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Detection of Genetically Modified Soybeans and Maize by the Polymerase Chain Reaction Method

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ABSTRACT

The feasibility of detecting genetically modified (GM) soybeans and GM maize by a polymerase chain reaction (PCR) method is determined. Primers specific for inserted genes and crop endogenous genes in Roundup Ready soybeans (Monsanto company) and Event 176 GM maize (Novartis/Ciba-Geigy company) were applied. Four pairs of primers, namely, 35S (35S-promoter, originated from cauliflower mosaic virus), NOS (nopaline synthase-terminator, derived from *Agrobacterium tumefaciens*), EPSPS (5-enolpyruvylshikimate-3-phosphate synthase, obtained from *A. tumefaciens* strain CP4) and LE (endogenous gene lectin) were used to identify the GM soybeans. An additional three pairs of primers, including CDPK-cry (pollen-specific calcium-dependent protein kinase promoter - delta-endotoxin, acquired from maize and *Bacillus thuringiensis* subsp. *kurstaki*, respectively), cryIA(b) (delta-endotoxin, evolved from *B. thuringiensis* subsp. *kurstaki*) and ivr (endogenous gene invertase) were directed to confirm the GM maize. Using 35S and EPSPS as primers, the method showed a limit of detection for samples containing 0.1% (w/w) of GM soybeans and containing 1% (w/w) of GM soybeans when NOS primers were applied. All soybean samples were evidenced by LE primer-PCR as soybean products. Detection limits of 0.1% (w/w) of GM maize in raw material using CDPK-cry primers and 2% (w/w) of GM maize with cryIA(b) were established. The maize products were also approved by Invertase primer-PCR. To further confirm detection of the target GM soybean by PCR, the 195 bp fragment, amplified from 35S-PCR, digested with endonuclease *Xmn*I resulted in 80 and 115 bp fragments, while digesting the 180 bp amplified products from NOS-PCR using endonuclease *Nsi*I yielded 96 and 84 bp fragments. The data further revealed that the PCR method can sufficiently differentiate GM soybeans and maize from non-GM products.

Key words: GM-soybean, GM-maize, PCR

INTRODUCTION

Genetically modified foods (GMF) are foods or additives that are produced or treated via a gene modification technique[1]. Genetically modified organisms (GMO) are the organisms in which genes are altered by a gene modification technique but not by natural mating or DNA recombination[1]. In recent years, global gene modification techniques have been greatly improved. Some insect-resistant and herbicide-tolerant genetically modified-crops (GM-crops) have been successfully developed and widely cultivated. The United States is presently the most advanced country in R & D and commercialization of GM-crops. So far, there are more than 50 GM-crop products approved by the United States government[2]. According to data from the OECD, the most popular GM-crops for field trials are: soybeans, tomatoes, maize, potatoes, wheat, cotton, sugar beets, oilseed rape, cucumbers, melons, alfalfa, lettuce, sunflowers, rice, and tobacco[2]. Cultivation of GM-crops in Taiwan has not yet been employed, however, field trials for GM-papayas and GM-tomatoes have been permitted[3]. The purpose of developing genetically modified crops is to produce desired results by altering specific traits of crops. In general, the research objectives of producing GM-crops can be classified into four categories: (1) to improve product quality: color, tomato ripening delay, and high starch maize production; (2) pest resistance: moth-resistant cotton, virus-resistant tobacco, and insect-resistant maize; (3) agronomic traits: drought resistant maize, and herbicide-tolerant soybean or maize; (4) others: heavy metal tolerance[4]. According to statistical data in 1998, 99% of GM-crops are cultivated in America, with United States accounting for 74%. This statistical data excludes the Asia region such as mainland China[2]. There is no international consensus of support for GMF. Some countries such as India and China encourage farmers to produce GM-crops; however, others, especially German-speaking countries, are opposed[4].

Up to 1999, 3 varieties of GM-soybeans have been approved for commerce by some countries. One is approved by the European Community, one is approved by Japan and the United States government approves all 3 varieties[2]. The traits of soybeans have been modified to become more herbicide tolerant and have a high content of oleic acid[2,5]. They are continually modified to produce lower saturated fatty acids and to increase stearic acid[6]. In 1995, global production of soybeans was 123.65 million tons and the United States accounted for 47.3% of the total. In 1999, 18.4 million hectares of farmland in the United States were cultivated with GM-soybeans or 65% of the total amount of soybeans. The Roundup Ready GM-soybean is now the major GM-soybean in the United States[5]. According to data from the ROC Council of Agriculture, Taiwan imports about 200 tons of soybeans annually, 95% of which are from the US[3]. Based

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Journal of Food and Drug Analysis, Vol. 8, No. 3, 2000

on this data, it is estimated that about 50% of commercially available soybeans in Taiwan are GM-soybeans.

