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Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon Port in South Korea

Bumkyu Lee, Chang-Gi Kim *, Ji-Young Park, Kee Woong Park, Hyo-Jeong Kim, Hoonbok Yi, Soon-Chun Jeong, Won Kee Yoon, Hwan Mook Kim *

Bio-Evaluation Center, KRIBB, Cheongwon 363-883, Republic of Korea

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ABSTRACT

In South Korea, imported genetically modified (GM) soybean and maize have been approved for both human consumption and use in animal feed, but not for use in cultivation in fields. This study was conducted to survey the spread of GM soybean and maize in South Korea using multiplex-PCR analysis methods. Cultivated soybean, wild soybean, and maize leaf samples were collected from 26 major areas of soybean cultivation throughout eight provinces. Roadside areas near a major grain port in Incheon were also surveyed to investigate the escape and spread of GM seeds and plants. Amplification results showed that no GM soybean or maize was collected from cultivated fields. However, four GM maize plants were found in samples collected from the roadside near a grain transporting company at the Incheon Port. Based on PCR analysis using GM maize event-specific primers, it was suggested that a maize plant may be Mon810, while the other plants may be stacked events: Mon863 × Mon810 or Mon88017 × Mon810.

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1. Introduction

Twenty-three countries worldwide have planted commercialized, genetically modified (GM) crops in 2007, and an additional 29 countries have approved GM crop importation for use in human food and animal feed, as well as for release into the environment (James, 2007). With food self-sufficiency estimated to be 29% in 2005, South Korea is a major food-importing country (Ko & Lee, 2007). Self-sufficiency rates of soybean and maize in South Korea are only 9.7% and 0.9%, respectively (Korea National Statistical Office, 2007); therefore, the demand is satisfied almost entirely from imported supplies. From 2001 to 2005, 77% of soybeans were imported from the USA, and 55% and 26% of maize was imported from China and the USA, respectively (Korea Agro-Fishery Trade Corporation, 2007). In South Korea, imported soybeans are predominately used for oil extraction, and the residual products are then used for animal feed. About 80% of the imported soybeans for oil extraction have been reported to be GM (Yoo, 2004). The proportion of GM maize among the imported maize for food was less than 0.01% in 2006 (Korea Agro-Fishery Trade Corporation, 2007). However, no statistics for the proportion of GM maize in the maize imported for animal feed is currently available. By the end of 2005, one GM soybean event and 15 GM maize events, including five stacked events, had been approved for consumption by the Korean Government (Korean Agricultural Biosafety Information Center, 2006) (Table 1).

Transgenes from GM crops can be transferred to their wild relatives by pollen and seed flow, and are a potentially serious problem when introduced into the natural environment (Hancock, Grumet, & Hokanson, 1996; Raybould & Gray, 1993; Snow & Morán-Palma, 1997). In South Korea, GM soybean and maize have not been approved for field cultivation. However, such cultivation can occur accidentally when seed lots become contaminated, as evidenced by the unapproved sale of Bt10 maize to farmers (Macilwain, 2005). It was also reported that abundant contamination occurred in pedigreed Canadian canola seed lots, which gained GM herbicide tolerance traits (Friesen, Nelson, & Van Acker, 2003). In South Korea and China, GM soybean, which is not approved for cultivation, was found available at local open markets (Shim, Nam, Choe, Jeong, & Chung, 2006; Zhou, Liu, Lian, & Zhang, 2007). Seed spillage during the transportation process may also lead to accidental release of GM soybean and maize. In Japan, herbicide-resistant GM oilseed rapes were found around ports and roadsides (Saji et al., 2005). Kim et al. (2006) have reported the cultivation of a GM maize plant around a grain-receiving port in South Korea.

* Corresponding authors. Tel.: +82 43 240 6520; fax: +82 43 240 6519 (H.M. Kim), tel.: +82 43 240 6543; fax: +82 43 240 6549 (C.-G. Kim).
E-mail addresses: cgkim@kribb.re.kr (C.-G. Kim), hwanmook@kribb.re.kr (H.M. Kim).

