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Regulation and detection methods for genetically modified foods in Korea*

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Abstract: Genetically modified organisms (GMOs) have been developed and commercialized in many countries for the past decade. The regulations on these GMOs in Korea have been established through the labeling and safety evaluation systems for management of genetically modified (GM) foods under the Food Sanitation Act enacted by the Korea Food and Drug Administration (KFDA). To manage these regulations effectively, several methods for GMO detection, including polymerase chain reaction (PCR) methods and immunoassay, are applied. For detection of GMOs at the level of DNA, primers from the promoter, terminator, other marker genes, and expressed proteins used in a wide range of GMOs were designed for single, multiplex, real-time PCR, and microarray applications. In Korea, multiplex PCR methods specific to GM soybean, maize, canola, and cotton were designed. The limit of detection (LOD) value was determined to be 1 % of GM mixtures, which is a significant value for the PCR method used for the labeling threshold in Korea. A DNA microarray chip was also developed for the detection of 24 GMOs, including GM soybeans, 13 GM maizes, 3 GM canolas, 5 GM cottons, and 1 GM rice. This DNA chip was proven to successfully detect GMOs from raw and processed foods. In the near future, more powerful screening and detection methods are needed for handling many kinds of GMOs and unauthorized GMOs.

Keywords: detection; genetically modified foods; labeling; microarray; multiplex polymerase chain reaction; safety assessment.

INTRODUCTION

In 2007, genetically modified (GM) crops were cultivated on 114.3 million hectares worldwide, and there has since been a rapid increase in GM crop cultivation [1]. Until December 2008, 132 genetically modified organisms (GMOs) have been offered for commercial cultivation [1]. While GM crops were developed and grown commercially in 23 countries in 2007, 52 countries including Korea, China, and Japan, have approved GM crops for import as foods and feed [1].

In recent years, special concerns have been raised about the safety assessment of foods and food ingredients derived from GMOs. Korea has established regulations and laws for GMOs in order to allow consumers to make an informed choice. As of December 2008, the Korea Food and Drug Administration (KFDA) has approved 54 events of GM crops (Table 1). GMOs will be continuously developed and cultivated worldwide. Korea may import more GMOs for food purposes in the near future. Therefore, detection methods for newly developed GMOs should be continuously developed to fulfill

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the requirements of the GMO labeling system legislation. Reliable and sensitive methods to detect GMOs in foods are of great importance.

Table 1 GMOs approved for food use in Korea.

Crop	Events	Company	Trait
Soybean (1)	RRS	Monsanto	HT
Maize (28)	MON810	Monsanto	IR
	1507	DuPont	HT & IR
	GA21	Monsanto	HT
	NK603	Monsanto	HT
	Bt11	Syngenta	HT & IR
	T25	Bayer CropScience	HT
	MON863	Monsanto	IR
	Bt176	Syngenta	IR
	DLL25	Monsanto	HT
	DBT418	Monsanto	HT & IR
	MON863xNK603	Monsanto	HT & IR
	MON863xMON810	Monsanto	HT & IR
	MON810xGA21	Monsanto	HT & IR
	MON810xNK603	Monsanto	HT & IR
	1507NxK603	DuPont	HT & IR
	MON810xMON863xNK603	Monsanto	HT & IR
	DAS-59122-7	DuPont	HT & IR
	MON88017	Monsanto	HT & IR
	Bt10	Syngenta	HT & IR
	MIR604	Syngenta	IR
	DAS-59122-7x1507xNK603	DuPont	HT & IR
	1507xDAS-59122-7	DuPont	HT & IR
	DAS-59122-7xNK603	DuPont	HT & IR
	Bt11xGA21	Syngenta	HT & IR
	MON88017xMON810	Monsanto	HT & IR
	Bt11xMIR604	Syngenta	HT & IR
	Bt11xMIR604xGA21	Syngenta	HT & IR
	MIR604xGA21	Syngenta	HT & IR
Canola (6)	GT73	Monsanto	HT
	MS8xRF3	Bayer CropScience	HT & MS
	T45	Bayer CropScience	HT
	MS1xRF1	Bayer CropScience	HT & MS
	MS1xRF2	Bayer CropScience	HT & MS
	Topas19/2	Bayer CropScience	HT
Cotton (13)	531	Monsanto	IR
	757	Monsanto	IR
	1445	Monsanto	HT
	15985	Monsanto	IR
	281/3006	DOW AgroScience LLC	HT & IR
	15985x1445	Monsanto	HT & IR
	531x1445	Monsanto	HT & IR
	LLcotton25	Bayer CropScience	HT
	MON88913	Monsanto	HT