In total, 14 varieties of GM-crops have been approved for commerce as of 1999. The European Community has approved 4, Japan has approved 4, and the United States has approved all 14 varieties[2]. The major traits of GM-maize are insect resistance, herbicide tolerance, and male-sterility[2,4,7]. Research continues for improving the following characteristics of GM-maize: (1) adaptability to harsh growing conditions, such as drought and soil with high salt content or heavy metals; (2) alteration of compositions, such as increasing specific amino acids (lysine, methionine, and tryptophan), and altering protein and fatty acid compositions[7]. The United States and China were ranked as the top two leading countries in maize production in 1998; 42% and 21% of global maize production, respectively[2]. In 1998, the global area for GM-maize production was 7.84 million hectares. Among those areas, 7.5 million hectares (95.6%) were in the US, while 300,000 hectares (3.8%) were in Canada. GM-maize cultivated in the US is mostly insect-resistant (6 million hectares) and some varieties are herbicide-tolerant or other (1.5 million hectares)[7]. In 1999, the areas for cultivating GM-maize in the US were further expanded to 10 million hectares; accounting for 31% of total maize production in that year[6]. Statistical data from the ROC Council of Agriculture shows that Taiwan imports about 600 tons of maize annually, 96% from the US[3]. Based on this data, about 30% of commercial maize in Taiwan is estimated to be GM-maize.

Methods for the identification of GMF can be divided into 3 categories[4]. The first category is nucleotide-based amplification methods, which include the polymerase chain reaction (PCR), ligase chain reaction (LCR), nucleic acid sequence-based amplification (NASBA), fingerprinting techniques (RFLP, AFLP, and RAPD, etc), probe hybridization, self-sustained sequence replication (3SR), and Q replicase amplification. The second category is protein-based methods including one-dimensional SDS gel electrophoresis, two-dimensional SDS gel electrophoresis, Western-blot analysis, and ELISA. The third category includes methods based on the detection of enzymatic activities. Every detection method has its own specificity and limitations. For example, the detection of enzymatic activities method is not recommended for processed foods, where proteins may be denaturalized. The methods based on PCR are not suitable for detection of highly processed foods because DNA fragments in foods could be broken into pieces[4]. Nevertheless, PCR is the most popular method used worldwide among the above 3 method categories. Using the PCR method to identify GM products, a primer is designed based on the regulatory sequence or structural gene in the inserted gene fragment. These designed primers possess some specific characteristics and can be used for product screening and product-specificity detection[4]. The PCR products need to be further confirmed by the following methods: nucleic acid sequencing, endonuclease mapping, and probe hybridization[4]. The PCR method is not only used for identification of GM products, but also for quantification purposes[8]. A new regulation that requires labeling of GMF has become mandatory in some European countries (such as Germany and Switzerland). Research on the PCR method for identification and quantification of GMOs in foodstuffs has been extensively carried out by a number of European countries[8]. In 1997, 29 laboratories in 13 European countries performed a collaborative trial study for detection of GM-maize and GM-soybeans. Results showed that using a PCR method with 35S-promoter primer was capable of detecting 2% of Event 176 GM-maize or Roundup Ready GM-soybeans in maize or soybean flours, respectively[9]. Some German researchers (Ehlers *et al*.) have reported that the selected primers derived from *ivr1*, *cry* IA(b), *bar*, 35S-*bar*, and ampR genes can be successfully used to detect Event 176 GM-maize[10,11]. Some European countries such as Switzerland have developed a process to detect GMOs in foods using the PCR method as shown in Figure 1. Food samples are screened by a 35S-promoter primer-PCR test. The samples with positive reaction are further confirmed by a PCR test using a primer with a specific characteristic to samples. In addition, some ELISA kits for screening Event 176 GM-maize and Roundup Ready GM-soybeans are now commercially available [12].