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Table 1
Characteristics of GM soybean and maize events approved for consumption in South Korea

Crops	Events	Promoters	Introduced genes	Terminators
Soybean	GTS40-3-2	e35S, FMV, *mas*	*CP4EPSPS*	*nos*, 7S
Maize	Bt11	35S	*cry1Ab*, *pat*	*nos*
	Bt176	35S, PEPC, PCDK	*cry1Ab*, *bar*	35S
	Das-59122-7	35S, ubiZM1, TA peroxidase	*cry34Ab1*, *pat*,*cry35Ab1*	35S, PINII
	GA21	ract	*mEPSPS*	*nos*
	Mon810	e35S	*cry1Ab*	
	Mon863	35S, 4AS1	*cry3Bb1*	*nos*, *tahsp17*
	Mon88017	e35S, ract	*cry3Bb1*,*CP4EPSPS*	*nos*, *tahsp17*
	NK603	e35S, ract	*CP4EPSPS*	nos
	T25	35S	*pat*	35S
	TC1507	ubiZM1, 35S	*cry1F*, *pat*	ORF25, 35S
	Mon863 × Mon810			
	Mon810 × GA21			
	Mon810 × NK603			
	Mon88017 × Mon810			
	Mon810 × Mon863 × NK603			

PCR analysis is the most widely used method for detecting the presence or absence of transgenes due to its high sensitivity and capacity to discriminate specific transgenic events (García-Cañas, González, & Cifuentes, 2002). Multiplex-PCR exhibits adequate sensitivity to simultaneously detect various GM crops in a single reaction without loss of specificity.

The present study was conducted to investigate the release of GM soybean and maize into cultivated fields as well as in areas at and surrounding a major port for grain importation in South Korea through the use of multiplex-PCR.

2. Materials and methods

2.1. Collection of soybean and maize tissue samples

To investigate the presence of genetic modifications in cultivated crops, we monitored major soybean production areas throughout eight provinces of South Korea in July 2006. Twenty-six sites met the requirement of having a cultivation area greater than ten ha and were therefore included in the present study. At each site, one soybean field close to the road was randomly selected, and leaf samples were collected from ten cultivated soybean plants (*Glycine max*), totalling 260 samples from 26 areas (Fig. 1A). Thirty-two leaf samples were collected from wild soybean plants (*Glycine soja*) that were found in the vicinity of seven soybean fields. We also collected 73 maize samples from maize fields close to the soybean fields (Fig. 1A).

In addition, we investigated GM soybean and maize plants along the roadsides near Incheon Port, South Korea's major grain-receiving port. Roadsides within a 3 km radius from the fifth pier at Incheon Port were investigated on foot to find seed spillages and feral soybean and maize. The fifth pier was selected as the centre of the investigation because a major grain transportation company and an animal feed production company are located close to this pier. We found 18 roadside maize plants in the vicinity of the company (Fig. 1B).

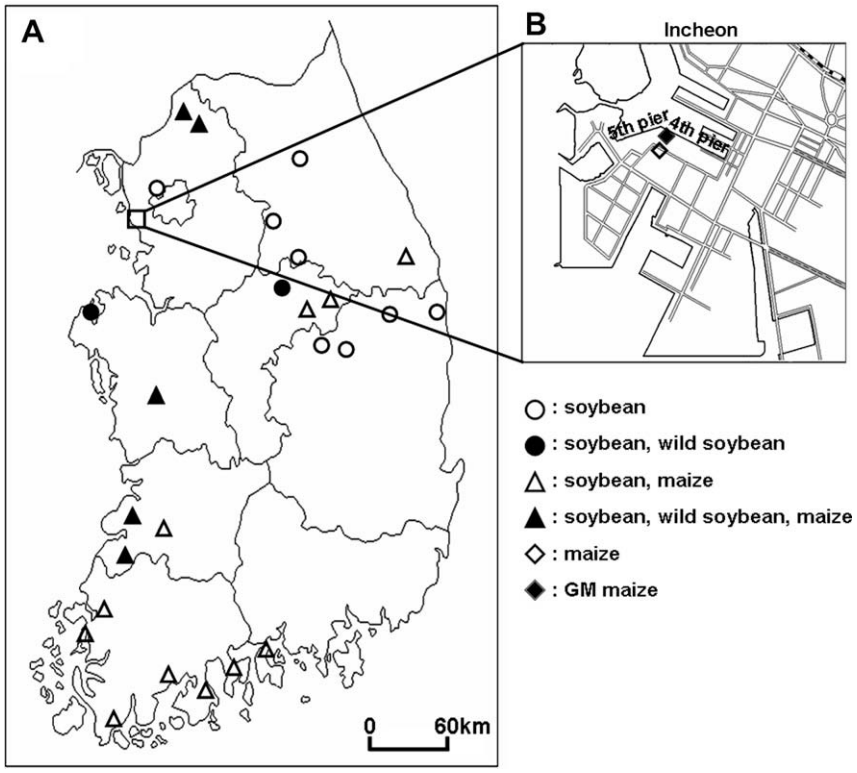

Fig. 1. (A) Monitoring sites at the cultivation fields. (B) Roadside sampling sites around Incheon Port. ♦, indicates location of GM maize plants found in the present study.

