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The significance of food safety in trade and banning the importation of GMO products into Iran

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professional paper

Summary

According to legislation in Iran, the importation of transgenic products should be banned due to the lack of strong evidence for the safety of genetically modified foods. Therefore, the detection of genetically modified on importing products should perform by food control laboratories. In this study, specific primers were designed for 35S promoter (500 bp), NOS terminator (253 bp), NPTII (470 bp) and GUS (443 bp) for the detection of GMO in 134 imported samples by Polymerase chain reaction. The results showed the identification of GMO in food has become an important issue in food control, and Iranian Government has not adopted to import any GMO products into Iran. Therefore, this could indicate the significance of food safety and low confidence of people on the safety of these products in Iran. In fact, this protocol can be used for detection of GM products and for the labeling GM samples in order to ensure human health safety and protect the environment.

Keywords: GMO, PCR, imported products, food safety, food control

Introduction

The technology used to transfer a specific gene from one organism to another organism through a process that has called gene transformation and genetic engineering. The produced transgenic organisms have called GMO (genetically modified organism) (Celec et al., 2005). Concerning the fact that GMO samples may have negative impacts on the environment and human health, it increases public concerns about GM food safety. According to legislation in Iran, the importation of transgenic products should be banned for reducing unknown hazards. As a whole, rice, wheat and maize are the country's major crops, and rice exclusively used for human consumption in Iran (Mohammadi et al., 2012). Moreover, rice produces approximately 2.2 million tons per annum while annual consumption is about three million tons. Therefore, the average per capita consumption of rice in Iran is 45.5 kg, and Iran is the 13th biggest rice consumers in the whole world (Mohammadi et al., 2012). Consequently, Iran has to import rice and other cereals such as soybean, sunflower, maize and canola from India, Pakistan Bangladesh and Thailand (Mohammadi et al., 2012). It reinforces the need for the development of the labeling system and various procedures for detection of GMO food in Iran. As a result, Iranian's government should pay serious attention to the nutritional status of Iranian people for omitting any real or potential hazards. Therefore, these concerns have led to perform strategies to control importing products, and GMO labeling system has become a vital requirement for all food containing any amount of GMO products in this country. The purpose of the labeling system is to inform the consumers of the presence of GMOs in the products. In fact, customers' right to know about food safety gives freedom of choice to them to choose between GM and non-GM foods. Besides, there are several factors that have impact on the view of people of GMO product consumption, such as age, education level and other cases. These factors have a direct relation with the attitude of people about GMO products and biotechnology. Unfortunately, many surveys illustrates that people have low information about biotechnology in Iran. It influences on opinion of people for acceptance or rejection of GMO products in Iran.

But they were more interested to be performed more research about these products, even if it increases their cost.

As a consequence, it is illegal to import unapproved products into Iran, and importing samples must test for the identification of GMO products. Therefore, the Food Control organization supervises on importation of GMO foods into Iran.

In this study, specific primers were designed for the 35S promoter (500 bp), NOS terminator (253 bp),

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NPTII (470 bp) and GUS (443 bp) for the detection of GMO in 134 imported samples by Polymerase chain reaction.
There are many reports that PCR method has been used in order to detect genetically modified organisms by using CaMV 35S promoter, NOS terminator, NPTII and GUS (Cankar et al., 2008; Shrestha et al., 2008; Sieradzki et al., 2008; Wen-Tao et al., 2009; Zhu et al., 2008).

Materials and methods

Genomic DNA was extracted from imported products into Iran, and PCR was performed in order to detect genetically modified products.
In total 134 imported products, including a variety of processed products containing rice, maize, soybean, sunflower and rapeseed ingredients and cereal seeds, were used for GMO detection. The analyzed products were as follows: 118 rice, 5 maize seeds, 2 frozen maize, 4 soybean seeds, 1 canned corn, 3 sunflower seeds and 1 rapeseed seeds. Approximately, the most of these samples imported from India and Pakistan in within time span of 2010 to 2013.

Reference material

Reference materials were used for positive and negative controls and for the validation of analytical methods. The following commercially available Certified Reference Materials were used: maize powder containing 5 % of Bt-11 maize (ERM-BF412f), soybean powder containing Roundup Ready® soybean (ERM-BF410e), rapeseed (Oxy235), Bt-rice and Bt-sunflower.