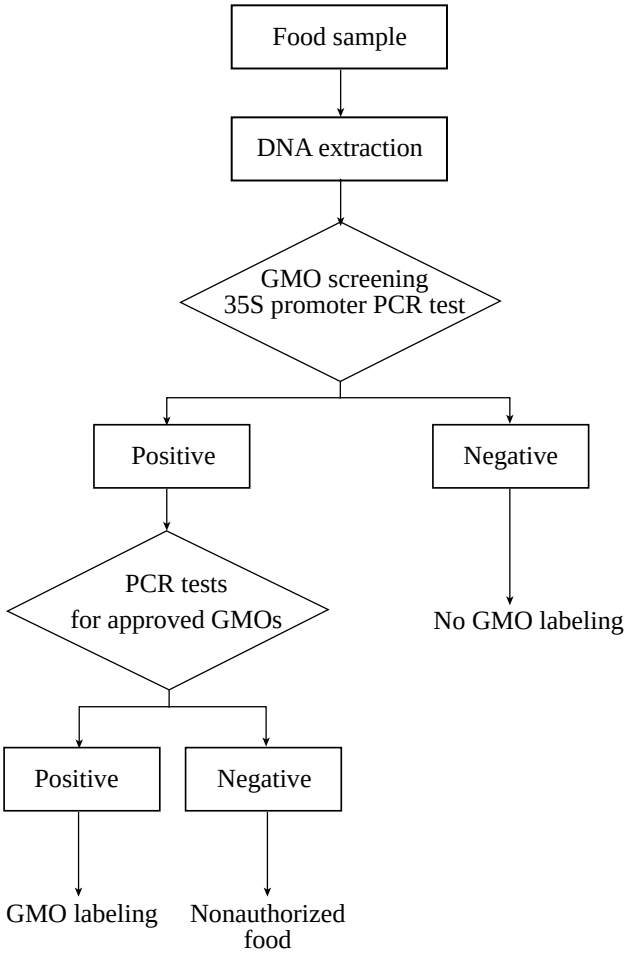

Figure 1. Flow chart of PCR method for detection of GMO in foods. (adapted from Huebner, P. etc. 1999)

According to reports, the primer used for PCR detection of GMO in foods is designed for one specific purpose only, such as screening[9] or specific trait detection[10,11,13]. The development of suitable primers for the above two purposes in one test has not been reported yet. Neither have their been research reported comparing the 2 primers, CDPK-*cry* and *cry*IA(b), for specific trait detection of GM-maize products. In this study, several PCR primers were selected, according to the literature, for the purpose of screening as well as specific trait detection to study the feasibility of detecting GM-soybeans and GM-maize products. The developed method could be a reference method for detection of GMF in Taiwan. The results of this study could also provide useful information for related authorities to regulate the labeling of GM food products. (Portions of this work were presented at the 38th Annual Meeting of the Chinese Agricultural Chemical Society, Taipei, Taiwan, June 16, 2000.)

MATERIALS AND METHODS

I. *Chemicals*

Chloroform and isopropanol were purchased from Merck Co. (Darmstadt, Germany). Hexadecyltrimethyl ammoniumbromide (CTAB) was obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Restriction enzymes *Xmn*I and *Nsi*I were purchased from Boehringer Co. (Mannheim, Germany) and Agarose was obtained from Amresco Co. (Solon, Ohio, USA).

II. *GM-Soybean and GM-Maize Standards*

Roundup Ready GM-soybean (Monsanto, U.S.A.) with 0%, 0.1%, 0.5%, 1%, 2%, and 5% (w/w) GMO contents and Event 176 GM-maize (Novartis/Ciba-Geigy, U.S.A.) with 0%, 0.1%, 0.5%, 1%, 2%, and 5% (w/w) GMO contents were obtained from Fluka Chemical Co. (Switzerland). The characteristics of these two standards are listed in Table 1.

III. *Equipment*

PCR thermal controller model PTC-100 with programmable thermal controller was purchased from MJ Research Co. (Water Town, MA, USA).

IV. *PCR Primers and Reagents*

Seven pairs of PCR primers as listed in Table 2 were used in this study. The primers of 35S, NOS, EPSPS, and LE were used to detect 35S-promoter, NOS-terminator, EPSPS structure gene region, in enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene existed in Roundup Ready GM-soybean, and Lectin gene, respectively. The primers of CDPK-cry, cryIA(b), and ivr are specific to regulatory sequence-structure gene, structure gene region, in Delta-Endotoxin gene existed in Event 176 GM-maize, and Invertase gene. These primers were synthesized by TIB Molbiol Co. (Berlin, Germany). DynaZyme DNA Polymerase kit was obtained from Finnzymes Co. (Espoo, Finland).

V. *DNA Preparation and Purification*

An extraction and purification method reported by Lipp *et al*. in 1999[9] was adopted in this study. DNA in test samples (25 mg) was extracted with CTAB. After precipitation, DNA was purified with chloroform and precipitated out with isopropanol.

VI. *PCR Reactions, and Products Analysis and Confirmation*

PCR reagent was prepared by mixing 25 μL of water with DynaZyme DNA Polymerase kit, which is composed of 5 μL of 10-folds buffer solution containing 1.5 mM Mg^{2+}, 1.5 μL of dNTP (200 μM), DNA polymerase (2.5 unit), and 1 μL

Table 1. Characteristics of Roundup Ready Soybean and Event 176 Bt-Maize (modified from http://www.bats.ch.)