Table 2
Oligonucleotide primers for detecting GM soybeans and maize

Gene	Orientation	Sequence (5′–3′)	Product size	Reference
35S	Sense	TGC CTC TGC CGA CAG TGG TC	83 bp	Trapmann et al. (2002)
	Antisense	AAG ACG TGG TTG GAA CGT CTT C		
nos	Sense	GAA TCC TGT TGC CGG TCT TG	180 bp	Lipp, Brodmann, Pietsch, Pauwels, and Anklam (1999)
	Antisense	TTA TCC TAG TTT GCG CGC TA		
lectin	Sense	TGC CGA AGC AAC CAA ACA TGA TCCT	414 bp	Pauli et al. (1998)
	Antisense	TGA TGG ATC TGA TAG AAT TGA CGT T		
zein	Sense	AGT GCG ACC CAT ATT CCA G	277 bp	Studer et al. (1997)
	Antisense	GAC ATT GTG GCA TCA TCA TTT		

Table 3
Oligonucleotide primers used to detect GM maize events

Event	Orientation	Target gene	Sequence (5′–3′)	Product size	Reference
Mon810	Sense	HSP70	AGT TTC CTT TTT GTT GCT CTC CT	193 bp	Matsuoka et al. (2000), Heo et al. (2004)
	Antisense	cry1A(b)	GAT GTT TGG GTT GTT GTC CAT		
NK603	Sense	HSP70	AGT TTC CTT TTT GTT GCT CTC CT	328 bp	Matsuoka et al. (2000)
	Antisense	CTP	ATC GGA TAA GCT CGT GGA TG		
TC1507	Sense	cryFa2	ACA AGT TCA GTA ATT GAA GAT TCT C	173 bp	Heo et al. (2004)
	Antisense	cryFa2	CGT GAA CTA ACT AAG TGT CCT		
GA21	Sense	OTP	GAA GCC TCG GCA ACG GCA	133 bp	Heo et al. (2003)
	Antisense	mEPSPS	ATC CGG TTG GAA AGC GAC TT		
Bt176	Sense	PEPC pro	GGT TAC CGC CGA TCA CAT GC	248 bp	Matsuoka et al. (2000), Heo et al. (2004)
	Antisense	cry1A(b)	GAT GTT TGG GTT GTT GTC CAT		

Leaf samples were collected and processed by crushing two young leaves per plant on a PlantSaver™ FTA card (Whatman, USA). All FTA cards containing plant sample DNA were stored at room temperature until PCR was completed in September 2006.

2.2. Detection of GM soybean and maize plants by multiplex-PCR

Two 2-mm disks were removed from each FTA leaf sample card by micro-punch and were placed in individual 1.5 ml tubes. A volume of 400 μl of FTA purification buffer (Whatman, USA) was added to each tube, and the tubes were incubated for 5 min at room temperature. The buffer was removed and discarded by micropipette, the incubation was repeated, and the buffer was removed again. Samples were washed with 400 μl TE buffer (10 mM Tris–HCl; pH 7.5, 0.1 mM EDTA; pH 8.0) for 5 min, and the FTA card disks were dried at room temperature for 1 h.

The multiplex-PCR protocol was performed according to Forte et al. (2005) with some modifications. Touchdown PCR was carried out in a final volume of 20 μl containing two prepared disks taken from a single FTA card, 10 mM Tris–HCl (pH 9.0), 40 μM KCl, 1.5 mM $MgCl_2$, 250 μM dNTPs mixture, 1 unit Taq DNA polymerase (Bioneer, Korea), and 0.5 μM of each specific primer (35S, *nos*, and *lectin* for soybean; 35S, *nos*, and *zein* for maize). Primer sequences are shown in Table 2. The 35S primer used in the present study can detect both the 35S promoter and the enhanced double 35S (e35S) promoter, which is frequently introduced into GM soybean and maize. The primers targeting the endogenous *lectin* and *zein* genes were used to confirm the presence of amplifiable soybean and maize DNA, respectively.

