensuring safety and quality of food. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 248−251.

Gasser, C. S.; Winter, J. A.; Hironaka, C. M.; Shah, D. M. Structure, Expression, and Evolution of the 5-Enolpyruvylshikimate-3-phosphate Synthase Genes of Petunia and Tomato. *J. Biol. Chem.* **1988**, *263* (9), 4280−4289.

Greilhuber, J.; Obermayer, R. Genome size and maturity group in *Glycine max* (soybean). *Heredity* **1997**, *78*, 547−551.

Hassan-Hauser, C.; Mayer, W.; Hörtner, H. Detection of the starch modifying *gbss*-antisense construct in transgenic potatoes. *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 83−87.

Hupfer, C.; Hotzel, H.; Sachse, K.; Engel, K.-H. Detection of the genetic modification in heat-treated products of Bt maize by polymerase chain reaction. *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 203−207.

Koziel, M. G.; Beland, G. L.; Bowman, C.; Carozzi, N. B.; Crenshaw, R.; Crossland, L.; Dawson, J.; Desai, N.; Hill, M.; Kadwell, S.; Launis, K.; Lewis, K.; Maddox, D.; McPherson, K.; Meghji, M. R.; Merlin, E.; Rhodes, R.; Warren, G. W.; Wright, M.; Evola, S. V. Field Performance of Elite Transgenic Maize Plants Expressing an Insecticidal Protein Derived from *Bacillus thuringiensis. Biotechnology* **1993**, *11*, 194−200.

Meyer, R. Detection of genetically engineered plants by polymerase chain reaction (PCR) using the FLAVR SAVR tomato as an example. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 583−586 (German).

Meyer, R.; Chardonnens, F.; Hübner, P.; Lüthy, J. Polymerase chain reaction (PCR) in the quality and safety assurance of food: detection of soya in processed meat products. *Z. Lebensm. Unters. Forsch.* **1996**, *203*, 339−344.

Michaelson, M. J.; Price, H. J.; Ellison, J. R.; Johnston, J. S. Comparison of Plant DNA Contents Determined by Feulgen Microspectrophotometry and Laser Flow Cytometry. *Am. J. Bot.* **1991**, *78* (2), 183−188.

Padgette, S. R.; Kolacz, K. H.; Delannay, X.; Re, D. B.; LaVallee, B. J.; Tinius, C. N.; Rhodes, W. K.; Otero, Y. I.; Barry, G. F.; Eichholtz, D. A.; Peschke, V. M.; Nida, D. L.; Taylor, N. B.; Kishore, G. M. Development, Identification, and Characterization of a Glyphosate-Tolerant Soybean Line. *Crop Sci.* **1995**, *35*, 1451−1461.

Pietsch, K.; Waiblinger, H. U.; Brodmann, P.; Wurz, A. Screeningverfahren zur Identifizierung "gentechnisch veränderter" pflanzlicher Lebensmittel. *Dtsch. Lebensm. Rundsch.* **1997**, *93*, 35−38 (German).

Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

Foods Produced by Means of Genetic Engineering, 2nd Status Report; Schreiber, G. A., Bögl, K. W., Eds.; Federal Institute for Health Protection of Consumers and Veterinary Medicine: Berlin, 1997; BgVV Hefte 1.

Studer, E.; Rhyner, C.; Lüthy, J.; Hübner, P. Quantitative competitive PCR for the detection of genetically modified soybean and maize. *Z. Lebensm. Unters. Forsch.* **1998**, *207*, 207−213.

Vollenhofer, S.; Burg, K.; Schmidt, J.; Kroath, H. Short Communication: Detection of Genetically Modified Organisms in Food. *Dtsch. Lebensm. Rundsch.* **1999**, *95*, 275−278.

Wurz, A.; Willmund, R. Identification of Transgenic Glyphosate-resistant soybeans. In *Foods Produced by Means of Genetic Engineering, 2nd Status Report*; Schreiber, G. A., Bögl, K. W., Eds.; Federal Institute for Health Protection of Consumers and Veterinary Medicine: Berlin, 1997; BgVV Hefte 1, pp 115−117.

Zimmermann, A.; Lüthy, J.; Pauli, U. Quantitative and qualitative evaluation of nine different extraction methods for nucleic acids on soya bean food samples. *Z. Lebensm. Unters. Forsch.* **1998**, *207*, 81−90.