Tradename	Characteristic	Inserted gene		
		Promoter	Structure	Terminator
Roundup Ready Soybean (Monsanto)	Gly[a]	P-35S[b]	CP4EPSPS[c]	nos 3'[d]
Event 176	ECB[e]	1) P-PEPC[g], P-CDPK[h]	1) two synthetic,	1) I9[l], T-35S[m]
Bt- Maize	GA[f]	2) P-35S	truncated *cryIA(b)*[i]	
(Novartis)		3) bacterial	2) *bar*[j]	
(Ciba-Geigy)			3) *bla*[k]	

[a] Gly: glyphosate herbicide tolerant.
[b] P-35S: promoter from the cauliflower mosaic virus.
[c] CP4EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium tumefaciens strain* CP4.
[d] nos 3': terminator of nopaline synthase gene from *Agrobacterium tumefaciens.*
[e] ECB: european corn borer (lepidopteran insect resistant).
[f] GA: phosphinothricin (glufosinate ammonium) herbicide tolerant, GA as selective trait.
[g] P-PEPC: green tissue-specific phosphoenolpyruvate carboxylase (PEPC) promoter from maize.
[h] P-CDPK: pollen-specific calcium-dependent protein kinase (CDPK) promoter from maize.
[i] *cryIA(b)*: delta-endotoxin from *Bacillus thuringiensis* subsp. *kurstaki.*
[j] *bar*: gene coding for a phosphinothricin acetyltransferase from *Streptomyces hygroscopicus.*
[k] *bla*: beta-lactamase gene; conveys resistance to beta-lactam antibiotics; from Tn3.
[l] I9: intron 9 from corn PEPC gene.
[m] T-35S: terminator of the 35S gene from the cauliflower mosaic virus.

Journal of Food and Drug Analysis, Vol. 8, No. 3, 2000

Table 2. Primers used in this study

Primer	Sequence 5'-3'	Gene	Amplicon (bp)	Reference
35S-1	GCT CCT ACA AAT GCC ATC A	35S		
35S-2	GAT AGT GGG ATT GTG CGT CA	promoter	195	(9)
NOS-1	GAA TCC TGT TGC CGG TCT TG	NOS		
NOS-3	TTA TCC TAG TTT GCG CGC TA	terminator	180	(9)
EPSPS-B1	TGA TGT GAT ATC TCC ACT GAC G			
EPSPS-B2	TGT ATC CCT TGA GCC ATG TTG T	CP4EPSPS[a]	172	(13)
LE103	GCC CTC TAC TCC ACC CCC ATC C			
LE104	GCC CAT CTG CAA GCC TTT TTG TG	Lectin	118	(13)
CDPK-cry 03	CTC TCG CCG TTC ATG TCC GT	CDPK-[b]		
CDPK-cry 04	GGT CAG GCT CAG GCT GAT GT	Delta-Endotoxin	211[c]	(14)
cryIA(b)-1	ACC ATC AAC AGC CGC TAC AAC GAC C	Delta-Endotoxin		
cryIA(b)-2	TGG GGA ACA GGC TCA CGA TGT CCA G		184	(11)
ivr1-1	CCG CTG TAT CAC AAG GGC TGG TAC C			
ivr1-2	GGA GCC CGT GTA GAG CAT GAC GAT C	Invertase	226	(11)

[a] CP4EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase from *A. tumefaciens* strain CP4.

[b] CDPK: calcium-dependent protein kinase promoter.

[c] The amplicon includes the last 73 bp of the CDPK promoter and the first 138 bp of the N-terminus of the *cryIA(b)* gene.

of each primer (100 μM). PCR reaction was performed by spiking 10 μL of DNA extracts into a centrifugation tube where the PCR reagent was then added. The DNA in centrifugation tube was incubated in a PCR thermocycler under the following program: 95˚C for 5 min followed by 95˚C for another 20 sec, 57˚C for 40 sec (57˚C for 35S, NOS, EPSPS, LE, and ivr primers; while 63˚C for CDPK-cry and cryIA(b) primers), and 72˚C for 1 min (in total, 40 cycles of above program was performed), and finally at 72˚C for 3 min. The PCR products were analyzed using a 2% agarose gel electrophoresis and confirmed using a specific restriction enzyme cleavage[9]. The restriction enzymes *Xmn* I and *Nsi* I were used for cleavage of 35S and NOS amplified products, respectively. The cleavage test was carried out by mixing 15 μL of reaction solution with 10-units restriction enzyme. The mixture was then incubated at 37˚C for 2 hr and analyzed using a 3% agarose gel electrophoresis. After reaction, the product of 35S primer (195 bp) was cleaved into two fragments (115 bp and 80 bp) and the product of NOS primer (180 bp) was cleaved into pieces of 96 bp and 84 bp.

