Central Asian Journal of Environmental Science and Technology Innovation **1**(6): 291–297 (2020)

RESEARCH PAPER

Identification of GMO food products of Plant origin: environmental risks and agrobiodiversity perspective

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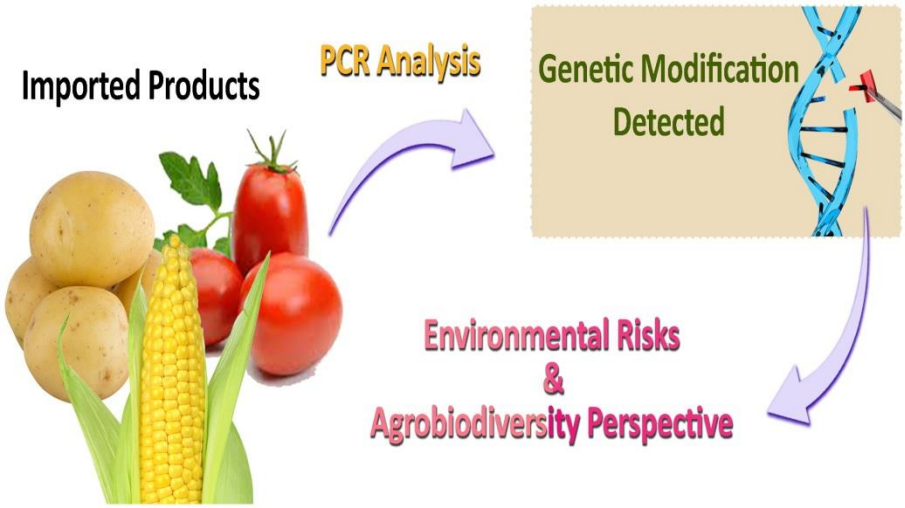
Highlights

- Identification of genetically modified sources in food with plant origin performed by PCR analysis.
- The indicator genetic modification seen in corn, potatoes and tomatoes samples from imported products.
- According to results, it is possible to assess the risk of GM (genetic modification) crops for the environment, bio- and agrobiodiversity risks.
- Development and improvement of the regulatory and legislative framework, as well as control in the field of GMO, strictly recommended.

Graphical Abstract



Article Info

Receive Date: 19 October 2020
Revise Date: 17 December 2020
Accept Date: 27 December 2020
Available online: 30 December 2020

Keywords:
GMO food
Risk assessment
Food safety
Agrobiodiversity

Abstract

The results of PCR analysis for the identification of genetically modified sources in food of plant origin, presented in this paper.The genetic modification in samples of corn, potatoes and tomatoes has been found in imported products from abroad. The results obtained make it possible in the future to assess the risk of GM (genetic modification)crops for the environment, bio- and agrobiodiversity, and will also be recommended to the relevant state bodies for the development and improvement of the regulatory and legislative framework, as well as control in the field of GMO (genetically modified organism) biosafety.

10.22034/CAJESTI.2020.06.02

E-ISSN: 2717-0519
P-ISSN: 2717-4034

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1. Introduction

The use of advanced methods of genetic engineering in agriculture has led to an increase in the production of genetically modified crops and food products made from them, an increase in production efficiency, including an increase in yield and fertility, an increase in nutritional value, an improvement in the appearance and safety of products, resistance to adverse environmental factors, which can be of great importance for ensuring food security of the growing world population, especially developing countries such as the Republic of Tajikistan. However, the use of complex molecular genetics technologies for their creation, which in fact are nanobiotechnology manners that used today to obtain genetically modified organisms, are not yet perfect, which causes a number of risks arising from the creation and use of such organisms (Mahdewi and Banjarani, 2020; Chaghakaboodi et al., 2021; Zeidali et al., 2021b; Haghshenas and Ghanbari, 2021).

To increase the productivity of agricultural crops, using genetic engineering methods (for example, transgenesis), plants are given new improved qualities and properties; facilitate the process of processing raw materials, etc. Such transgenic plants (or GM plants, GMOs) are obtained by introducing foreign genetic information into the genome of the recipient plant. It should be noted that the presence of transgenic constructs in the genome can lead to unpredictable changes in the composition of nucleic acids and the balance of gene expression, as a result of which the following can increase in transgenic cultures: toxicity and allergenicity; the danger of becoming a harmful weed; manifest biological aggressiveness (displacement of valuable and rare aboriginal species by them or the loss of the latter as a result of contamination with genes transferred from a transgenic plant); toxic effects on non-target species (for example, bees, butterflies, ants, ground beetles, etc.), accumulation of toxins of transgenic plants in soil and water, and other adverse effects on the environment (Parrott, 2010; Saini et al., 2020). Transgenic crops (GM crops) are grown in open ground and interact with the environment, are a product that is consumed by humans, animals, or used as raw materials for the production of food and feed. Therefore, the use of genetically modified crops raises questions about food safety, potential risks of GM crops for the environment and human health (Anklam et al., 2002; Farokhian et al., 2021; Bakhshi et al., 2021; Zeidali et al., 2021a).

