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https://doi.org/10.5109/1526339

出版情報：九州大学大学院農学研究院紀要. 60 (2), pp.285-290, 2015-09-18. Faculty of Agriculture, Kyushu University

J. Fac. Agr., Kyushu Univ., **60** (2), 285–290 (2015)

Monitoring the occurrence of genetically modified maize in Korea: A 3–year observations

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(*Received April 14, 2015 and accepted May 19, 2015*)

The cultivation area of genetically modified (GM) crops is increasing all over the world. Currently, GM crops are not allowed to grow in Korea but have officially been allowed to import as food and feed purposes. In recent years, concerns regarding the unintentional escape of GM crops during transport and manufacturing, and the subsequent contamination of the local, non–GM plants have been raised. In this connection, monitoring was executed around grain–receiving ports and feed–manufacturing plants at 31 sites in six provinces of Korea from July to September 2010–2012. We found spilled maize grains and established plants around open storage areas of ports and along transportation routes near feed–manufacturing plants. A total of 151, 191, and 21 maize plants were found in 2010, 2011, and 2012, respectively. Based on PCR analyses, 11, 35, and 5 plants were detected as GM in 2010, 2011, and 2012, respectively. Where the plants were confirmed as GM maize, we revisited the sites several times every year until the end of maize growing season. In most cases, the established maize plants found in this study were at the vegetative stage and failed to reach up to reproductive stage. Moreover, the established maize plants found during the year of 2010 and 2011 were not found at the same sites thereafter. Based on the results of this study, we conclude that, although GM maize plants could escape during transport or storage, their infestation by overwintering or pollen flow of GM maize is not likely to occur in the environment of Korea.

Key words: GMO, Monitoring, Environmental risk assessment

INTRODUCTION

Genetically modified (GM) crops were cultivated in 25 countries in 2008 after the commercialization of these crops in 1996. In 2009, it was registered that a total area of 134 million hectares were used for GM crops, which implied a 7% increase from that of 2008. Also, a recent study recorded 14 million small and large farmers in Egypt, Bolivia and other countries beside major cultivating countries such as America, Argentina and Brazil (James, 2009).

Alongside this rapid increase of the cultivation of GM crops, the contamination of natural land and environment has become an increasingly alarming issue. For an example, Syngenta reported that accidentally a variety of corn (maize) called Bt10 between 2001 and 2004 (Macilwain, 2005) has been released. Moreover, in 2006, it was reported that the monitoring of pedigreed canola seed lots in west Canada showed contaminations that displayed herbicide–resistant traits (Friesen *et al.*, 2003).

There is a ways to release GM crops through movement during the transportation process. Adventitious spillage of GM crops will likely lead to environmental risks of gene flow to allied species, which will ultimately affect plant colonies and even the insect ecosystem (Snow and Moran–Palma, 1997). For instance, the monitoring of import grain ports and along transportation routes in Japan have led to the discovery of wild transgenic canola in 2005 (Saji *et al.*, 2005). Furthermore, survey reports suggested that transportation routes, that include roadsides and railways between Saskatchewan and Vancouver, now had more than 60% of transgenic herbicide resistant oilseed rapes (Yoshimura *et al.*, 2006).

Previous reports in Korea announced that there were no risks to GM contamination or adventitious spillage. Furthermore, it is even more imperative that a local assessment be conducted because the maize is not native to the grower (Shim *et al.*, 2001). To escape from the harmful effects of GM crops on the local environment and human health, Korea needs to conduct a risk assessment for GM crops (Korea Biosafety Clearing–House, 2007). The rate of total food self–sufficiency in Korea was only 26.7%. This implies that a majority of the crops in Korea are imported. But, much of what is imported to Korea are GM maize, as estimated by the 0.3 million tons of the total importation of 1.4 million tons being GM maize products in 2009 (Korea National Statistical Office, 2012).

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Seed spillage and unintentional movement of maize during the transportation process is highly likely with the increasing GM Maize importation ratio (Kim *et al.*, 2006; Lee *et al.*, 2009).