RESULTS AND DISCUSSION

As of 1999, the US government had approved 3 varieties of GM-soybeans and 14 varieties of GM-maize for commerce. However, only two varieties, Roundup Ready GM-soybeans and Event 176 GM-maize, are now the major products on the market[8]. Roundup Ready GM-soybean and Event 176 GM-maize products with different contents of GMO were used as standards in this study and the specific characteristics of the genes in the above products were detected using the PCR method. The purpose of this study was to evaluate the feasibility of the PCR method for detection of GMF.

I. *Application of PCR Method on Screening of GM-Soybeans and GM-Maize*

Two primers, 35S and NOS, which are specific to the 35S-promoter and NOS-terminator, respectively, of EPSPS inserted gene in herbicide-tolerant Roundup Ready GM-soybeans and to the 35S-promoter of *bar* inserted gene in herbicide-tolerant Event 176 GM-maize were selected for PCR analysis[9]. In 1997, 22 out of 28 commercially available GM-crops were inserted with the 35S-promoter or NOS-terminator[4]. Theoretically, using the primers specific to the above two genes for PCR analysis allows the identical PCR products to be amplified and therefore the GMO in foods can be detected by using this method[9]. So far, 35S and NOS primers are the only two primers used to screen GM-soybeans or GM-maize according to the literature[9]. Results showed that two PCR products with 195 bp (from 35S) and 180 bp (from NOS) were amplified from GM-soybean standard containing 0.1%, 0.5%, 1%, 2%, or 5% GMO. While no PCR product was found from the regular soybean products (0% GM-soybean) as shown in Figure 2, the PCR analysis with 35S and NOS primers could detect as low as 0.1% and 1% of GMO, respectively, in the GM-soybean standard. The 35S- and NOS-PCR analysis conducted by a 29 laboratory

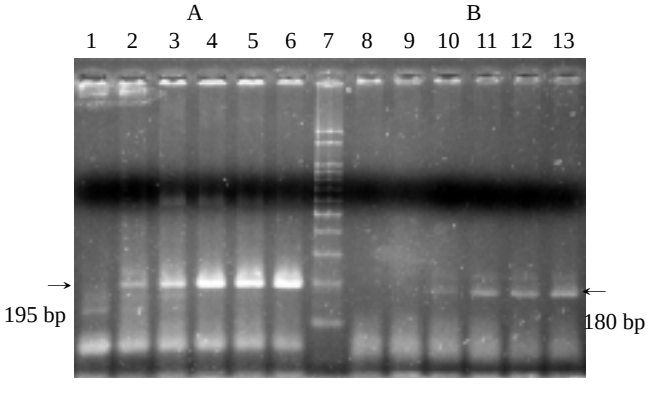

Figure 2. PCR products amplified from 35S and NOS-gene regions of Roundup Ready GM-soybean with primers 35S-1/35S-2(lane 1-6) (A) and NOS-1/NOS-3(lane 8-13) (B).
Lane 1 & 8: 0% GM-soybean (negative control) ; Lane 2 & 9: 0.1% GM-soybean ; Lane 3 & 10: 0.5% GM-soybean ; Lane 4 & 11: 1.0% GM-soybean ; Lane 5 & 12: 2.0% GM-soybean ; Lane 6 & 13: 5.0% GM-soybean ; Lane 7: 100 bp DNA ladder.

collaborative study in Europe showed that the detection limit for GMOs in GM-soybeans was only 2%. A possible false negative reaction could happen when less than 2% of GMO exists in the GM-soybean product[9]. However, in Switzerland, where GMO labeling is required, the GMO content in GM-soybeans is higher than 1%[8]. Thus, the detection technique developed in this study allowing as low as 0.1% of GM-soybeans to be detected (using 35S primer) is capable of achieving the European standard.

Event 176 GM-maize contains only 35S-promoter but no NOS-terminator[4]. In this study, the DNA in Event 176 GM-maize standard was extracted repeatedly and many PCR reactions were conducted to optimize the reaction conditions. However, the results were unsatisfactory. Unstable results were also reported by other laboratories using the 35S-primer for PCR analysis[9]. This could be due to the genome in maize, which is much bigger than in soybeans resulting in less DNA copy number extracted from maize using the same extraction technique[9]. According to the results in this study and other literature[9], the products amplified by 35S-primer-PCR from GM-soybeans are quantitatively more than those amplified by the NOS-primer-PCR (Figure 2). The mechanism is unclear, although, a diverging sensitivity possibly exists between these two primers[15]. A CTAB method used for DNA extraction in this study has been reported to yield a higher quality DNA extract but lower DNA recovery[16]. According to Lipp *et al*.[9], a 100-mg GM-soybean or GM-maize sample is required to perform this test. However, we have found that a sample of 25 mg is enough to conduct a PCR test as using the established method in this study.

