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Biotechnology

Detection of Genetically Modified Corn Allergens in Food Products Using PCR-Methods

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Corn (*Zea mays* L.) is one of the most important cereal crops used for human and animal food all over the world. With the widespread use of corn products, it is becoming more and more difficult to prevent corn allergies. In addition, corn is one of the most common transgenic crops. Detection of corn allergens is important for food safety and health protection, accurate labeling, and consumer information. This study aims to evaluate allergens of genetically modified (GM) corn in food by PCR-based methods. Three important allergens were investigated, namely corn zein, *Zea m* 8 (chitinase), and GMO-specific Cry1Ab delta-endotoxin. Various corn foods were tested, such as flour, chips, puffcorns, popcorn kernels, roasted popcorn, and crispbread. Agarose gel electrophoresis of PCR products revealed allergen genes in all tested products. However, the amplified fragments were degraded according to the forms and severity of food processing. The obtained results indicate that the PCR methods described in this study can reliably detect GM corn allergens in food. © *2024 Bull. Georg. Natl. Acad. Sci.*

corn, allergen detection, polymerase chain reaction, GM maize

Corn (maize, *Zea mays* L.) is a high-yielding and an essential food crop available globally. It is widely utilized as raw material, additive and ingredient in food and feed production. A big variety of products are derived from corn, including porridge, flour, baby powder, corn starch, oil, sauce, syrup, etc. Corn is often used in cooking. It is a staple in some baked goods such as corn bread, corn muffins, cakes, tortilla, etc. [1]. In Georgia, corn ranks second after wheat in terms of cultivated area and food produced.

Allergy to corn has commonly been reported in countries, such as southern Europe and Mexico, where its consumption is popularly high. Oral ingestion of maize can lead to IgE-mediated allergic reactions, like oral food allergy syndrome and even severe reactions, like anaphylaxis [2]. Due to the widespread availability of maize-based foods, it is becoming more and more difficult to prevent maize-induced allergies. It is worth noting that corn allergens maintained their stability even after cooking.

Special proteins of maize trigger allergic reactions. Different types are included in maize allergenic proteins. Among them are *Zea m* 1, *Zea m* 8, zein, etc. They have diverse structures and funct-

ions [3]. Based on the existing literature, it is clear that there is still much to be learned about corn allergens. Allergies to corn have received more attention since genetically modified maize was developed and used in food production. Moreover, it is one of the two most common transgenic crops [4].

To assess the safety of genetically modified food according to international law both plant species-specific and GMO-specific allergens should be tested. In addition, regulations require food to be labeled if it contains allergens and/or genetically modified ingredients [5,6]. The implementation of these regulations need to develop an accurate, fast and affordable methods of allergen and GMO detection.

DNA-based polymerase chain reaction (PCR) is recognized as the most effective technique for food analysis as DNA is the most stable molecule during food processing. Several PCR-based methods were developed for detection of GM maize allergens. Several publications have described PCR methods targeting the maize zein and *Zea m* 8 as well as the GMO-specific Cry1Ab delta-endotoxin genes [7-9].

Despite the research done, the existing methods do not meet the requirements and the accurate analysis of GM corn allergens in processed foods is highly relevant. In this study, several PCR methods were validated to detect maize allergens, namely zein and *Zea m* 8 (chitinase) as well as GMO-specific potential allergen Cry1Ab delta-endotoxin in a various processed food.

Materials and Methods

Food and GMO reference materials. Food products containing corn as a main or additional ingredient were selected for the study. They were purchased at the local markets of Tbilisi, Georgia. Food products chosen for research are: corn flour, nachos round chips, tortilla chips, sweet puffcorn, popcorn kernels, fire roasted popcorn, crunchy puffcorn snack and crispbread. Corn is the main ingredient in these foods, with the exception of crispbread, which contains only 30% corn. As GMO reference material, maize GMO standards (ERM-BF413) containing 2% and 10% MON 810 were commercially purchased from Fluka, Biochemika. MON 810 has been approved as an insect-resistant GM maize event where the cry1Ab gene of the soil bacterium, *Bacillus thuringiensis* (Bt), has been inserted into the maize genome.

DNA Extraction

All food samples (except corn flour) were ground using an electric grinder (IKA TUBE-MILL 100) to achieve a flour-like consistency, which enhances the isolation of genomic DNA. DNA was extracted from 100 mg of each product using the cetyltrimethyl ammonium bromide (CTAB)-based method [10]. GMO certified reference materials were obtained in dried powder form and DNA was extracted from them using the DNeasy Plant Mini Kit (Qiagen). The resulting DNAs were assessed via agarose gel electrophoresis, with 7 µl aliquots of each sample analyzed on a 1% agarose gel.

