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Monitoring of Genetically Modified Rice among Some Imported Rice Samples in Iran

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HIGHLIGHTS

- Genetically Modified (GM) rice was not found among some bulk and retail imported rice samples in Iran.
- Absence of GM rice in the samples was in accordance with the Iranian national regulation.
- More comprehensive studies are needed to find the situation of other GM foods in the Iranian markets.

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Acronyms and abbreviations
GM=Genetically Modified
PCR=Polymerase Chain Reaction

ABSTRACT

Background: Genetically Modified (GM) foods are produced using genetic engineering. This survey attempted to identify the presence of GM rice varieties among some imported rice samples in Iran.

Methods: From May to July 2016, a total of 50 samples of the imported rice to Iran were obtained, including 20 bulk rice samples from Bandar Abbas custom, Southern Iran and 30 retail rice samples from some commercial brands sold in the local markets of Yazd, Iran. Polymerase Chain Reaction was used to assess the GM varieties.

Results: None of the studied rice samples had the target genes related to GM products.

Conclusion: This study indicated no evidence for presence of GM rice among some bulk and retail imported rice samples in Iran. Since marketing of GM rice is not legally permitted in Iran, more comprehensive studies must be designed with higher sample size in various provinces of country to achieve more detailed data about situation of GM rice in the Iranian markets.

Introduction

Rice (*Oryza sativa* L.) is the second most consumed cereal in the world after maize and is cultivated solely for the human consumption (Choi et al., 2012). Rice is the staple food in more than 100 countries which comprises about half of the world's current population. This crop consists of a considerable energy supply needed for the humans and is consumed as the main food in the developing countries (Bhullar and Gruissem, 2013).

The main purposes of production of Genetically Modified (GM) foods produced through genetic engineering are to optimize the product and create desirable characteristics, such as increase of the economic efficiency, improving nutritional value, as well as resistance to herbicides and pests (Kramkowska et al., 2013; Nicolia et al., 2014; Snell et al., 2012). Among various types of GM foods in the world, more than 80% of the area under cultivation of GM foods is dedicated to the canola, soybean, and corn (Lee, 2014). The production of GM food is a recently developed science, so that the first GM plant was produced in 1996. However, due to the great effect

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of this new technology on the agro-food trade market, the production of GM foods grew rapidly in some parts of the world (Bawa and Anilakumar, 2013; James, 2015; McHughen, 2013).

It has been stated that production of GM foods could play an important role in reducing the malnutrition and providing food security in the world. Consequently, GM products are officially and legally distributed and consumed in some countries (Blair and Regenstein, 2015). However, the safety considerations led to the necessity of using labeling measures on the GM foods' packages. According to the laws set by the European Union, labeling is mandatory for a food product with more than 0.9% of GM compounds (Ceccoli and Hixon, 2012; Price and Underhill, 2013; Taski-Ajdukovic et al., 2009).

The GM rice has been cultivated on experimental scales in the countries such as China, United States, Philippines, Pakistan, Spain, and India (Blair and Regenstein, 2015; Yuan et al., 2013). The first Iranian pest-resistant GM rice, named Tarom Mola'i was produced at a trial scale and limited level in the last decade (Afraz et al., 2009; Shirdeli et al., 2019). Although it seems that GM rice has not yet reached the mass production stage at the international trade market, a potential exists for the presence of this product in markets of Iran due to the entry of GM plants into the international trade. On the other hand, labeling of the GM foods imported to Iran is not fully legislated in the national markets.

In this survey, we attempted to identify the presence of some GM varieties among the imported rice samples in Iran. To achieve this goal, the conventional Polymerase Chain Reaction (PCR) method was applied as a highly accurate and sensitive method as a standard tool for detection of GM products by European Union (Tengel et al., 2001; Wen-Tao et al., 2006).

Materials and methods

Sampling

From May to July 2016, 50 samples of the imported rice to Iran were obtained using the random cluster sampling method, including 20 bulk rice samples from Bandar Abbas custom, Southern Iran and 30 retail rice samples from some commercial brands sold in the local markets of Yazd, Central Iran.

All the rice samples were thoroughly homogenous and kept in a dark place. Furthermore, 15 mg of each sample was selected and grounded in thrice according to the standard regulation (European Commission, 2004, 2014).

DNA extraction

DNA was extracted using the DNeasy Plant Mini kit (GeneAll, #117-101, Korea) according to the instructions. Quality and quantity analyses of the extracted DNA were performed using 0.8% agarose gel electrophoresis (Fanavaran Sahand Azar, Iran) as well as spectrophotometry (BioTek instruments, USA), respectively.

Confirmation of O. sativa species

In order to confirm of *O. sativa* species in the samples, PCR technique was applied using specific primer pair for the target gene of *PLD* (Table 1) and thermal cycler (ABI, simpliAmp, USA). The reaction for all targets was performed in 20 µl of the final concentration of 1x master mix (Amplicon, Denmark), 0.5 µM of each primer, and 100 ng of the extracted DNA. The amplification program consisted of the first denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60.9 °C for 30 s, and elongation at 72 °C for 30 s. The last elongation was carried out at 72 °C for 5 min. Finally, the amplicons were assessed using 3% agarose gel electrophoresis. The fragment with the size of 80 bp in length was related to conventional rice (*O. sativa*).

GM identification

In order to identify the genes of the GM products in rice samples, the conventional PCR technique was applied by specific primers for the target genes of MON810, Bt11, T25, and Bt176 (Table 1). Amplification was performed using thermal cycler in 20 µl volume reaction with 1x PCR master mix (Amplicon, Denmark), 0.5 µM of each primer, and 100 ng extracted DNA. The amplification program is represented in Table 2. The results achieved from the amplification were assessed using agarose gel electrophoresis alongside by 50 bp DNA ladder.