II. *Application of PCR Method on Detection of SpecificTraits of GM-Soybeans and GM-Maize*

The primers used for detection of specific traits of Roundup Ready GM-soybeans were specific to the structure gene of herbicide-tolerant EPSPS inserted gene[13]. In total, five primers, which could yield five products with 169 bp, 172 bp, 179 bp, 447 bp, and 508 bp, have been used for the above purpose[17]. However, they are only for identification. The detection limit by using these primers has not been documented. A PCR product between 150 and 300 bp was suggested to be suitable for GMO detection[4]. A primer capable of producing 172 bp PCR product was selected in this study because it is shorter than 300 bp and is the first primer used for detection of specific traits of GM-soybeans. The results showed that a PCR product with 172 bp appeared after PCR reaction of GM-soybeans with different GMO contents (0.1%, 0.5%, 1%, 2%, and 5%), but no 172 bp product was found in the negative control as shown in Figure 3. The detection limit reached 0.1% showing this method is capable of performing a routine analysis. The test samples were also confirmed to be soybean products by using a Lectin gene primer[13], a endogenous gene of soybeans (Figure 3 B).

Four primers, which could produce PCR products with 184 bp, 211 bp, 420 bp, or 1914 bp, have been reported to be specific to Event 176 GM-maize products[17]. The primer producing PCR product with 1914 bp is not suitable for detection of processed GM-maize products; however, it can be used to study the fragmentation of DNA in those products[14]. A comparison of detection limits between the 2 primers capable of producing PCR products of 184 bp and 211 bp has not yet been conducted. In this study, two primers, cryIA(b) and CDPK-cry capable of generating PCR products with 184 bp and 211 bp, respectively, were tested. A PCR product with 184 bp was amplified from the *cry*IA(b) structure gene region of the GM product with primer cryIA(b), while the products with 211 bp were amplified from a 73 bp fragment from promoter region of *cry*IA(b) gene and a 138 bp fragment from the structure gene in GM products with primer CDPK-cry. Results showed that the PCR product with 211 bp appeared after the PCR reaction of GM-maize with 0.1%,

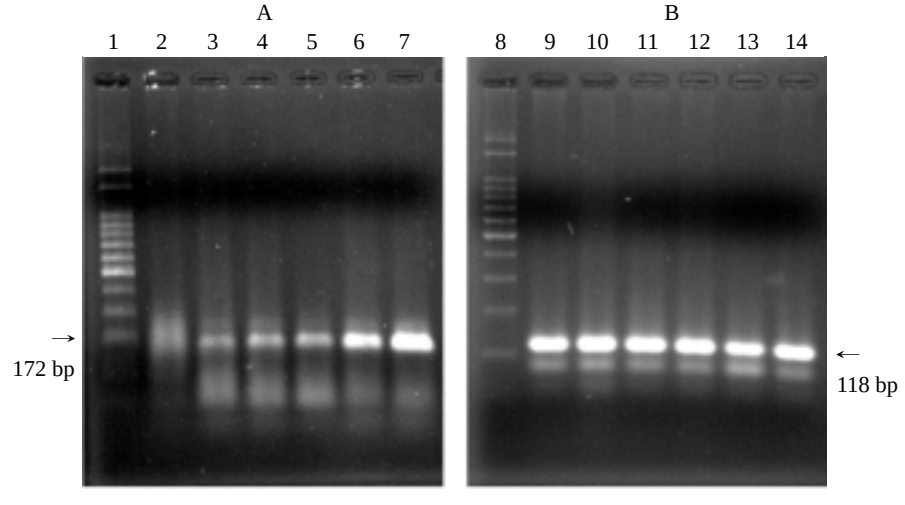

Figure 3. PCR products amplified from EPSPS and lectin-gene regions of Roundup Ready GM-soybean with primers EPSPS-B1/EPSPS-B2 (lane 2-7) (A) and LE103/LE104 (lane 9-14) (B).
Lane 1 & 8: 100 bp DNA ladder; Lane 2 & 9: 0% GM-soybean (negative control); Lane 3 & 10: 0.1% GM-soybean; Lane 4 & 11: 0.5% GM-soybean; Lane 5 & 12: 1.0% GM-soybean; Lane 6 & 13: 2.0% GM-soybean; Lane 7 & 14: 5.0% GM-soybean.