(*continues on next page*)

Table 1 (*Continued*).

Crop	Events	Company	Trait
	BG2xLL(Bollgard II 15985xLLcotton25)	Bayer CropScience	HT & IR
	Bollgard II 15985xRound Ready Flex MON88913	Monsanto	HT & IR
	281/3006x88913	DOW AgroScience LLC	HT & IR
	281/3006x1445	DOW AgroScience LLC	HT & IR
Potato (4)	SPBT02-05	Monsanto	CPBR
	RBBT06	Monsanto	CPBR
	Newleaf Y (RBMT15-101, SEMT15-02, SEMT15-15)	Monsanto	CPBR & PVYR
	Newleaf PLUS (RBMT21-129, RBMT21-350, RBMT22-82)	Monsanto	CPBR & PLRVR
Sugar beet (1)	H7-1	Monsanto	HT
Alfalfa (1)	J101/J1633	Monsanto	HT

HT, herbicide tolerance; IR, insect resistance; MS, male sterility; CPBR, Colorado potato beetle resistance; PVYR, potato virus Y; PLRVR, potato leafroll virus resistance

Most countries have recognized that consumers are concerned about GMO products, and, therefore, it is necessary to determine the threshold level of GMOs. Labeling policies for GM foods differ from country to country. For instance, the labeling threshold was defined as 0.9 % in the European Union [2], 3 % in Korea [3], and 5 % in Japan [4]. Therefore, GMO labeling regulations have necessitated the development of reliable and sensitive methods for GMO detection. Currently, DNA and protein-based methods, which have been developed by many researchers, are applied to GMO detection. For the detection of GMOs at the level of DNA, PCR-based methods are mainly used, whereas for protein-based detection, immunoassays such as enzyme-linked immunosorbent assay (ELISA) and lateral flow strip tests are predominantly used. The aim of this review is to systematically examine and describe the regulation and detection methods for GM foods in Korea.

REGULATION OF GM FOODS IN KOREA

Since the increasing production and spread of GM foods to Korea, systematic regulations for GM foods were required. To date, the regulation of GM foods and food additives has been promulgated by the Food Sanitation Act, which mandates the safety announcement of such food to ensure safety to human health. In the following section, we describe the safety assessment and guideline approval process for the labeling of GM foods in Korea.

Approval process for safety assessment of GM foods

The current safety assessment system in relation to GM foods in Korea is carried out according to the Safety Evaluation Guidelines by Notification 1999-46 of the KFDA. The subjects of application are GMOs used in food ingredients and food additives. The purpose of the guideline is to ensure the biosafety of foods and food additives containing GMOs. The application procedures for the safety evaluation of GM foods are as follows: the director of the KFDA fully evaluates the appropriateness of the safety assessment within 90 days from full data presentation by the applicant. If further scientific proof to reach agreement on the safety evaluation is needed, the additional data is requested from the applicant. This period is exempted from the total number of evaluation days. Upon completion of the safety

