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Monitoring the Occurrence of Genetically Modified Soybean and Maize around Cultivated Fields and at a Grain Receiving Port in Korea

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Increased imports of genetically modified (GM) soybean and maize might cause genetic contamination of those crops that are conventionally bred, as well as wild soybeans within Korea. Leaves of maize and both cultivated and wild soybeans were sampled in and near rural fields to detect the presence of transgenes. Roadsides around a major grain port in Incheon were also surveyed to monitor the occurrence of incoming GM soybean and maize. The amplificability of DNA extracted from the collected samples was determined by PCR using soybean- or maize-specific primers: *lectin* and *zein* genes, respectively. The presence or absence of transgenes was detected by primer sets for the 35S and *nos* genes. Transgenes were not found in the cultivated or wild soybean or in the maize collected from cultivated fields. However, we obtained one GM maize plant among seven along the roadsides around Incheon Port. Although the effect of a single GM maize plant would be negligible and would not pose any threat to natural environments, an increase in the import of GM plants might lead to future, unapproved cultivation of GM crops. Therefore, appropriate monitoring is necessary to detect the occurrence of GM plants in areas around grain receiving ports and within agroecosystems.

Keywords: detection, genetically modified plant, maize, monitoring, PCR, soybean

The availability of soybean (*Glycine max* L. Merr.) and maize (*Zea mays* L.) for consumption in Korea depends almost entirely on imports because their self-sufficiency rates are only 7.3% and 0.8%, respectively (Korea National Statistical Office, 2005). Between 2000 and 2004, Korea annually imported 1.3 to 1.6 million tons of soybean and 8.5 to 8.8 million tons of maize (Korea National Statistical Office, 2005). About 80% of the soy imported for oil production is from genetically modified (GM) plants (Yoo, 2004). Most GM soybean and maize that are approved for Korean consumption have been engineered for herbicide tolerance (i.e., gluphosinate and glyphosate) or insect resistance (Bruderer and Leitner, 2003; Jun et al., 2005; Agricultural Biosafety Clearing House, 2006; Table 1).

Cultivation of GM soybeans in the U.S. is considered safe in terms of its gene flow because it is largely a self-pollinated species and because wild soybeans, such as *Glycine gracilis* Skvortsov and *Glycine soja* Sieb. et Zucc., do not occur naturally in the U.S. However, East Asia is the center of origin for *G. soja*, which is the wild ancestor of cultivated soybeans (OECD, 2000; Dorokhov et al., 2004). Kwon et al. (1972) have reported gene flow between cultivated and wild soybean plants. Moreover, Nakayama and Yamaguchi (2002) have found that the rate of soy hybridization due to gene flow ranges from 0% to 5.89%, and have suggested that, because of that rate, the environmental release and cultivation of GM soybeans in East Asia may cause ecological risks. In Korea, Kim et al. (2003) have examined 243 sampling sites but have not found any glyphosate tolerance in wild soybeans.

Although the GM soybean and maize imported for human consumption or animal feed are currently not allowed to be planted in Korean fields, such cultivation could accidentally happen if seed lots became contaminated, as evidenced by the unapproved sales of *Bt10* maize to farmers (Macilwain, 2005). Friesen et al. (2003) also have discovered abundant contamination in pedigreed Canadian canola seed lots with GM herbicide tolerance traits.

Seed spilled during the transportation process also may lead to the growth of GM soybean and maize in normally uncultivated habitats. Crawley and Brown (1995) have investigated the dynamics of oilseed rape populations along the verges of a motorway around a main oilseed-crushing factory in southern England. There, seed that falls from passing lorries could contribute to the establishment of such a population on disturbed soil. Therefore, the potential exists for GM crop plants to become established if their seeds are spilled after arriving at nearby grain ports.