Journal of Food and Drug Analysis, Vol. 8, No. 3, 2000

0.5%, 1%, 2%, or 5% GMO content, but no 211 bp product was found in the negative control sample. The PCR product with 184 bp only appeared on the GM-maize containing 2% and 5% GMO as shown in Figure 4 A&B. These results indicate that using these two primers allows the regulatory and structure gene regions of *cry*IA(b) gene to be detected. The detection limits for primers CDPK-cry and cryIA(b) are 0.1% and 2%, respectively. A primer with an invertase gene, a endogenous maize gene, was used to confirm whether the test samples were maize products. Results showed that a maize characteristic PCR product with 226 bp was found as shown in Figure 4C, confirming the test samples were maize products.

Seven primers tested in this study were capable of generating the PCR products with a size less than 226 bp. Based on this character, they are all suitable for detection of raw materials of GMO products and may also be used to detect the processed GMO products. Four primers were tested for detection of GM-soybean products. Among them, NOS primers are less sensitive in GMO detection; while the primers of 35S and EPSPS are recommended to be used for GM-soybean screening and specific traits detection of GM-soybean products. Likewise, four primers were tested for GM-maize detection. Among them, the 35S primer was used for screening purposes but the test results were unsatisfactory. The primers of CDPK-cry and cryIA(b) were used for specific traits detection. The CDPK-cry primer showed a satisfactory sensitivity and its detection covered both regulatory and structure gene regions allowing the double-checking of these two gene regions. Therefore, it is appropriate for Event 176 GM-maize detection. The other two primers of LE and ivr demonstrated satisfactory detection of endogenous gene of soybeans and magize.

III. *A Flow Model for PCR Detection of GMO in Foods*

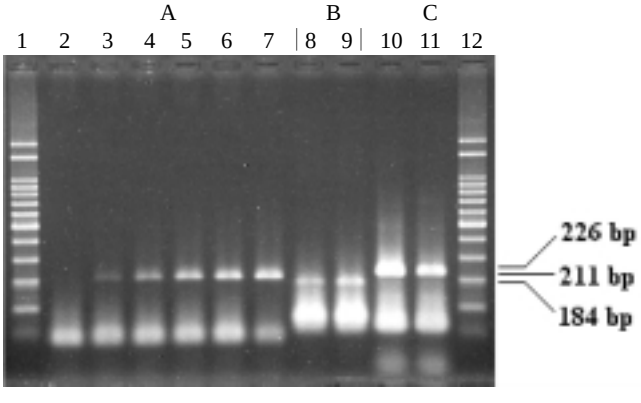

Figure 4. PCR products amplified from delta-endotoxin and invertase gene regions of Event 176 GM-maize with primers CDPK-cry 03/04 (A), cry IA(b)-1/2 (B) and ivr1-1/2 (C).
Lane 1&12: 100 bp DNA ladder; Lane 2&10: 0% GM-maize (negative control); Lane 3: 0.1% GM-maize; Lane 4: 0.5% GM-maize; Lane 5: 1% GM-maize; Lane 6&8: 2% GM-maize; Lane 7, 9&11: 5% GM-maize; Lane 2-7: with primers CDPK-cry 03 and CDPK-cry 04; Lane 8-9: with primers cry IA(b)-1 and cry IA(b)-2; Lane 10-11: with primers ivr 1-1 and ivr 1-2.

The purposes of using PCR method for detection of GMO in foods (to take the GM-soybean as an example) are as follows: (1) to identify if the GM products are inserted with other genes that are not allowed for use; (2) to identify if the test samples are exact soybean products; and (3) to count fragment varieties of inserted genes.

A flow diagram is shown in Figure 1. The regulatory gene in extracted DNA is selected to be a target gene for screening purposes. The samples with negative reactions are samples containing no target gene. The positive samples, which contain the regulatory gene to be tested, were further confirmed by testing the specificity of the structure gene and detecting a endogenous gene. The samples with positive reaction were designated as GM-soybean products. However, the samples with negative reactions in detection of the structure gene were classified as non-authorized GM-soybean products. Nevertheless, the above flow could be rearranged if necessary. The endogenous gene can be tested initially to confirm if the test samples are soybean products. Screening and confirmation tests are further performed in case the test samples are confirmed to be soybean products. Otherwise, the further tests are unnecessary. Furthermore, the counting of fragment varieties in inserted genes was not discussed in this study because no definite fragment variety number was concluded according to the literature. Based on the described above, we have concluded that detection of the regulatory gene, structure gene, and endogenous gene needs to be carried out for performing PCR detection on GM-soybean products. By using the above detection method, the test samples conducted in this study were confirmed to be Roundup Ready GM-soybeans and Event 176 GM-maize.