assessment evaluation, the safety of the new product is officially announced on the KFDA's homepage (<http://gmo.kfda.go.kr>) and the completion of the evaluation is conveyed to the applicant. To ensure expertise and objectivity during the safety evaluation progress, a "Committee for Evaluation of the Safety Assessment Data of GM Foods and Food Additives" comprised of 20 experts from the academic and research fields, and related government research workers from five divisions review the data. The five divisions represented are: (a) general division, which evaluates the general information on GMOs as foods; (b) molecular characterization division in the fields of botany, plant genetics, molecular biology, and microbiology; (c) toxicology division, which focuses on toxicity of recombinant protein; (d) allergy division, which reviews the allegenicity of protein derived from gene insertion; and (e) gene stability division, relating to safety issues upon gene transfer. For transparency in the safety evaluation process, an expert recommended by a consumer group also participates in the review.

Guidelines for labeling of GM foods

At present, about 20 countries including Korea, the European Union, Japan, Australia, and New Zealand have a labeling system for GMOs. This labeling system is managed by both the scientific data obtained from GMO detection and a "certificate for an identity preserved (IP) handling system". The certificate for an IP handling system describes information on the management of non-GMO production, including the purchase of seeds, production, storage, transport, sorting, and shipping to prevent the adulteration of GMO products.

As for processed goods made from GM soybean and maize, a labeling system enabled by notification of the KFDA was activated in July 2001. Canola, cotton, and sugar beets were added in June 2007. According to the KFDA regulations, processed foods require GMO labeling for GM soybean, maize, canola, cotton, or sugar beets if one or more are the top five ingredients of the final product, and if a foreign protein or DNA is still present in the final product. However, there is no specific label system for soybean source, oils, sugars, and alcohol products. The threshold of unintentional GMO mixing in Korea is 3 %. The labeling standard of GM crops over 3 % is "GM crop", "containing GM crop", or "may contain GM crop".

DETECTION TECHNOLOGY FOR GM FOODS

Detection methods of GM foods are based on the examination of either the GM gene or foreign protein from the gene in foods. Overall, there are two different GMO detection methods. The first is polymerase chain reaction (PCR), which is the analysis method used for detecting the GM gene. The second is an immunological method, which is based on the antigen–antibody reaction, and is the analysis method used for detecting the foreign protein. Both PCR and immunological assays can be used for agricultural products. However, in the case of processed foods, immunological assays cannot be used for the detection of GMOs due to protein denaturation and degradation during processing. Therefore, PCR methods can only be used for processed foods. A protein-based lateral flow strip test and ELISA are used for determining GMO content in seeds or foods. General PCR and real-time PCR systems are used as qualitative and quantitative assays for analysis of GMOs, respectively. Qualitative and quantitative analyses of GMOs can be used for the analysis of intact and simply ground agricultural products. However, for processed foods, a qualitative assay can only be applied because a quantitative method is not yet established. Here, we introduce and briefly describe the GMO detection techniques used in Korea.

Qualitative PCR

For GMO detection, it is very important to know information about the gene cassette consisting of a promoter, terminator, and structural gene (encoding region) inserted in GMOs.

Qualitative PCR uses certain oligonucleotide primers derived from the gene cassette inserted in GMOs. A PCR method that can distinguish one particular variety from all other varieties using primers is described as being "gene-specific", "construct-specific", or "event-specific" [5,6]. An event-specific detection system has been promoted for many years in Korea, as well as in other countries, because of its high specificity based on the junction between the transgenic insert and the host genome. This DNA detection method has a wide range of applications involving raw materials and processed foods compared to the protein-based detection methods.