To monitor for these GM plants, a number of methods are available for properly detecting the presence or absence of transgenes, e.g., herbicide bioassays, immunoassay, ELISA, and PCR. Of these, PCR is the most widely used (Lübeck, 2002) because of its high sensitivity, fast analysis time, and its capability of discriminating specific transgenic events (Querci et al., 2004). It is also the only method that can be applied to field samples. A qualitative and quantitative screening PCR-based method

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has been proposed by food researchers for specific detection of GM soybean and maize (Jankiewicz et al., 1999; Yamaguchi et al., 2003; Yun et al., 2004).

The objective of the present study was to monitor the occurrence of GM soybean and maize in cultivated fields, as well as in areas at and surrounding a major port for grain importation in Korea.

MATERIALS AND METHODS

Collection of Soybean and Maize Tissue Samples

We monitored 47 major areas of soybean cultivation throughout 8 provinces of Korea for the presence of GM crops (Fig. 1A). From 11 to 26 July 2005, leaf samples were collected at each site from 10 cultivated soybean (*G. max*) and 3 to 6 wild soybean (*G. soja*) plants by crushing two young leaves per plant on an FTA Classic card (Whatman, USA). In addition, 54 DNA samples were gathered from the leaves of 2 to 4 maize plants in each of 25 areas within 4 provinces (Fig. 1A). All FTA cards containing the DNA were stored at room temperature (RT) until PCR was completed in January 2006. In a second experiment, we investigated the occurrence of GM soybean and maize along the roadsides around Incheon Port, the major grain port in Korea. Our survey, conducted on 8 July 2005 (Fig. 1B), focused on seed spillage on the main roads that run from the piers to major oil and animal feed companies nearby. Soybean and maize plants were investigated by researchers walking or driving around the areas presented in Figure 1B. Because PCR analysis results indicated that one of the maize samples collected on FTA cards at Incheon was possibly transgenic, this sampling site was revisited on 2 August 2005, and an entire leaf was taken from the suspected plant. Two other maize plants and some spilled seeds not found on 2 July were also collected on that second date. All samples were then stored in a deep freezer before analysis.

Detection of Transgenes by PCR

The primers designed to detect plant-specific genes and transgenes (Table 2) were synthesized by Bioneer (Korea). For GTS40-3-2 soybean, the cauliflower mosaic virus (CaMV) 35S promoter and chloroplast transit peptide 4 (CTP4) were used as target sequences for transgenes. Because all the GM maize in Table 1 contain either a 35S promoter or nopaline synthase (*nos*) terminator, both were used as target sequences for maize detections. Amplificability of the extracted DNAs was verified with plant-specific primer pairs that targeted the *lectin* gene for soybean or the *zein* gene (Forte et al., 2005) for maize. Two 2-mm disks were removed from each FTA leaf-sample card and were placed in micro-