Touchdown PCR was performed as follows: initial denaturation at 94 °C for 2 min followed by denaturation at 94 °C for 15 s, annealing for 30 s at 65 °C initially, and then at temperatures decreasing 1 °C/cycle to a final temperature of 50 °C, and extension at 68 °C for 1 min, for 15 cycles. After the touchdown program was completed, the reactions were cycled 20 times at 94 °C for 15 s, 50 °C for 30 s, 68 °C for 1 min and finished by a final extension for 7 min at 72 °C. The PCR product (10 μl) was separated and visualized on a 1.8% agarose gel containing ethidium bromide. Genomic DNA from certified reference materials (CRMs) containing 5% GTS40-3-2 soybean and Bt11 maize (Institute of Reference Materials and Measurements; IRMM, Geel, Belgium) was extracted by the cetyltrimethylammonium bromide (CTAB) method (Meyer, 1999) and served as positive controls for the detection of GM soybean and maize, respectively.

2.3. Identification of a GM maize event

To determine a transgenic event in GM maize collected in the Incheon area, PCR analysis was performed with five transgenic event-specific primer pairs: Mon810, NK603, TC1507, GA21, and Bt176 (Heo, Kim, Park, Woo, & Kim, 2004; Matsuoka et al., 2000). Oligonucleotide primer sequences are shown in Table 3. PCR analysis was performed in a final volume of 20 μl containing two prepared disks from an FTA card, 10 mM Tris–HCl (pH 9.0), 40 μM KCl, 1.5 mM $MgCl_2$, 250 μM dNTPs mixture, 1 unit Taq DNA polymerase (Bioneer, Korea), and 1 μM of each specific primer. PCR conditions for the amplifications were as follows: initial denaturation at 95 °C for 10 min; denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, for 37 cycles; and a final extension at 72 °C for 5 min. The PCR product (10 μl) was separated and visualized on a 1.8% agarose gel. DNA from the CRMs containing 5% of each maize line (IRMM, Geel, Belgium) was extracted by the CTAB method (Meyer, 1999) and served as positive controls for detection.

3. Results and discussion

Multiplex-PCR was conducted to identify GM soybean from the cultivated and wild soybean leaf samples collected at 26 sites in South Korea. The GTS40-3-2 soybean (Roundup™ Ready Soybean), the only GM soybean approved for consumption in South Korea, contains the 35S promoter and *nos* terminator; hence, we used primers to detect both and the endogenous *lectin* gene (Table 1). Based on the multiplex-PCR analysis, neither the 35S promoter (83bp) nor the *nos* terminator (180bp) was detected in the cultivated and wild soybean samples (Fig. 2A and B).

Although the outcrossing rates in soybean plants are very low, gene flow between cultivated soybeans ranging from 0% to 5.89% has been reported (Nakayama & Yamaguchi, 2002). Kwon, Im, and Kim (1972) also reported gene flow between cultivated and

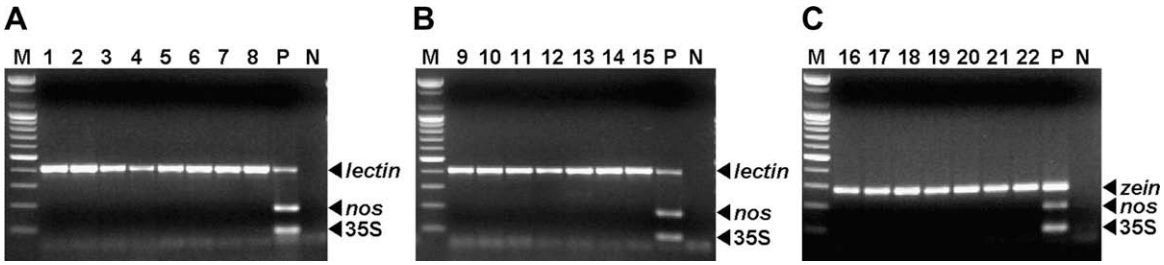

Fig. 2. Agarose gel electrophoresis of multiplex-PCR products from DNA samples collected from major soybean and maize fields in eight South Korean provinces. (A) cultivated soybean leaf samples; (B) wild soybean leaf samples; (C) maize leaf samples. M, 100-bp DNA ladder; Lanes 1–22, collected DNA samples; P, positive control (A and B: GTS40-3-2 soybean; C: Bt11 maize); N, negative (no DNA template) control.

wild soybean plants. Cultivated soybean varieties originate from East Asia, and diverse wild soybeans are distributed all over the Korean Peninsula (OECD, 2000). Therefore, the escape of GM soybean would be a potential risk to the local ecosystem. In fact, it was reported that unintentional mixtures of GM soybean have been found in the local open markets (Shim et al., 2006; Zhou et al., 2007). Moreover, it has been reported that GM soybeans were found in soybean cultivated fields in South Korea. Therefore, although GM soybean was not found in this survey, continuous monitoring should be conducted to cope with the unintentional release of GM soybean in the future.