Therefore the movements of GM maize during transportation as well as the travel route area require monitoring for the adventitious spillage of GM maize contamination problem and environment risks. Gene flow (Crop to crop) study reported that cross pollinations occurred at a maximum distance of 200 m from the GM source (Weekes *et al.*, 2007). Genetic contaminations may also happen in the conventional maize fields due to its high rate of outcrossing (about 5% self–pollination).

The objective of this study was to detect the presence of GM maize from major grain receiving ports and feed processing plants in Korea.

MATERIALS AND METHODS

Monitoring site

Monitoring was conducted at 31 different sites throughout six provinces of Korea from July to September 2010 to 2012. We monitored two major grain receiving ports, Incheon and Gunsan, and the surrounding areas. Gunsan and Incheon are one of the major ports in Korea, located west of Korea, and the Gunsan and Incheon coastal industrial region has developed around the port (Fig. 1). Some of the cargoes discharged at the port of Gunsan and Incheon are transported to the capital region near the food and feed manufacturing plant. We started our roadside survey at port in Incheon City and Gunsan City, about three kilometres around of the port, where numbered 7th and 3rd. The roadside survey extended about three kilometres from the starting point at Incheon 7th port and Gunsan 3rd port to the industrial that around the way of ports (Fig. 1). We surveyed the side of the road leading to ports of 7th and 3rd to detect the presence of maize plant and seed. The areas adjacent to the monitoring zone have various land–use types, including urban districts, compost processing plants, and a steel bridge. Maize sampling was mainly conducted around the grain storage area and roadsides. Maize plants and seeds around feed manufacturing plants were collected along roadsides and transportation routes throughout six provinces of the Republic of Korea. The locations of monitoring sites and number of collected samples are shown in Table1.

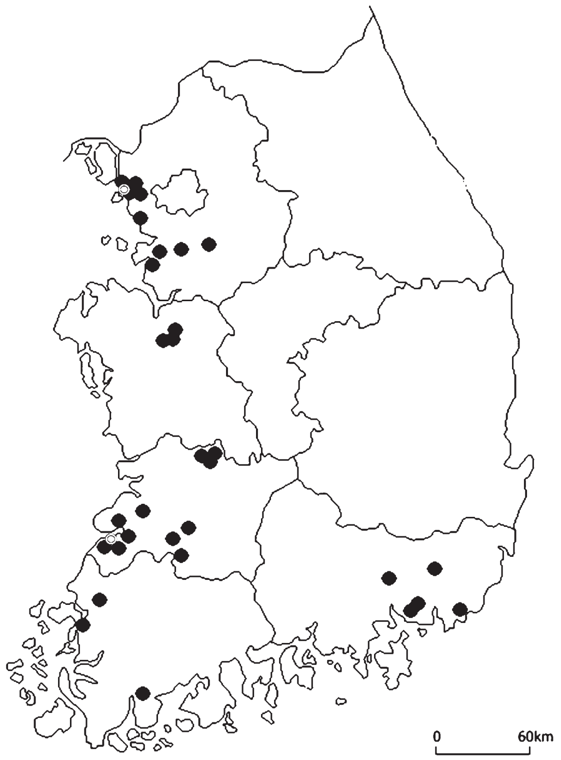

Fig. 1. Monitoring sites in grain receiving ports and feed manufacturing plants in Korea (◎: Port, ●: Manufacturing plant).

Roadside survey and sampling of maize leaf tissue and seed

The roadside surveys were carried out at the time of growing of maize, from July to September, in 2010, 2011, and 2012. We performed the census of maize individuals and sampled their leaf materials on foot along the same stretch of road around port and manufacturing plant in all three years. Live maize plants were collected near roadsides, transportation routes, and around the grain storage area. Leaf materials were harvested along the same stretch of roads. About 10 cm^2 of young leaves were collected from each plant and inserted in a 50 mL tube and transferred to the laboratory on dry ice. Maize seeds were collected at one to three meters from the road edge, a zone that contained sidewalk, drains, and flowerbeds. Spilled seeds were collected and inserted in a 50 mL tube and brought to the laboratory. Collected seeds were sown in the pot (45 × 27 cm) filled with commercial potting mix in a greenhouse maintaining at 25 ± 5°C with a 16–h photoperiod. An area of 10 cm^2 of young leaves were collected from the seedlings emerged in the tray at 14 days after sowing.