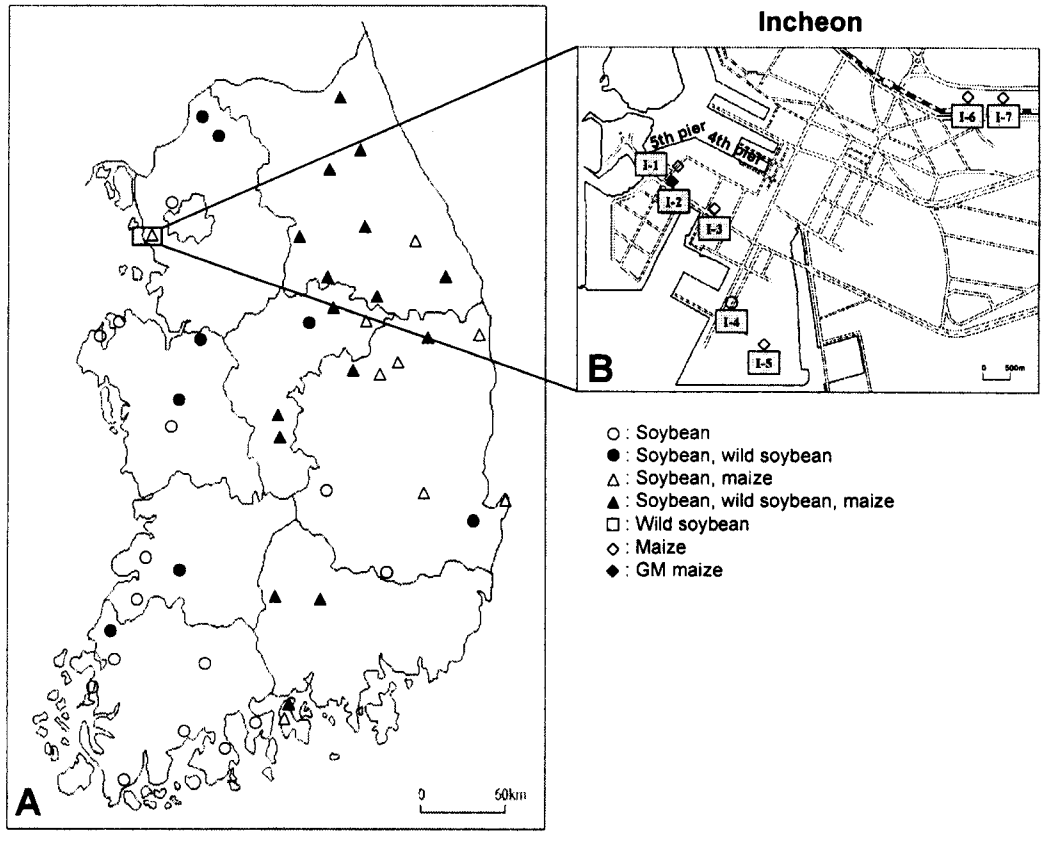


Figure 1. A, Soybean and maize monitoring sites; **B**, Roadside sampling sites around Incheon Port. ◆ , indicates location of GM maize plant found in the present study.

Table 1. Characteristics of some genetically modified soybean and maize approved for consumption in Korea (Agricultural Biosafety Clearing Houses, 2006).

Events	Novel traits	Promoters	Introduced genes	Terminators
GTS40-3-2 soybean	Tolerance to glyphosate	35S	*epsps*	*nos*
Bt11 maize	Resistance to European corn borer; tolerance to gluphosinate ammonium	35S	*cry1Ab, pat*	*nos*
Event176 maize	Resistance to European corn borer; tolerance to gluphosinate ammonium	35S	*cry1Ab, bar*	35S
GA21 maize	Tolerance to glyphosate	*P-ract*	*epsps*	*nos*
MON810 maize	Resistance to European corn borer	35S	*cry1Ab*	*nos*
MON863 maize	Resistance to corn rootworm	4-AS1, 35S	*cry3Bb1*	*tahsp17, nos*
NK603 maize	Tolerance to glyphosate	*P-ract*, 35S	*epsps*	*nos*
T25 maize	Tolerance to gluphosinate ammonium	35S	*pat*	35S
TC1507 maize	Resistance to European corn borer; tolerance to gluphosinate ammonium	35S, *ubiZM*	*cry1Fa2, pat*	35S

Table 2. Primers used for the detection of genetically modified soybean and maize.

Plants	Primers (sense/antisense)	Sequences (5′ → 3′)
Soybean	Lectin (sense)	TTAGATGGCCTCATGCAACAC
	Lectin (antisense)	TCTTGGGATTTGGCCAACAATA
	35S (sense)	TTCATGTGATATCTCCACTGAC
	CTP4 (antisense)	CAAAACCAACATAGAATTTGC
Maize	Zein (sense)	AGTGCGACCCATATTCCAG
	Zein (antisense)	GACATTGTGGCATCATCATTT
	35S (sense)	ATTGATGTGATATCTCCACTGACGT
	35S (antisense)	CCTCTCCAAATGAAATGAACTTCCT
	Nos (sense)	GTCTTGCGATGATTATCATATAATTTCTG
	Nos (antisense)	CGCTATATTTTGTTTTCTATCGCGT