All GM maize events approved for consumption in South Korea contain the 35S (or the e35S) promoter, the *nos* terminator, or both (Table 1). Therefore, multiplex-PCR was used to detect the 35S promoter, *nos* terminator, and endogenous *zein* genes, which would identify the presence of GM maize. Based on the PCR, neither the 35S nor the *nos* sequences were detected in the samples collected from maize-cultivated fields (Fig. 2C).

To investigate kernel escape during grain transportation, we surveyed for GM plants around the Incheon Port. We collected 18 maize samples from the roadsides near the entrances of a grain transportation company and animal feed production companies (Fig. 1B). Based on PCR analysis, neither the 35S promoter nor the *nos* terminator was detected in seven samples collected near one of the animal feed production companies. However, four out of 11 samples collected from the roadside near the entrance of the grain transportation company were confirmed to be GM maize. PCR products for the 35S and the *nos* sequences were obtained from three maize samples, while the 35S promoter only appeared in another maize sample (Fig. 3A).

Of the GM maize events approved for consumption in South Korea, Bt11, Mon863, Mon88017, and NK603 contain both the 35S promoter and the *nos* terminator, while Bt176, Das-59122-7, Mon810, T25, and TC1507 contain the 35S promoter but not the *nos* terminator (Table 1). To identify the four GM maize events that we harvested, PCR analysis was performed using event-specific primers for Mon810, NK603, TC1507, GA21, and BT176. Of the four maize samples, a 200-bp single band was only detected from PCR reactions that used the Mon810-specific primers (Fig. 3B). Based on these results, the maize plant that only contained the 35S promoter is Mon810. The three maize plants containing both 35S and *nos* sequences are likely stacked traits and are either Mon863 - Mon810 or Mon88017 × Mon810 of the GM maize events approved for consumption in South Korea (Table 1). However, it is also possible that the maize events which are unapproved by the South Korean government might have been mixed elsewhere and imported to South Korea.

The four GM maize plants found in the present study may be a result of kernel escape during transportation. Kernel escape in the present study may not be considered a serious problem due to the lack of habitats surrounding the investigated roadsides. Maize is also known to be a non-invasive plant in natural habitats (OECD, 2003), besides which, wild relatives of maize are absent in South Korea. However, seed spillage during transportation may cause the spread of GM maize to cultivated fields and, consequently, gene flow from GM maize to non-GM maize.

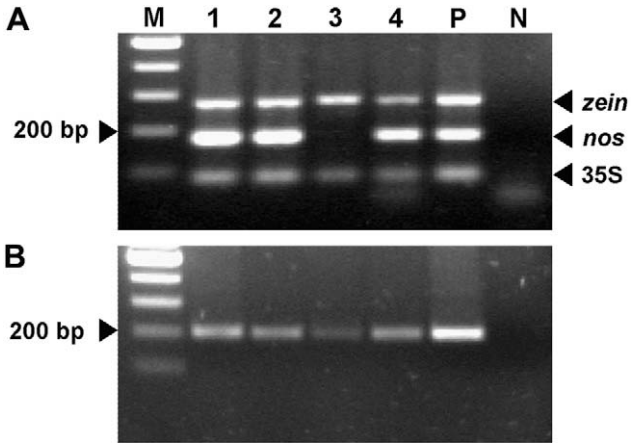

Fig. 3. Agarose gel electrophoresis of PCR products from maize DNA samples collected from roadsides near Incheon Port. (A) Amplification by multiplex-PCR; (B) Amplification using Mon810-specific primers. M, 100-bp DNA ladder; Lanes 1–4, maize samples; P, positive control (A: Bt11 maize; B: Mon810 maize); N, negative (no DNA template) control.

Although the cultivation of GM crops has not yet been approved in South Korea, their importation is expected to increase, and unforeseen cultivation may occur. In fact, a single GM maize plant growing in a small vegetable garden around Incheon Port has been reported in South Korea (Kim et al., 2006). Therefore, continuous monitoring for the presence of GM plants along transportation routes and roadsides around grain transportation companies and animal feed production companies at the Incheon Port will be important to regulate the spread of GM plants. We also verified that a two-step PCR approach (multiplex and event-specific PCR) was useful in identifying most GM soybean and maize events imported to South Korea.

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