In Tajikistan, control over imported crops and food is mainly concerned with the determination of nutrients and the presence of fungal toxins, without taking into account possible genetic manipulation. As a result, there are few reports and information about the existence of GM crops, feed, and food containing genetically modified sources (GMOs), their impact on the environment and human health. The purpose of this study is to identify and identify genetically modified sources in food of plant origin, such as potatoes, corn and tomatoes, sold in the domestic food market for planning follow-up activities to assess the risk of GM crops to the environment, agro- and biodiversity.

2. Material and methods

Depending on the type of genetically modified sources, three methods of GMO identification are used. The first involves the use of polymerase chain reaction (PCR), in which transgenic DNA is identified. The second method is based on detection by enzyme-linked immune sorbent assay (ELISA), in which the modified protein is identified, and the third is the determination of enzymatic activity, in which a change in the chemical composition of the product is identified (Chiueh et al., 2001; Deisingh and Badrie, 2005). PCR methods are widely applicable and can be applied to unprocessed and heavily processed foods.

There are two types of PCR for detecting GMO content: qualitative PCR analysis, which is able to confirm the existence of GMO using gel electrophoresis (Common PCR) and real-time PCR (q-Real time PCR), which can be used to quantify its amount. Real-time PCR has been applied to characterize many crops such as corn, soybeans, and GM potatoes (Chiueh et al., 2001). The method for the determination of GMOs provided by the European standard is based on the identification of recombinant DNA using the polymerase chain reaction (PCR) method followed by electrophoresis and staining of the amplification products. The identification of the 35S promoter and the NOS terminator from *Agrobacterium tumefaciens* is a so-called "screening method" for

identifying foods from GM plants. The use of the 35S promoter and the NOS terminator as target sequences allows the detection of most food products from genetically modified sources, since these regulatory elements are currently present in almost all genetically modified plants that are allowed practically all over the world. Plant material of agricultural crops was used as the test samples: potatoes - three varieties, one of them is of local origin, two other samples of potatoes, as well as two varieties of corn and one variety of tomato, imported from selected countries of East and South Asia. All these samples were purchased on the capital market, from the seeds of which DNA was isolated and the detection and identification of GM sources was carried out (Cardarelli et al., 2005). Extraction of genomic DNA was carried out according to the method: DNA extraction kit for deep processed food product Cat. No: DP326, TIANGEN Biotech (Beijing) CO. LTD. DNA extraction and purification were performed from the test samples based on the complexity of their composition and technological process. DNA samples generally contain ingredients that act as inhibitors of the PCR reaction, such as fatty acids, polysaccharides, polyphenols, and other compounds that can interfere with DNA extraction or even degrade DNA quality. Qualitative determination of DNA was carried out on PCR equipment in real time for all samples in order to detect a DNA fragment corresponding to genomic DNA. The primers and probes used in this study, together with their target group in the studied DNA, are shown in Table 1.

Table 1. Regulatory sequences of transgenic inserts: the 35S promoter of the cauliflower mosaic virus and the NOS terminator from *A. tumefaciens.*

Genes identification	Sequence of primers	Sequence of probes (fluorescence)	Notes
CaMV35S	F:5′-CGACAGTGGTCCCAAAGA-3′	P:5′-FAM-TGGACCCCCACCCACGAGGAGCATC-BHQ1-3′	Screening and detection of transgenic maize, tomatoes
	R:5′-AAGACGTGGTTGGAACGTCTTC-3′		
NOS	F:5′-ATCGTTCAAACATTTGGCA-3′	P:5′-FAM-CATCGCAAGACCGGCAACAGG-BHQ1-3′	Screening and detection of transgenic maize, tomato and potato
	R:5′-ATTGCGGGACTCTAATCATA-3′		

PCR was performed on Rotor-Gene Q equipment in a volume of 20 μl for 7 samples, including standard and negative samples containing 90 μl 2 × SuperRealPreMix (SYBR Green Probe), 5.4 μl Forward Primer (10 μM), 5.4 μl Reverse Primer (10 μM), 3.6 μl fluorescence probe (10 μM), 7 μl isolated DNA and 66 μl nuclease-free water. Table 2 shows the conditions for real-time PCR (time / temperature profiles) used for each pair of primers.