centrifuge tubes, to which 200 μL of FTA purification buffer was added. The tubes were incubated at RT for 5 min and the wash solution was discarded. The wash was repeated with 200 μL of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA; pH 8.0), and the FTA card disks were then dried at RT for 1 h. PCR was performed with a final volume of 20 μL that contained two disks, 0.5 μL of a 10 mM dNTP mixture, 0.3 μL of Taq DNA polymerase, and 2 μL of each specific primer listed in Table 2. PCR conditions for the amplifications included: initial denaturation at 95°C for 10 min; then 37 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min. DNAs from the maize leaf and seed samples collected on 2 August were extracted by the cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). PCR was conducted as described above.

Agarose Gel Electrophoresis

The PCR product (10 μL) was separated and visualized on an 1% agarose (Qbiogene, USA) gel containing ethidium bromide in 0.5X TAE buffer (20 mM Tris acetate, 5 mM EDTA; pH 8.0). GTS40-3-2 soybean and Mon810 maize plants that had been grown in a glasshouse at the Korea Research Institute of Bioscience and Biotechnology served as positive controls for the detection. A 100-bp DNA Ladder (Bioneer) was used as a marker.

TA Cloning

PCR amplification with the *nos* primers yielded a 152-bp PCR product, which was then cloned into the pCR2.1TOPO (Invitrogen, USA) vector using standard procedures. After a clone was selected, full-length sequencing of the insert was carried out by Genotech (Korea) with a 3730 XL DNA Analyzer (Applied Biosystems, USA).

RESULTS AND DISCUSSION

Monitoring of GM Soybean and Maize Contamination in Cultivated Fields

The amplificability of DNA extracted from 470 cultivated and 111 wild soybean samples was verified using

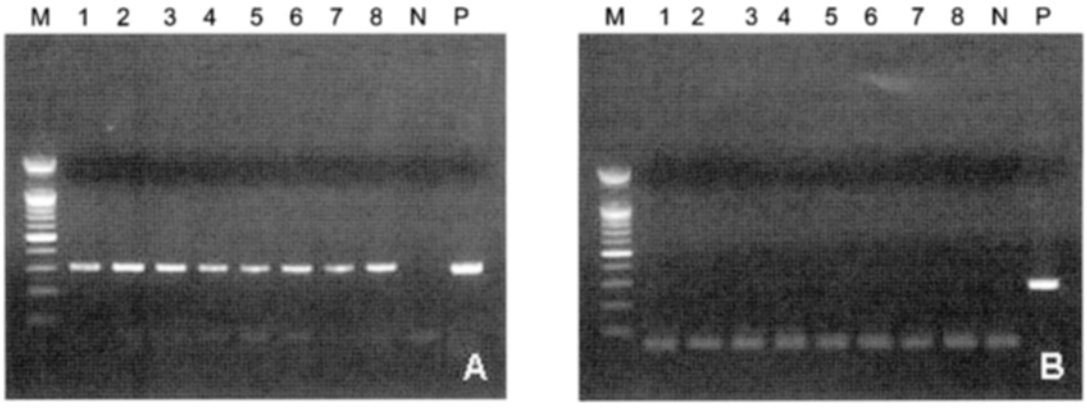


Figure 2. Agarose gel electrophoresis patterns of PCR products from DNA of cultivated soybean samples collected in 8 provinces of Korea. **A**, amplification of *lectin* gene; **B**, amplification of 35S gene. M, 100-bp DNA ladder; Lanes 1-8, cultivated soybean samples from Yanggu, Yeoncheon, Boeun, Taean, Gochang, Goheung, Mungyeong, and Namhae, respectively; N, negative control; P, positive control (GTS40-3-2 soybean).