In Korea, the KFDA and the National Institute of Agricultural Science & Technology have used certified primers to detect GMOs in accordance with the standards of the American Organization of Analytical Chemists Research Institute (AOAC) [7,8]. By December 2008, official detection methods for GM crops using qualitative PCR have been authorized in Korea. One GM soybean (RRS), 10 GM maizes (MON810, Bt11, Bt176, T25, GA21, NK603, TC1507, Bt10, MON863, and DAS-59122-7), 2 GM potatoes (NewLeaf plus and NewLeaf Y), 2 unapproved GM maizes (CBH351 and Event 32), and 2 unapproved GM rices (Bt rice and LL601) can be analyzed by qualitative PCR. Qualitative and quantitative PCR for analyzing the extracted DNA from processed food products are used as approved analysis methods of GM foods, and the results of the analysis are assessed for approval of the tested foods. The qualitative analysis procedure of a GMO is illustrated in Fig. 1. DNAs were extracted twice from two different sample lots, and then analyzed by the PCR method. If the analysis of the endogenous gene in the food sample shows a negative result when compared to the control, the sample can be labeled as "GMO analysis is impossible". However, if PCR shows a positive result, further analysis methods, including screening PCR and event-specific PCR, should be performed to assess the presence/absence of a GMO. If the qualitative analysis result shows the presence of GMO, then the supplier should verify the labeling system according to the KFDA regulation, including a certificate for an IP handling system. Also, in the case of agricultural products, quantitative analysis should be performed to confirm the GMO content, which should be less than 3 % total. However, because CBH351, Bt10, and LL601 are not approved, further analysis to improve their quantitative detection is not required.

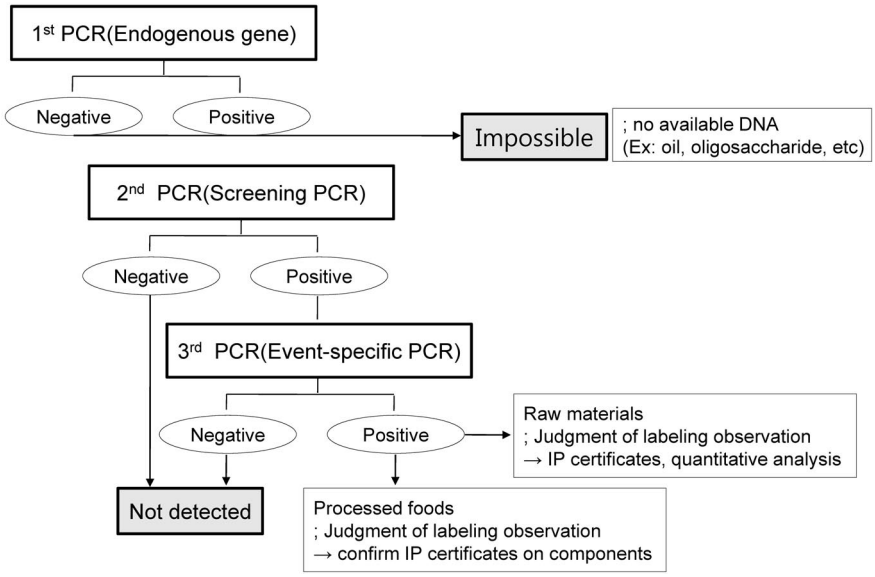

Fig. 1 Qualitative analysis of GMOs.

Quantitative PCR

The advantage of real-time PCR is that the amount of target sequences can be directly monitored by measuring a fluorescence signal produced during the course of the reaction. This is done by the fluorometric measurement of an internal probe during the reaction. This probe is labeled with a fluorescent reporter dye and a corresponding quencher dye. When the reporter dye and quencher dye are in close proximity, the quencher dye absorbs the fluorescence from the reporter dye; therefore, no fluorescent signal is emitted. The DNA sequence of the probe is designed to anneal exactly in the region to be amplified. In the extension step, Taq DNA polymerase has 5'–3' exonuclease activity, which breaks down the probe, thereby physically separating the quencher dye from the reporter dye. The reporter dye becomes capable of emitting a fluorescent signal, since it is no longer suppressed by the quencher dye [9]. The amount of fluorescence is proportional to the amount of PCR products. Therefore, it is possible to measure the exact number of cycles that are needed to produce a certain amount of PCR product through real-time PCR. After real-time PCR analysis, the threshold line is set in the region associated with exponential PCR product growth, and then a Ct-value is determined. The Ct-value is defined as the cycle number at which the fluorescence signal crosses the threshold line.