Extraction DNA

A sample of 50 mg seeds from each plant were used for extracting DNA following the method of Murray and Thompson (1980). The extracted DNA samples were resolved on 0.7 % Agarose gels. The ratio of the absorbance at 260 and 280 nm (A260/280) is used to assess the purity of nucleic acids.

Sequence alignment and Primer Design

All retrieved sequences from GenBank were aligned and compared with Mega ver. 4 software. PCR primers were designed based on the most conserved regions of known sequences available from DDBJ/GenBank with Oligo ver-5 software. Primers were capable of amplifying PCR products based on conserved regions in 35S promoter, NOS terminator, NPTII and Gus. Also, primers that amplify *actin, zein, lectin*, *acc,* and *11s storage protein* genes as endogenous reference genes (Table 1). Furthermore, designed primers in semi-nested PCR technique were capable of amplifying PCR results (Table 2).

Table 1. Sequences of Primers are used in This Study

Primer	Sequence 5′-3′	Specificity	Amplicon (bp)
Rice	CTCTCCCTgTATgCCAg CATACGGTCAGCAATACCAG	Actin/sense Actin/anti-sense	522
Soybean	TCgTCAggCTTAgATgTgCTAgA CTgCATTTgTCACAAATCATgAA	Lectin/sense Lectin/anti-sense	515
Maize	CTAgCAACATAgAAAgCACA ggCTgAATggTAgTAgTTgT	Zein/sense Zein/anti-sense	616
Rapeseed	ATgATgATgATgAggTTAgg TCTgCgTCTACCAATAATAAC	Acc/sense Acc/anti-sense	314
Sunflower	CACCTATTCACCACATCACA AAgCTggTTCTCATggTTC	11SSP/sense 11SSP/anti-sense	667
35S promoter	CATggAgTCAAAgATTCAAA ATATAgAggAAgggTCTTC	CaMV/sense CaMV/anti-sense	500
NOS terminator	CgTTCAAACATTTggCAATA CCCgATCTAgTAACATAgAT	NOSt/sense NOSt/anti-sense	253
NPTII	TCCggCCgCTTgggTggAgAg CTggCgCgAgCCCCTgATgCT	NPTII/sense NPTII/anti-sense	470
GUS	TCACCgAAgTTCATgCCAgTCCAg CTgCgACgCTCACACCgATACC	GUS/sense GUS/anti-sense	443

Table 2. Sequences of Primers are used for semi-nested PCR

Primer	Sequence 5′-3′	Specificity	Amplicon (bp)
GUS	CgATTATCATCACCgAATAC	GUS/anti-sense	289 bp
NPTII	TCATCATCATCATAgACACA	NPTII/anti-sense	332 bp
NOS terminator	AACCCATCTCATAAATAAC	NOSt/anti-sense	141 bp
35S promoter	ggCAATggAATCCgAggAgg	CaMV/anti-sense	236 bp

PCR amplification and DNA analysis

DNA amplification reactions were performed in a thermal cycler (Mastercycler, Eppendorf). Reactions were performed in a volume of 25 µL containing 2.5 µL of 10x buffer, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.4 µM of each primer, 0.2 unit/µL Taq polymerase and approximately 0.4 ng/µL genomic DNA. The thermocycler program included 5 minute for initial strand separation at 94 °C; followed by 35 cycles of 30 seconds at 94 °C, 45 seconds at 52 °C, 30 seconds at 72 °C, and a final 7 minute step at 72 °C. Furthermore, above-mentioned reaction was used for semi-nested PCR, as well.

PCR product confirmation

PCR products were resolved on a 0.7 % Agarose electrophoretic gel and visualized by ethidium bromide staining (0.5mg/ml in Deionized water) under an UV-transilluminator.