Table 2. Relevant conditions and technical parameters for real-time PCR.

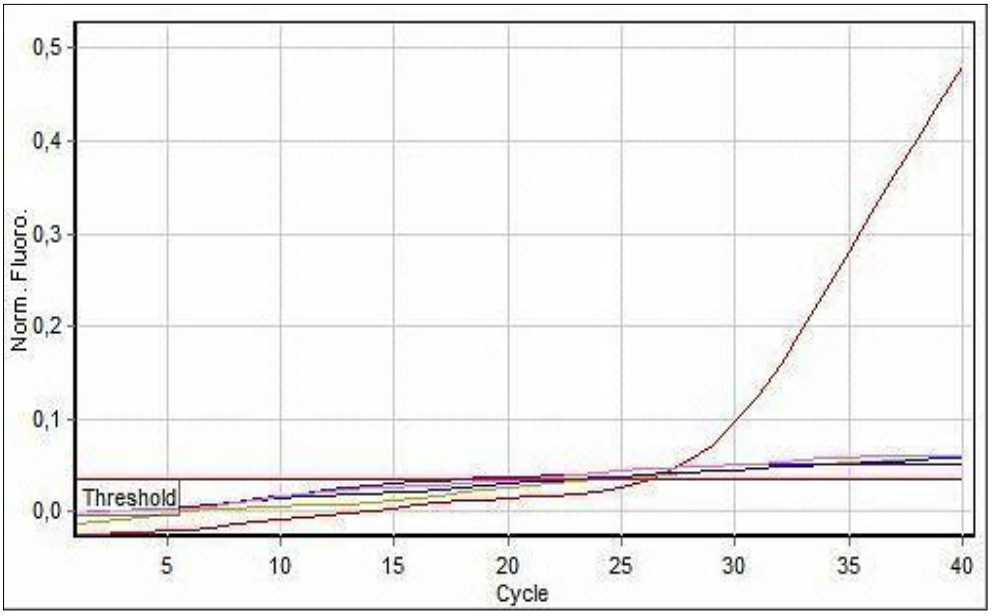
Pairs of primers	Stage	Cycle	Temperature (°C)	Time	Step	Collecting signals
CaMV35S-F	Initial denaturation	1x	95	15мин	Initial denaturation	none
CaMV35S-R.	PCR	40x	95	3 с	denaturation	none
NOS – F			60	20-32 с	Annealing	yes
NOS – R						

Two pairs of primers were used to detect genetic modification in the test samples. The sequences of pairs of oligonucleotide primers and their target groups are shown in Table 1. A pair of primers CaMV35S-F / CaMV35S-R was used to detect the CaMV35S promoter gene. The NOS - F / NOS - R primer pair was used to detect the NOS promoter gene, which increases resistance to fungal and bacterial infections in genetically modified potatoes.

3. Results and Discussion

One of the most widely used general purpose constitutive promoters in plant genetic engineering is the CaMV35S promoter. It is responsible for the transcription of the entire genome of the cauliflower mosaic virus.