In real-time PCR, reference materials with a defined copy number or content of GM-derived DNA are used to construct a standard curve, which is used to determine the proportion (%) of GMO in unknown samples [10]. A limited range of commercial reference materials is available for production of standard curves, so some researchers have produced their own calibration standards using purified genomic DNA or target DNA sequences cloned into plasmids [11,12]. Some Korean researchers have also investigated real-time PCR using plasmids as reference materials [13–18].

Real-time quantification offers several advantages over end-point quantification; namely, it is well suited to automation and high-throughput screening. Currently, real-time PCR is considered to be the most powerful tool for the detection and quantification of GMOs in foods and feeds.

In Korea, 1 soybean (RRS), 11 maizes (MON810, Bt11, Bt176, T25, GA21, NK603, TC1507, MON863, DAS-59122-7, MON88017, and MIR604), 4 canolas (T45, GT73, Ms8, and Rf3), 6 cottons (531, 1445, 15985, Mon88913, LLcotton25, and 281/3006), 2 alfalfas (J101 and J163), and 1 sugar beet (H7-1) can be quantified using real-time PCR with TaqMan chemistry.

Multiplex PCR

Multiplex PCR is currently regarded as a rapid and convenient screening assay for GMO detection because it allows for the simultaneous amplification of multiple organisms. However, it is difficult to distinguish between PCR products of similar length and to cover many GM events due to the limitations of gel electrophoresis. Multiplex PCR is generally suitable for detecting several genes simultaneously if samples represent raw material. In the case of processed foods, the application of multiplex PCR is limited due to the poor quality of target DNAs resulting from food processing. Therefore, amplicon size should be considered when detecting GM materials in processed foods using PCR. Large amplicons may not be suitable for detecting the presence of GMO derivatives in processed foods because food processing often results in DNA degradation due to various chemical, physical, and enzymatic factors. In this respect, a multiplex PCR-based method may be more suitable for the detection of GM materials from raw materials than processed foods. The design of primers is also a very important part of multiplex PCR, because primer specificity and melting temperature (T_m value) are more critical than in conventional PCR. Although the specific primers amplify target DNA sequences, they may have different amplification rates. In order to amplify specific PCR products with equal efficiency, the concentrations of individual primer pairs should be optimized.

Several multiplex PCR methods for GMO detection have been developed in Korea. Heo et al. [19] reported the detection of GM maize using multiplex PCR (MON810, GA21, NK603, TC1507, and Bt176). A multiplex PCR method of eight different events of GM maize (Bt176, Bt11, MON810,

MON863, NK603, T25, TC1507, and GA21) was also reported by Kim et al. [20]. Recently, multiplex PCR methods of three events (DAS-59122-7, TC6275, and MIR604) and four events of GM maize (Event3272, LY038, MIR162, and MON88017) were reported by Ji et al. [21] and Kim et al. [22], respectively. In addition, multiplex PCR methods of three events of GM canola (GT73, MS8xRF3, and T45) [23] and four events of GM cotton (MON1445, MON15985, MON88913, and LLcotton25) were developed by Kim et al. [24]. Currently, these methods are partially used as general test methods for the detection of GMOs in the Korean Food Code.

Microarray

A DNA microarray is important as a high-throughput assay, and has already been used for GMO detection [25–29]. In Korea, a DNA microarray chip was developed for the detection of 24 GMOs, which include 2 GM soybeans (GTS-40-3-2 and A2704-12), 13 GM maizes (Bt176, Bt11, MON810, MON863, NK603, GA21, T25, TC1507, Bt10, DAS59122-7, TC6275, MIR604, and LY038), 3 GM canolas (GT73, MS8xRF3, and T45), 5 GM cottons (MON1445, MON 15985, MON 531, MON 88913, and LLcotton25), and 1 GM rice (Shanyou 63). The principle of DNA microarray analysis based on PCR is shown in Fig. 2.

