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# Detection of unapproved genetically modified potatoes in Korea using multiplex polymerase chain reaction

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ABSTRACT

Several genetically modified (GM) potato cultivars with improved traits such as increased amylopectin levels, decreased asparagine levels, and reduced incidence of black spot have been developed. In this study, we describe a multiplex polymerase chain reaction (PCR) method specific for four GM potato events (EH92-527-1, AM04-1020, PH05-026-0048, and E12) that are unauthorized in Korea. The UDP-glucose pyrophosphorylase (*UGPase*) gene was used as the endogenous reference gene. The specificity of the primer sets was evaluated using GM potatoes and other GM crops. The limit of detection in the developed multiplex PCR was confirmed to be approximately 0.04% (w/w). Thirty-three commercial products containing potato ingredients were tested by the multiplex PCR assay, and GM potato event E12 was found in one of the 33 samples. This result suggested that the developed PCR method could be used to effectively identify unauthorized GM potatoes in Korea.

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## 1. Introduction

Many types of genetically modified (GM) crops have been developed in recent years, and various GM ingredients have been used in commercial foods and feed products around the world. Many GM cultivars with characteristics of herbicide tolerance, insect resistance, disease resistance, and abiotic stress tolerance have been developed for crops such as maize, soybean, cotton, potato, and canola (James, 2015). The International Service for the Acquisition of Agri-biotech Applications (ISAAA) indicates that 29 crops and 406 GM events have been authorized for planting as of November 2016 (ISAAA, 2016). In Korea, seven GM crops (maize, soybean, cotton, potato, canola, alfalfa, and sugar beet) and 145 GM events have been authorized for use in foods by the Ministry of Food and Drug Safety (MFDS, 2016).

Potato (*Solanum tuberosum*) is the fourth most important food crop in the world and has been widely used for human food and animal feed products (Solomon-Blackburn & Barker, 2001; Watanabe et al., 2004). GM potatoes with new qualities, such as increased amylopectin levels, decreased asparagine levels, and reduced incidence of black spot, have also been developed (Toevs, Guenthner, Johnson, McIntosh, & Thornton, 2011). Asparagine, an amino acid present in potatoes, produces the known carcinogen acrylamide during frying. Innate™ potato events (E12, E24, F10, F37, J3, J55, J78, G11, H37, and H50) with low acrylamide potential were developed by the J.R. Simplot Company and were approved for commercial use by the U.S. Department of Agriculture in 2014 and the Canadian government in 2016 (Baker, 2013, pp. 97–110; Waltz, 2015).

Since the development of New Leaf potatoes with coleopteran insect resistance by Monsanto (St. Louis, MO, USA), several GM potatoes have been developed for commercial use (Jaccaud, HÖhn, & Meyer, 2003). EH92-527-1 (Amflora) and AM04-1020, developed by the BASF Plant Science Company GmbH (Ludwigshafen, Germany), are transgenic lines that exhibit increased levels of amylopectin in potato starch through silencing of the expression of granule-bound starch synthase (GBSS) for industrial applications (Gao et al., 2016; Tilocca et al., 2014). PH05-026-0048 (Fortuna), which exhibits increased resistance to *Phytophthora infestans*, a pathogen causing late blight and tolerance to imidazolinone herbicides, was also developed by BASF (Storck, Bohme, & Schultheiss, 2012).

According to the ISAAA (ISAAA, 2016), 47 GM potato events had been developed by 2016. However, only four GM potato events, i.e., SPBT02-05, RBBT06, New Leaf Y, and New Leaf Plus, all produced by Monsanto (MFDS, 2016), are authorized for use in Korea.

Because various GM events have been developed and commercialized, effective detection methods are required to identify GM

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ingredients. At present, polymerase chain reaction (PCR) is one of the most effective tools for identification of GMO in both, raw materials and processed foods (Querci, Van den Bulcke, Žel, Van den Eede, & Broll, 2010). In particular, event-specific multiplex PCR, which exhibits high sensitivity and specificity, can be used for simultaneous identification of several target events in a single reaction (Guo et al., 2011; Kim et al., 2013, 2014; Randhawa, Sharma, & Singh, 2009; Randhawa, Singh, & Sood, 2016; Yang, Guo, Zhang, Liu, & Zhang, 2008).

In this study, a multiplex PCR system using event-specific primers was established for detection of unauthorized GM potato events in Korea and was used to analyze commercial potato products. The developed method may help researchers identify unauthorized GM potato events in food and feed supply chains.