The positive and negative controls were used in all amplification reactions. The positive controls were the samples gifted from the Agricultural Biotechnology Research Institute, Karaj, Iran. However, the negative controls included distilled water. Moreover, all reactions were repeated in triplicate.

Statistical analysis

The collected data were analyzed by SPSS version 16. The *p* value of less than 0.05 was used to declare significant correlation.

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Table 1: The primer pairs used in this study

Target gene	Primer name	Primer sequence (5'-3')	PCR product (bp)
PLD	PLD 3959F	GCTTAGGGAACAGGGAAGTAAAGTT	80
	PLD 4038R	CTTAGCATAGTCTGTGCCATCCA	
MON810	VW01	TCGAAGGACGAAGGACTCTAACG	170
	VW03	TCCATCTTTGGGACCACTGTCG	
Bt11	IVS2-2	CTGGGAGGCCAAGGTATCTAAT	189
	PAT-B	GCTGCTGTAGCTGGCCTAATCT	
T25	T25-F7	ATGGTGGATGGCATGATGTTG	209
	T25-R3	TGAGCGAAACCCTATAAGAACCC	
Bt176	Cry03	CTCTCGCCGTTCATGTCCGT	211
	Cry04	GGTCAGGCTCAGGCTGATGT	

Table 2: PCR program for VW01/VW03, T25, Cry03/Cry04, PAT-B/IVS2-2 primers

Stage	Temperature (˚C)		Time (s)	Cycle
First denaturation	94		300	**1**
Denaturation	94		30	**30**
Annealing	VW01/VW03	62	30	
	T25	57.4		
	Cry03/Cry04	60.5		
	PAT-B/IVS2-2	60.1		
Elongation	72		30	
Final elongation	72		300	**1**

Results and discussion

The amplification with PLD primer pair with the size of 80 bp in length used to confirm the presence of *O. sativa* (Figure 1). None of the samples were amplified with the primer pairs of the GM target genes, including MON810, Bt11, T25, and Bt176.

Considering the increasing production of GM foods, many researchers are still dealing with the dilemma of using or not using the mentioned compounds in foods. So, appropriate methods are required to detect the presence and measure the levels of GM compounds in foods. GM products have been supplied and consumed over the past 20 years in Iran; however, the National Food and Drug Organization of Iran has permitted for the food suppliers to use GM oilseeds in the production of edible oil. In other words, marketing of other GM products (except GM oilseeds) is not legally approved in this country. In this regard, the findings of our survey indicated that the absence of GM rice in markets of Iran is in accordance with the Iranian national law.

In this study, no GM rice was found among the investigated samples that is similar with the findings of Elsanhoty et al. (2013), who indicated that none of the rice samples distributed in Saudi Arabia were transgenic. Despite, Alaraidh et al. (2011) detected three GM rice samples among a total of 150 rice samples in Saudi Arabia. The data on monitoring of GM rice in international markets are rare. Also, we are still faced with some controversies about marketing and safety of GM foods. So, further investigations are required in this regard.

Nowadays, GM products have a prominent status in international food trade, although controversies exist between the proponents and opponents of the development and consumption of these products. Along with the massive and growing production of various GM foods, some researchers indicated several problems and worries considering the safety hazards and the unintended risks of GM products. Few authors have announced that consumption of GM products may endanger the human health, affect agriculture industry and environment, raise public concerns, and force the regulatory agencies to take authoritative measures (Bawa and Anilakumar, 2013; Kramkowska et al., 2013); however, this concept has not proven yet. According to a paper published previously in 2012, it was stated that consumption of Roundup-tolerant GM maize induces carcinogenic effects on rat (Séralini et al., 2012). But, the mentioned paper

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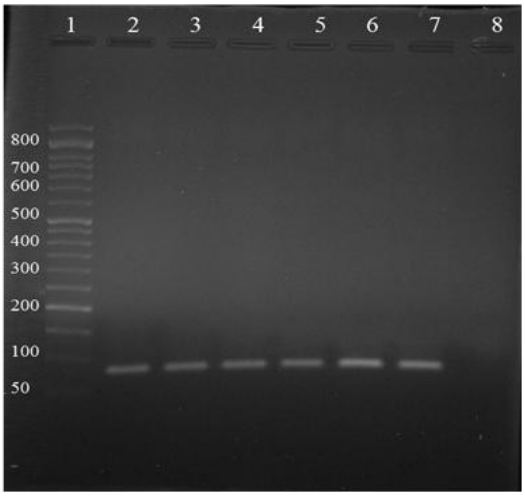

Figure 1: Agarose gel electrophoresis (3%) of the amplified region of *PLD*. Lane 1: 50 bp DNA ladder; lane 2: positive control; lanes 3-7: examined rice samples; lane 8: negative control. The desirable amplified fragment was 80 bp.

was later retracted by the journal because of some scientific errors (Séralini et al., 2014).

It should be noted that although some researchers found no adverse or toxic effects due to consumption of GM rice in animal models (Shirdeli et al., 2019; Yuan et al., 2013), but it may be not enough to make final decision about consumption or mass production of GM rice. Before any decision, safety of GM food products must be finally evaluated by official government organizations.

Conclusion

To the best of our knowledge, this study is the first of its kind indicating no evidence for presence of GM rice among some bulk and retail imported rice samples in Iran. Since marketing of GM rice is not legally permitted in Iran, more comprehensive studies must be designed with higher sample size in various provinces of country to achieve more detailed data about situation of GM rice in the Iranian markets.

Author contributions

M.S.H. gathered the samples and wrote the manuscript; H.F. analyzed the data; M.S.H and S.S.H did the laboratorial procedures. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest.

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