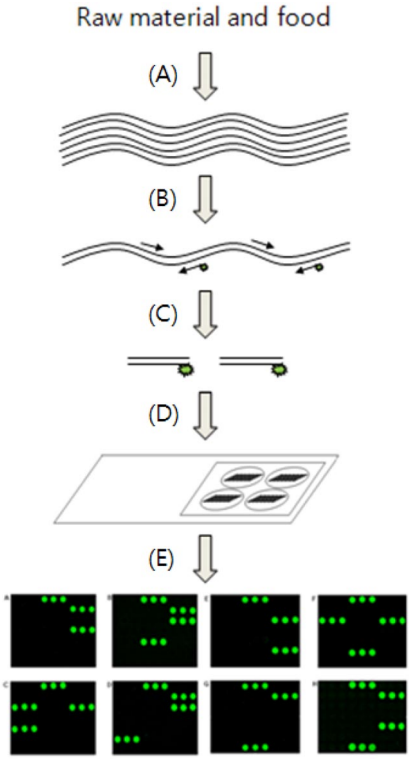

Fig. 2 Principle of the DNA microarray system for GMO detection. (A) Genomic DNA is extracted from raw material and food. (B) A PCR is performed with Cy3-labeled primers. (C) PCR products are labeled with the fluorescent dye Cy3. (D) The PCR products will hybridize with their complementary probes onto the glass slide. (E) After the washing steps, positive spots, which are hybridized by the probe, are detected by a microarray scanner.

The microarray includes a total of 32 oligonucleotide probes for endogenous reference targets, event-specific targets, screening targets (35S promoter and *nos* terminator), and internal targets (18S rRNA). The genes corresponding to *lectin*, *starch synthase* IIb (zSSIIb), *fatty acyl-ACP thioesterase* (FatA), *acyl carrier protein* (Acp1), and *sucrose phosphate synthase* (SPS) were chosen as endogenous reference genes of soybean, maize, canola, cotton, and rice, respectively. To simultaneously detect multiple target sequences in GMOs, multiplex PCR was coupled with a microarray, and the designed primer pairs successfully amplified the target sequences. Currently, the applicability of the microarray system for GMO detection in processed foods is being investigated in our laboratory.

The advantage of microarray technology is that screening and identification are carried out in a single step in contrast to the PCR-based approaches. Furthermore, because the microarray system is very flexible, new varieties can be included in the screening procedure by adding additional probe sequences to the array [30]. However, the microarray system requires additional equipment and trained specialists. Although microarray technology is only used as a first-line screening assay for GMOs, in the near future, it may be used to precisely quantify the amount of detected GMO varieties. It is believed that the microarray system will play an important role in the detection of GMOs in a variety of food ingredients.

CONCLUSIONS

To date, more than 100 transgenic species have been approved for commercialization. With the development of genetic technology, a greater variety of transgenic products will be produced. In order to satisfy the rapid detection requirement of a large number of GMO samples, it is essential to develop rapid, automated, and high-throughput analytical systems. Many GMO detection methods have been developed in Korea, and authorized detection methods were recognized by the Korean government among these developed methods. Approved GMOs, which are verified for their safety, and unapproved GMOs were divided according to the analysis results of those methods. In addition, food regulation organizations, such as KFDA and the rural development administration in Korea, have supported the research and development of new detection technology in respect to GMOs through research funding. Multiplex PCR detection methods have been developed to efficiently monitor different kinds of GM maize, cotton, and soybean events in a single reaction using event-specific primers. Also, research on a "simultaneous detection method of approved GMO using microchip" was performed, and the developed method may be used as an approved detection method if it passes the validation test. In conclusion, we introduced regulation and detection methods for GM foods in this review. Detection methods for examining GMOs should be continuously developed and validated through international exchange. This approach should also be incorporated into Codex guidelines on the validation of GMO analysis methods.

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