Results and discussion

DNA was extracted by the CTAB method from imported food samples provided a good quality and quantity of DNA which was checked by amplifying the endogenous rice *actin* (522 bp), maize *zein* (616 bp), soybean *lectin* (515 bp), rapeseed *acc* (314 bp), sunflower *11s storage protein* genes (667 bp) (Fig. 1). Agarose gel electrophoresis and spectrophotometric results revealed DNA of high purity. In addition, a binary vector *pCAMBIA-2301* that contains essential elements was used as positive control for validation in PCR assay. Following PCR, expected 500, 253, 470 and 443-base pair fragments were detectable for 35S promoter, NOS terminator, NPTII and GUS respectively, and expected 289, 332, 141 and 236 bp fragments were detectable for GUS, NPTII, NOS and 35S in semi-nested PCR for PCR confirmation, respectively (Fig. 2). This method could use for the detection of samples with Limit of detection (LOD) 1 % (w/w). In the present study, no GMO products were observed in different kind of imported samples into Iran during 4 years. Indeed, PCR amplification and semi-nested PCR presented in this study could be used as a very useful tool for GMO detection in foods and feeds. The results showed Iranian Government has not allowed no GMO products are imported into Iran. Therefore, it showed the significance of food safety and low confidence of people on the safety of these products in Iran. Besides, the importation of food products is increasing year by year, and most concerns about GMO products refer to unknown risks for health human. But, Iran's food safety may not damage by a dramatic surge in food product imports for two reasons. First, Iranian government implements a new policy for enhancing the country's food products and alleviating dependence on imports. Second, there are a lot of quality control laboratories for the control of imported food products.

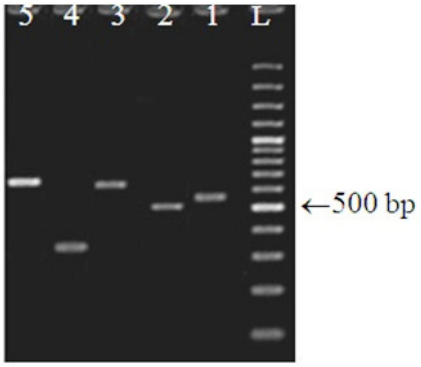

Fig. 1. Electrophoretic Agarose gel, stained with ethidium bromide, of the PCR products of Host specific genes, 1-rice *actin* gene (522 bp), 2-soybean *lectin* gene (515 bp), 3-maize *zein* gene (616 bp), 4-rapeseed *acc* gene (314 bp) and 5-sunflower *11s storage protein* gene (667 bp), M: Molecular marker (100 bp ladder)

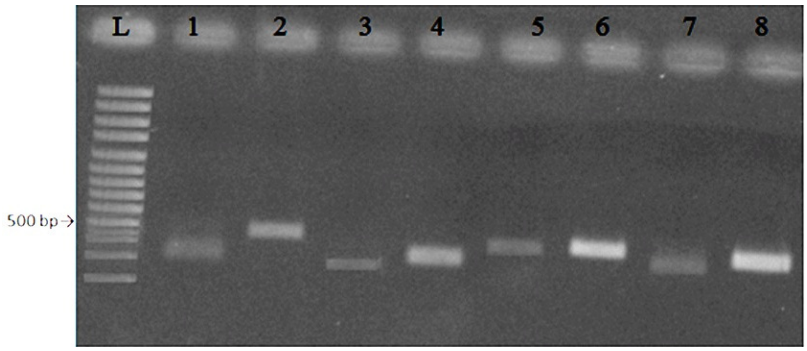

Fig. 2. Electrophoretic Agarose gel, stained with ethidium bromide, of the semi-nested PCR products and PCR products, 1- 35S (236 bp), 2-35S (500 bp), 3- NOS (141 bp), 4- NOS (253 bp), 5- NPTII (332 bp), 6- NPTII (470 bp), 7- GUS (289 bp) and 8- GUS (443 bp), L: Molecular marker (100 bp ladder)

Many surveys showed that government of Islamic republic of Iran has declared his obligation to observe biosafety standard in order to minimize the risks of GM products for human and the environment. Biosafety is a collection of rules and regulations. In other words, biosafety has been adopted by Iranian government (Hasheminya and Dehghannya, 2013). These regulations are aimed at reducing possible risks of GM products. Generally, no sample has been detected as GMO-positive in different kind of imported samples into Iran during 4 years, and it showed that Iranian Government has announced a policy on GM products and has banned the importation of GM crops entirely (Hasheminya and Dehghannya, 2013).

Conclusions

Iranian Government has lifted restrictions on imported samples. This result indicates Iranian policy towards GMO product and the significance of food safety in this country. With regard to the fact that the amount of rice cultivation and other major crops is not enough for domestic consumption and this country has imported a large portion of the rice and other crops from other regions, and these concerns have led to perform strategies to control importing products. Consequently, this protocol can be used for the detection of GM products and for the labeling GM samples in order to ensure human safety and protect the environment.

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