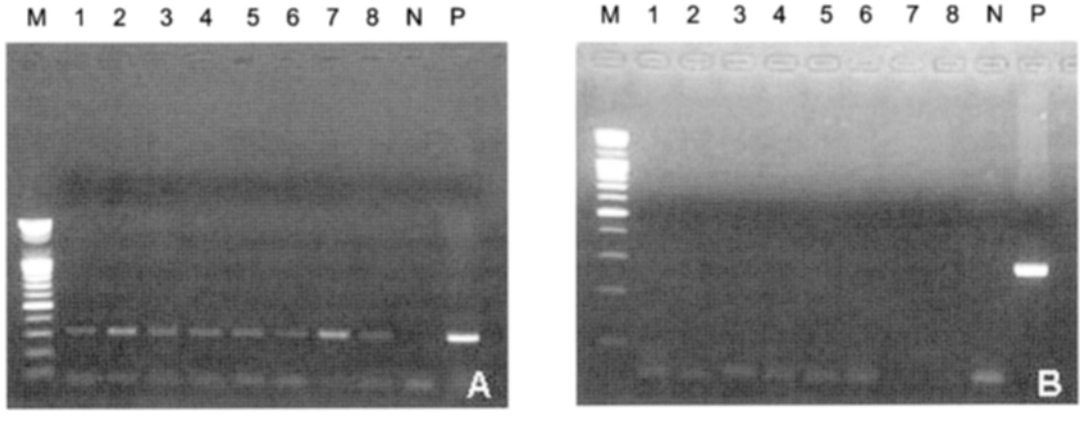


Figure 3. Agarose gel electrophoresis patterns of PCR products from DNA of wild soybean samples collected in 8 provinces of Korea. **A**, amplification of *lectin* gene; **B**, amplification of 35S gene. M, 100-bp DNA ladder; Lanes 1-8, wild soybean samples from Yanggu, Yeoncheon, Boeun, Taean, Gochang, Goheung, Mungyeong, and Namhae, respectively; N, negative control; P, positive control (GTS40-3-2 soybean).

a primer pair specific to soybeans (Fig. 2A, 3A). No 35S gene was detected from either the cultivated or the wild samples (Fig. 2B, 3B). Leaf DNA was also extracted from 54 maize samples in cultivated fields; neither the 35S nor the *nos* gene was detected from any of those samples.

FTA cards have been used to collect and store DNA samples from a wide range of animal (Smith and Burgoyne, 2004) and plant (Lin et al., 2000) species. Here, we found that DNA samples could be easily collected from field plants and stored for over six months at room temperature without deterioration. These samples were also successfully amplified by PCR, thus demonstrating that the use of FTA cards is suitable for monitoring the occurrence of GM plants.

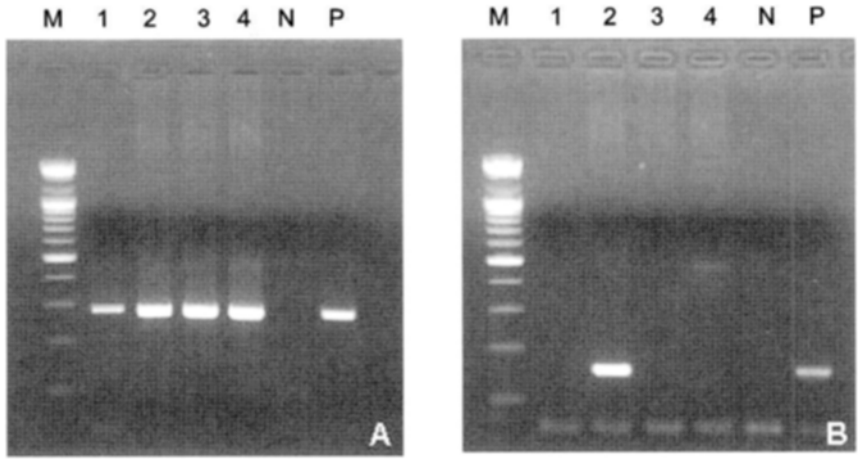
Monitoring of GM Maize Contamination around Incheon Port

Although soybean plants were found around the roadsides at the Incheon port, their source was a small vegetable garden (I-4 in Fig. 1B), and no transgenes were detected from five samples collected in that garden. Furthermore, we observed no cultivated or wild soybean plants that originated from spilled seed.