IV. *Confirmation Test of PCR Products*

As described above, PCR products can be confirmed using the following methods: nucleic acid sequencing, endonuclease mapping, probe hybridization, and nested PCR method[4]. After screening, the PCR products of 35S-promoter and NOS-terminator can be further cleaved by a proper endonuclease to confirm the final products[4,9]. In this study, GM-soybean samples were reacted with 35S- and NOS-primer-PCR to give the products 195 bp and 180 bp, respectively, which were further cleaved by endonuclease *Xmn* I and *Nsi* I, respectively, for confirmation. The 195 bp product was digested into 115 bp and 80 bp products; while the 180 bp product was cut into two products with 96 bp and 84 bp. PCR products were thus confirmed.

V. *The Possible Reasons for Causing False PCR*

A possible false positive reaction can occur if test plant samples are contaminated or infected by the *Cruciferae* group, since the 35S-promoter comes from the Cauliflower mosaic virus[4]. The non-GM plant contains no NOS-terminator, however, a NOS-terminator could exist in the roots of non-GM plants resulting in a false positive PCR. This is because the NOS-terminator originates from *Agrobacterium*

turmefaciens, which is a microorganism flora existing in soil and could contaminate the roots of the plant. In addition, because the PCR is a kind of enzyme reaction, any factors that affect an enzyme reaction could also result in a false PCR. These factors include improper preparation of the DNA template, primer and reagents, and existence of interference. A false negative could result from the above factors. These false reactions can be minimized by using a GMO standard as a positive control. The test samples used in this study were GMO standards. However, a possible contamination of negative control by standards should be avoided. We also used a GMO standard as a positive control to ensure proper PCR.

The aim of developing a method for GMF detection was to implement a food labeling system. However, due to technical or information limitations, some problems could arise in developing a PCR method for detection of GMO in foods. (1) Processed food: In highly processed or fermented foods, genes could be altered or proteins could be denatured, making detection difficult. (2) Varieties of GM products: a great quantity with different varieties of GM-crops are cultivated. (3) Mixture of GM products: different varieties of GM-crops are mixed either artificially or naturally. (4) Shortage of the information regarding the gene sequence of the inserted gene, which could result in difficulty in designing a suitable primer[4]. (5) Trade to countries where GMF are unregulated could create a problem in GMF detection.

We have developed a PCR method capable of identifying Roundup Ready GM-soybeans and Event 176 GM-maize from traditional products. The detection limit could reach as low as 0.1%. The future work in our laboratory will include evaluation of a DNA extraction method; and test of different primers, GMOs or processed foods; and other detection methods in addition to PCR method.

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以PCR方法檢測基因改良大豆及玉米

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摘　要

本研究以PCR方法探討鑑別檢測基因改良大豆與基因改良玉米之可行性。針對Roundup Ready (Monsanto公司) GM－大豆及Event 176 (Novartis/Ciba-Geigy公司) GM－玉米產品插入基因與品種特性基因選定不同引子，進行PCR方法檢測。用以鑑別GM－大豆之引子共四對，分別爲35S (35S-promoter, 源自cauliflower mosaic virus)、NOS (nopaline synthase-terminator,源自*Agrobacterium tumefaciens*)、EPSPS (5-enolpyruvylshikimate-3-phosphate synthase，源自*A. tumefaciens* strain CP4) 及LE (品種特性基因lectin)。而鑑別GM－玉米之引子則採用三對，分別爲CDPK-cry (pollen-specific calcium-dependent protein kinase promoter -delta-endotoxin，分別源自玉米及*Bacillus thuringiensis* subsp. *kurstaki*)、cryIA(b) (delta-endotoxin，源自*B. thuringiensis* subsp. *kurstaki*) 及ivr (品種特性基因invertase)。結果顯示，大豆檢體以35S及EPSPS引子檢測時，其最低檢測量均爲0.1% (w/w)，NOS則爲1% (w/w) ；檢體並以LE引子－PCR反應確定均爲大豆產品。至於玉米檢體之測試結果，使用CDPK-cry引子其最低檢測量爲0.1% (w/w)，cryIA(b)爲2% (w/w)，並經由Invertase引子－PCR反應確定爲玉米產品。此外，GM－大豆之35S-PCR產物，進一步以限制酵素*Xmn* I進行切割確認，195 bp產物切成80 bp與115 bp產物，而NOS之180 bp產物則以限制酵素*Nsi* I切爲84 bp 與96 bp，確認PCR產物。結果顯示本報告所使用之PCR方法能區分一般與基因改良之大豆及玉米。

關鍵詞： PCR，基因改良大豆，基因改良玉米