Detection of GM maize plants and event by PCR

GM maize was confirmed by PCR and was investigated to determine the specific GM maize event. Genomic DNA of leaf tissues of young maize was extracted using a DNA extraction kit (RBC YGP–100, Taipei, Taiwan). Nos terminator and 35S promoter–specific primers and internal primers specific to *SSIIb1* were used for detection of transgenes of the GM maize (Table 1). The PCR reaction mixture contained, 10 mM Tris. HCl (pH 9.0), 40 μM KCl, 1.5 mM MgCl2, 250 μM dNTPs mixture, 1 unit Taq DNA polymerase (Solgent, Republic of Korea), and 2 μM of each event–specific primer in a total volume of 20 μL. PCR was set up following: *SSIIb1*was pre–incubation for 3 min at 94°C; 35 cycles of denaturation for 30 s at 94°C; annealing for 30 s at 55°C and extension for 50 s at 72°C; then a final extension at 72°C for 7 min. nos was pre–incubation for 3 min at 94°C; 35 cycles of denaturation for 30 s at 94°C; annealing for 30 s at 58°C and extension for 50 s at 72°C; then a final extension at 72°C for 7 min. 35s was pre–incubation for 3 min at 94°C; 35

Table 1. Oligonucleotide primers for detection of genetically modified maize

Gene	Orientation	Sequence (5' – 3')	Product size	Reference
35S	Sense	TGC CTC TGC CGA CAG TGG TC	83 bp	Trapmann 2002
	Antisense	AAG ACG TGG TTG GAA CGT CTT C		
nos	Sense	GAA TCC TGT TGC CGG TCT TG	180 bp	Lipp 1999
	Antisense	TTA TCC TAG TTT GCG CGC TA		
SSIIb1	Sense	CTC CCA ATC CTT TGA CAT CTG C	151 bp	Yoshimura 2005
	Antisense	TCG ATT TCT CTC TTG GTG ACA GG		

Table 2. Oligonucleotide primers for detection of genetically modified maize events

Event	Orientation	Target gene	Sequence (5' – 3')	Product size	Gene
Mon810	Sense	*HSP70*	AGTTTCCTTTTTGTTGCTCTCCT	193 bp	Matsuoka 2000
	Antisense	*cry1Ab*	GATGTTTGGGTTGTTGTCCAT		
NK603	Sense	*HSP70*	AGTTTCCTTTTTGTTGCTCTCCT	328 bp	Matsuoka 2000
	Antisense	*CTP*	ATCGGATAAGCTCGTGGATG		
Bt176	Sense	*PEPC pro*	GGTTACCGCCGATCACATGC	248 bp	Matsuoka 2000
	Antisense	*cry1Ab*	GATGTTTGGGTTGTTGTCCAT		
Mon863	Sense	*cry3Bb1*	GATGACCTGACCTACCAGA	234 bp	Onishi 2005
	Antisense	tahsp17	GCACACACATCAACCAAATT		
GA21	Sense	*OTP*	GAAGCCTCGGCAACGGCA	133 bp	Heo 2004
	Antisense	*mEPSPS*	ATCCGGTTGGAAAGCGACTT		
Bt11	Sense	*adh1*–1S IVS6	GGTTACCGCCGATCACATGC	248 bp	Heo 2004
	Antisense	*cryIA(b)*	GATGTTTGGGTTGTTGTCCAT		
MIR604	Sense	*mcry3A*	CGCGGTGTCATCTATGTTAC	162 bp	Ahn 2008
	Antisense	*pmi*	AGGCTACATCCGTGCAGGAG		

cycles of denaturation for 30 s at 94°C; annealing for 30 s at 63°C and extension for 50 s at 72°C; then a final extension at 72°C for 7 min. The amplified products were then subjected to electrophoresis on 1.8% agarose gel containing ethidium bromide.