We found no established, transgenic maize populations in our survey. Although seven maize plants were discovered near the port, only three appeared to have germinated from seeds spilled from passing vehicles (I-3, I-6, I-7 in Fig. 1B). The other four plants may have been intentionally planted in a small vegetable garden in our monitoring area (I-2, I-5 in Fig. 1B). Some of the spilled maize seeds were collected for our analyses (I-1 in Fig. 1B).

DNA was extracted from three of the seven maize plants and seeds (Fig. 4). Although the 35S gene was not detected in those samples, agarose gel electrophoresis of the PCR products showed a band for the *nos* gene (Fig. 4B, Lane 2) from one of two maize plant samples collected in the vegetable garden (I-2). Further DNA sequence analysis confirmed its presence (Fig. 4C).

The presence of this *nos* gene in the absence of the 35S gene indicates that this sample was obtained from



C

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  1  CGCTATATTT  TGTTTTCTAT  CGCGTATTAA  ATGTATAATT  GCGGGACTCT  AATCATAAAA
 61  ACCCATCTCA  TAAATAACGT  CATGCATTAC  ATGTTAATTA  TTACATGCTT  AACGTAATTC
121  AACAGAAATT  ATATGATAAT  CATCGCAAGA  CA
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Figure 4. Agarose gel electrophoresis patterns of PCR products from DNA of maize samples collected from roadsides near Incheon Port. **A**, amplification of *zein* gene; **B**, amplification of *nos* gene; **C**, partial DNA sequences amplified by PCR using *nos* primers. M, 100-bp DNA ladder; Lanes 1-4, maize samples from I-1 (Hang-dong), I-2 (Hang-dong), I-6 (Sungui-dong), and I-7 (Sungui-dong), respectively; N, negative control; P, positive control (Mon810 maize).

a GM maize event that differed from Bt11, Event176, MON810, NK603, T25, and TC1507 (Table 1). Therefore, the GM plant that we found might be GA21 maize, which has the *nos* terminator without a 35S promoter. This maize plant appeared to have been planted by a local resident who could have obtained seeds from an employee of a transportation or animal feed company. Nonetheless, the definitive establishment of a GM maize population was not found in our study and the effect of this single GM maize plant on the environment would be negligible. Because maize plants are non-invasive in natural habitats (OECD, 2003), they cannot pose an environmental threat. The introgression of transgenes from GM maize to its wild relatives also would not have occurred because wild relatives of maize are not present in Korea (Shim et al., 2001). However, the transfer of transgenes to cultivated non-GM maize might be possible if a GM crop were to be grown in areas small enough to allow gene flow via pollen.

Although the cultivation of GM plants has not yet been approved in Korea, their import is expected to increase, and their unforeseen, intended or accidental cultivation may eventually occur. Therefore, Kjellsson and Strandberg (2001) have emphasized the importance of surveillance wherever GM plants are grown or transported, including not only in cultivated fields but also in unexpected places, e.g., wastelands, harbor surroundings, and roadsides. Such tactics are already being employed in Japan, where GM herbicide-tolerant rapeseed plants have been reported around receiving ports (NIES, 2005). Korea relies as greatly on food imports as Japan does. Appropriate monitoring is necessary to detect the possible dispersal of imported GM plant seeds in the transportation system, as well as through the transfer of transgenes to wild relatives via pollen flow.

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