## 2. Materials and methods

### 2.1. Samples

Certified reference materials (CRMs) of GM potato (EH92-527-1, PH05-026-0048, and AM04-1020), GM soybean (RRS, MON87701, A2704-12, A5547-127, and DP305423), and GM maize (MON810, NK603, DAS59122-7, and Bt11) were purchased from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). GM soybean MON89788, GM maize (GA21 and T25), and GM canola (RF1, T45, and MS8) were purchased from the American Oil Chemists' Society (AOCS, Urbana, IL, USA). GM potato E12 and GM cotton (LLCotton25, MON15985, and MON88913) were provided by the Ministry of Food and Drug Safety (MFDS) in Korea. Thirty-three processed products, including raw potatoes, frozen foods, snacks, fries, canned food, mashed potato, and potato starch, were purchased from food markets in Korea and the USA (Table 1).

**Table 1**
List of processed potato products used in this study.

| Sample No. | Sample type | Country of origin |
|---|---|---|
| 1 | Raw potatoes | Korea |
| 2 | | Korea |
| 3 | | Korea |
| 4 | | USA |
| 5 | Frozen foods | USA |
| 6 | | USA |
| 7 | | USA |
| 8 | | USA |
| 9 | | USA |
| 10 | | Belgium |
| 11 | Snacks | USA |
| 12 | Snacks | USA |
| 13 | | USA |
| 14 | | Malaysia |
| 15 | | USA/Poland |
| 16 | | USA/Belgium |
| 17 | | Malaysia |
| 18 | | USA |
| 19 | | Belgium |
| 20 | | Korea |
| 21 | | Korea |
| 22 | | Germany |
| 23 | Snacks | Italy |
| 24 | | Indonesia |
| 25 | | Italy |
| 26 | | Italy |
| 27 | | Italy |
| 28 | | China |
| 29 | | Korea |
| 30 | Fries | USA |
| 31 | Canned food | USA |
| 32 | Mashed potatoes | Korea |
| 33 | Starch | China |

### 2.2. DNA preparation

Each sample was ground into a powder in liquid nitrogen using a mortar and pestle. Genomic DNA extraction was carried out using a DNeasy Plant mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration was measured using a UV spectrophotometer (UV-1700; Shimadzu, Kyoto, Japan), and the purity of extracted DNA was evaluated by measuring the absorption at 260/280 and 260/230 nm.

### 2.3. Oligonucleotides

Four event-specific primer pairs were used in a multiplex PCR method for detection of four GM potato events. The primer pairs Event527-bf1/St527-R1 and 26-44-RB-393F/26-48-RB-473R based on the GMOMETHODS database (EU-RL GMFF, 2016) were used for detection of EH92-527-1 and PH05-026-0048, respectively. E12-aF5/aR5 and AM04-aF1/aR3 targeting E12 and AM04-1020, respectively, were designed based on a detection method for genetically modified DNA from the Ministry of Health, Labor, and Welfare (MHLW, 2016) and the GMOMETHODS database (EU-RL GMFF, 2016). The UDP-glucose pyrophosphorylase (*UGPase*) gene (GenBank Accession No. U20345) was used as the endogenous potato reference gene. The nucleotide sequences of primers are listed in Table 2, and all primers were synthesized by Bionics (Seoul, Korea).

### 2.4. Multiplex PCR condition

PCR assays were carried out in a final volume of 25 μL containing 2.5 μL of 10× buffer (Applied Biosystems, Foster City, CA, USA), 0.2 mM of each dNTP (Applied Biosystems), 1.5 mM of $MgCl_2$, 1 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems), and 100 ng of genomic DNA. The concentration of each primer pair was adjusted to show similar intensities on an agarose gel. PCR was performed using a thermal cycler (PC808; ASTEC, Kyoto, Japan) under the following conditions: pre-incubation for 5 min at 94 °C; 40 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C; followed by a final extension for 7 min at 72 °C. After PCR, the amplified products were analyzed on a 3% agarose gel stained with 0.5 μg/mL ethidium bromide.

### 2.5. Cloning of target sequences

Since the CRMs for GM potato were insufficient, standard plasmids were prepared for use as positive controls for detection of the four GM potato events. PCR products for each event-specific target and an *UGPase* fragment were purified with the QIAquick Gel Extraction kit (Qiagen, Valencia, CA, USA) and cloned into a commercial pGEM-T Easy vector (Promega, Madison, WI, USA). The cloned plasmids were isolated from bacterial cultures using a QIAamp DNA Mini kit (Qiagen) and confirmed by sequence analysis using an ABI PRISM 3700 DNA analyzer (Perkin Elmer, Boston, MA, USA) at GenoTech (DaeJeon, Korea).

### 2.6. Limit of detection of the PCR assay

Pure non-GM potato DNA was mixed with a DNA mixture extracted from pure samples of four GM potato events in order to determine the limit of detection (LOD) of the established multiplex PCR system at various levels. The mixtures of CRMs for individual GM potato events were prepared containing 25, 5, 1, 0.2, 0.04, and 0.008% (w/w) for each GM potato event, respectively.

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