PCR was carried out with event–specific primers for seven GM maize events, Mon810, NK603, MON863, GA21, Bt176, Bt11 and MIR604. Oligonucleotide sequences of the primers are shown in Table 2. The PCR reaction mixture contained, 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, Republic of Korea), and 2 μM of each event–specific primer in a total volume of 50 μl. PCR was programmed as follows: initial denaturation at 95°C for 10 min and 37 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, followed by a final extension for 5 min at 72°C. The amplified products were then subjected to electrophoresis on 1.8% agarose gel containing ethidium bromide. DNA of CRMs containing 5% of each maize event (IRMM, Geel, Belgium) was extracted by the CTAB method (Meyer, 1999), and served as a positive control for detection.

RESULTS

To investigate the escape of maize seeds escape during cargo work or storage, we surveyed two major grain receiving ports, Incheon and Gunsan of Korea. In Incheon, most of the imported grains are transported to oil companies and animal feed and grain transfer companies. Therefore, we surveyed around the grain storage areas and major oil and animal feed companies nearby. Based on the PCR analysis, 61 samples contained 35S promoter and nos terminator sequences. In 2010, we found a total of 89 maize seeds and 151 maize plants in our surveys area (Table 3). All the seeds were sown for checking, whether they are GM or not. Unfortunately, no seed was germinated from the collected seeds of 2010. So we were unable to detect the GM maize from the seeds. Among 151 maize plants, 11 maize plants were detected as GM. Among them, five from Nonsan, three from Kimje, one from Gunsan port and two from Mokpo (Table 3). From our study in 2011, a total of 25 maize seeds and 191 maize plants were collected from our study areas (Table 3). Among the 25 seeds, four seeds (two from Incheon port and two from Nonsan) were identified as GM. From 191 maize plants, 35 plants were detected as GM maize. Among 35 GM maize, most of the GM plants (28) were collected from Gunsan and the rest seven plants were detected from Jungep (Table 3). The number of seeds and maize plants were much lower than those collected in the year 2012. Only a total of 10 maize seeds and 21 maize plants were collected in 2012. Among the maize

Table 3. Monitoring sites and the number of collected samples for genetically modified maize plants and seeds collected from grain receiving ports and feed manufacturing plants in the Republic of Korea

Province	Site	Collected samples (GM)					
		2010		2011		2012	
		Plant	Seed	Plant	Seed	Plant	Seed
Gyeonggido	Inchun port	19	15	8	7(2)	0	0
	Inchun	10	0	0	0	0	0
	Ahnsan	0	0	0	0	0	1(1)
	Youngin	10	0	0	0	0	0
	Pyungtak	0	0	5	11	0	2(1)
Chungcheongnamdo	Nonsan	24(5)	40	0	4(2)	0	4(1)
	Suchun	0	3	0	0	0	2(2)
	Chunan	62	0	23	3	0	0
Gyeongsangnamdo	Yangsan	0	0	0	0	0	1(1)
	Kimhe	0	0	0	0	0	0
	Hamam	2	0	0	0	0	0
Busan	Busan	0	0	0	0	0	0
Jeollabukdo	Iksan	18	0	23	0	16(4)	0
	Jungep	0	0	11(7)	0	0	0
	Junju	0	0	0	0	0	0
	Kimje	5(3)	0	0	0	4	0
	Gunsan port	1(1)	31	42(28)	0	1(1)	0
Jeollanamdo	Hampyung	0	0	0	0	0	0
	Kangjin	0	0	0	0	0	0
	Mokpo	12(2)	0	79	0	0	0
Total		151(11)	89	191(35)	25(4)	21(5)	10(6)

seeds, six (one from Ahnsan, one from Pyungtak, one from Nonsan, two from Sunchun and one from Yangsan) were identified as GM and five maize plants (four from Iksan and one from Gunsan port) out of 21 were detected as GM maize (Table 3).

In 2010 and 2011, Event–specific PCR demonstrated that transgenes for four maize events, i.e. MIR604, GA21, Bt176, and Bt11 were not found in the maize samples (data not shown). We detected maize event like MON810 and Mon863 specific genes, resistant to insect andNK603 specific gene, resistant to glyphosate. The number of seeds and maize plants were much lower collected in the year 2012. Event–specific PCR demonstrated that transgenes for five maize events, MIR604, GA21, Bt176, Bt11, and Mon863 were not found in the maize samples (data not shown). We detected maize event of MON810and NK603 in 2012.