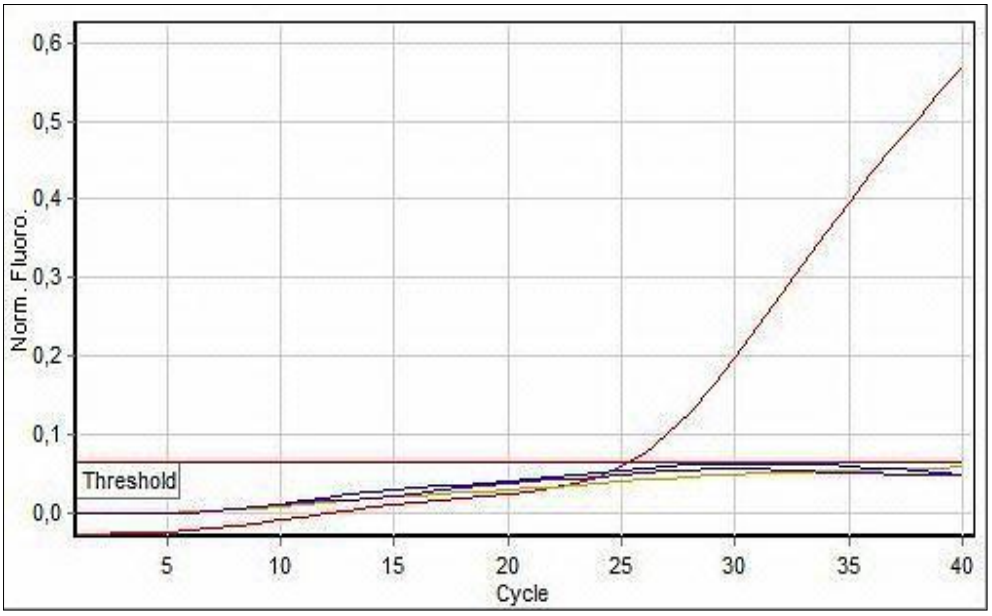
This is a very strong promoter that provides a high level of gene expression in the tissues of dicotyledonous plants. Constitutive promoters such as CaMV35S are actively and successfully used in experimental studies to assess the expression of transferred genes in a large number of plant species. In Fig. 1 shows the results of detecting the CaMV35S promoter sequence in imported potato, corn and tomato samples. Studies have shown that all samples contain the CaMV35S promoter sequence, which is consistent with the results obtained by Elsanhoty (Elsanhoty et al., 2002; Elsanhoty, 2004).



No.	Color	Name	Type	Ct	Ct Comment	Given Conc (Copies)	Calc Conc (Copies)
1		p	Positive Control	26,33			
2		n	Negative Control				
3		Potato (foreign)	Unknown	22,80			
4		Tomato (foreign)	Unknown	17,95			
5		Corn (foreign)	Unknown	20,96			

Fig. 1. Kinetic curves of the DNA of the amplified CaMV35S gene in potatoes, corn and tomatoes obtained on a Rotor-Gene Q device.

Studies have been carried out to detect the NOS gene in potato samples of local and Russian origin. The results showed that these samples do not contain the NOS terminator gene (Fig. 2).



No.	Color	Name	Type	Ct	Ct Comment	Given Conc (Copies)	CalcConc (Copies)
1		p	Positive Control	25,40			
2		n	Negative Control				
3		Potato, NOS gene (Tajik origin)	Unknown				
4		Potato, NOS gene (Russian origin)	Unknown				

Fig. 2. Kinetic curves of the DNA amplified terminator gene NOS in potatoes.

The specificity of the selected primers and probe was investigated by real-time PCR on DNA samples isolated from GM potatoes, maize, and tomatoes. An increase in the fluorescence signal was observed in the reaction with DNA samples isolated from agricultural crops from far abroad. For potato samples of local and Russian origin, no increase in the fluorescence signal was observed, which confirms the high specificity of the selected primers and the PCR probe (Collonnier et al., 2005).The results of the work showed that genetically modified sources were found in the studied samples of potatoes, maize and tomatoes imported from certain countries of East and South Asia and sold in the Tajik food market.

4. Conclusion

The analysis of the results showed that 80% of the studied samples gave a positive result for genetic modification in the CaMV35S promoter gene. Only 20% of the studied potato samples (of local and Russian origin) do not contain the NOS terminator gene. These test systems allow detecting GM crops imported and sold on the local food market, and also significantly expand the range of detectable GMO lines industrially grown in the world. Based on the study of methodological approaches and the use of these test systems, evidence was obtained (sensitivity, specificity and detection limit), which makes it possible to recommend this method for screening control of GMOs.

Also, these results will be further used to assess the risk of GM crops for the environment, bio- and agrobiodiversity, and will also be taken into account when working to improve legislation and control in the field of GMO biosafety. Of course, the future development of mankind is unthinkable without the latest genetic engineering technologies. Problems such as providing mankind with food, alternative bioenergy resources,

combating various types of pollution, bioremediation and reclamation of disturbed ecosystems can and will certainly be solved using nanobiotechnology, including those related to the production of GMOs. However, genetic engineering activities can carry serious risks that must be able to assess and eliminate. While these risks exist, certain biosafety measures need to be applied.

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How to cite this paper:

Sabzalievich, B.S., Dustmurodovich, S.S., Saidmurodovich, N.D., Abdumanonovna, A.F., Yusufovna, N.F., 2020. Identification of GMO food products of Plant origin: environmental risks and agrobiodiversity perspective. *Cent. Asian J. Environ. Sci. Technol. Innov.,* **1**(6), 291-297.