DISCUSSION

Korea could produce approximately 22.6% of food for its inhabitants (Korea National Statistical Office, 2012). The rest of the 77.4% of the food crops in Korea has to be imported from other countries. Similar to other food products, the maize self–sufficiency rate is very low in Korea. The amount of imported food showed an increasing trend every year. It is strictly prohibited to grow GM crops in Korea, but it can be imported for food products, or feed processing. Although there is no GM maize cultivation locally, every year there are reports of GM maize spill (Korea Biosafety Clearing–House, 2008).Hence, it is very important to monitor GM crops that can enter our local environment system and potentially alter local plants through unintentional gene flow.

The aim of this study was to monitor the existence of GM maize from the imported GM maize around the grain port to animal feed manufacturing plant and along the transportation route. Generally, GM maize is imported through ports of Incheon and Gunsan and the transported to animal feed manufacturing plants or food plants (Ministry of oceans and fisheries, 2008). We monitored both the spill of maize seed and the established maize seedlings around the investigation sites. The sites mainly consisted of grain storage areas and along the transportation route to animal feed manufacturing plant by grain transport vehicle. We found spilled maize grains and plants around open storage areas of ports and along transportation routes near feed–manufacturing sites.

Based on the PCR analyses of maize seeds and plants collected from around grain receiving ports and feed manufacturing plants, a considerable amount of maize imported for animal feed is likely to be GM. Previous studies demonstrated that no GM maize was found in cultivated fields in Korea. However, one plant and several

GM maize plants were found in a small vegetable garden in 2005 and along the roadside in 2006, respectively, in the Incheon port (Kim *et al*., 2006; Lee *et al*., 2009). In this study, the monitoring sites were focused more on the distribution routes of imported maize from grain receiving ports to feed manufacturing plants. Many spilled maize seeds were found around the grain storage areas in the Incheon and Gunsan ports, and along the transportation routes near feed manufacturing plants. The possibility that spilled seeds emerge and become established on the ground appears to be low, as these facilities are generally located inside industrial parks, in which most of the area is paved with block, concrete, or asphalt. Although several GM maize plants were found in these areas, most of these facilities were far from the cultivated fields. In fact, it is not difficult to eliminate spilled seeds or established plants on the pavement or roadside when compared to the clean–up of land under crop cultivation, and the impact of this GM maize on the natural environment may be negligible.

The monitoring of GM crops for three years in Japan (Nakajima *et al*., 2009) can be cited as a similar study to this local investigation attempt, as transportation routes were selected for monitoring. Our results were in consistent with the findings of where cultivated GM crops were reported to have been germinated along the road side during the six month of monitoring. In addition, it is not difficult to eliminate spilled seeds or established plants on the pavement or roadside when compared to the clean–up of land under crop cultivation, and the impact of this GM maize on the natural environment might be negligible. Nevertheless, the focus of this study was on the consistent presence of the GM maize plant and the spilled seed, regardless of the minimal amount. The study confirmed that GM crops always have the potential to inflict contamination on the environment due to gene–flow (Chandler and Dunwell, 2008). Therefore, to protect the possible environmental risks, people and offices that are involved directly or indirectly with GM crop should take necessary actions and precautions (monitoring or regulating) to prevent the inclusion of GM plants within the local ecosystem.

Although the cultivation of GM maize is not yet allowed in Korea, the importation of GM crops for food and foraging purposes is continuously increasing. Therefore, the escape of GM maize might increase with time. Agroecologists should play an important role in monitoring and regulating the escape of GM plants because of the possible environmental risks associated with such events. Furthermore, even though GM maize seeds or plants are found every 3 year of investigation near ports, routes and forage factories, adaptations of local or agricultural environment of the released GM maize seeds or plants were not investigated. Therefore, based on three years of research, we can arrive at a conclusion that the environmental impact by unintended released GM maize is insignificant.

ACKNOWLEDGMENTS

This study was financially supported by research fund of Chungnam National University in 2013.

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