PCR Analysis

Genes of common maize allergens, such as zein and Zea m 8 (Zea mays chitinase -chiA) were investigated. In addition, the Cry1Ab delta-endotoxin (cry1Ab) gene was studied because it is introduced into the insect-resistant GM maize event MON810. PCR primers for each gene were taken from previous publications and are presented in Table.

Table. Oligonucleotide primers used in this study

Target	Primers	Amplicon length (bp)	References
Zein	ZEINf/ ZEINr	102	[10]
Zea m 8	Zea8m130f / Zea8m130	130	[8]
cry1Ab	Cry102f / Cry102r	102	[8]

PCR amplifications were carried out in a thermal cycler Techne TC-412. The reactions were performed in a final volume of 25 µl containing 1.25 U

Taq DNA polymerase in standard Taq buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP (deoxynucleotide solution mix), 1 µl (60-70 ng) of genomic DNA and 0.5 µM of each primer.

The PCR cycling profile for primers Zea8m130f/Zea8m130r was as follows: 95°C initial denaturing for 3 min, followed by 40 cycles of 95°C denaturing for 30s, 60°C annealing for 30s, 72°C extension for 35s; 72°C final extension for 5 min. The PCR cycling profile for primers Zein102f/zein102r was as follows: 40 cycles of 95°C denaturing for 30s, 63°C annealing for 30 s, 72°C extension for 35s; 72°C final extension for 5 min. The PCR with GMO-specific primers Cry102f/Cry102r was carried out in the following conditions: denaturing at 94°C for 2 min, 35 cycles of 30s at 94°C, 30s at 60°C, 60s at 72°C; final extension at 72°C for 3 min. The amplification products were analyzed by electrophoresis in 2.0% agarose gels containing 1 µg/ml of Ethidium Bromide (EtBr).

Results and Discussion

In this study, a PCR-based method was used to detect allergen genes, as it is recognized as the most reliable means of food analysis [1]. Three important allergen genes, namely maize zein and zea m 8 (chitinase), as well as the GMO-specific Cry1Ab delta-endotoxin introduced into insect-resistant GM maize, were investigated.

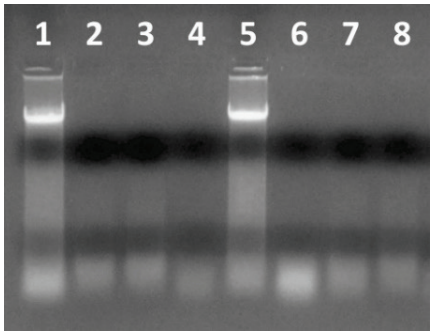

Fig. 1. Agarose gel electrophoresis of the Genomic DNAs.
Samples: lane 1. corn flour; lane 2. nachos round chips; lane 3. tortilla chips; lane 4. sweet puffcorn; lane 5. popcorn kernels; lane 6. Crispbread; lane 7. fire roasted popcorn; lane 8. crunchy puffcorn snack.

Fig. 1 shows an image of genomic DNA electrophoresis on an agarose gel. Comparison of the obtained results showed that corn flour and popcorn kernels yield visible high molecular weight genomic DNA with faint bands of degraded DNA (Fig. 1, lanes 1, 5). However, high degradation was observed in DNAs obtained from all other foodstuffs (Fig. 1, lanes 2-4, 6-8). The obtained results are explained by the fact that the samples of corn flour and popcorn kernels were processed only by mechanical impact (grinding), while all other products, along with mechanical, experienced thermal effects.

In order to evaluate the allergenicity of food products, two specific allergens of corn, namely chitinase and zein, were investigated. Chitinases are important from an allergological point of view, since they participate in the protection of plants against fungi and pathogens. It should be noted that the zein gene is used to detect the corn species, while the zein protein is considered an allergen.

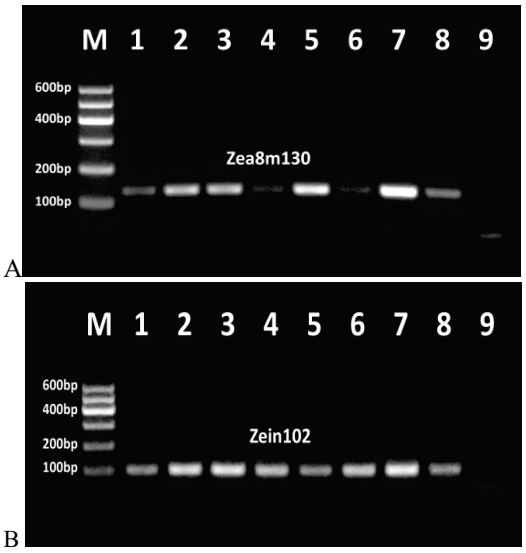

Fig. 2. PCR amplification of maize allergen genes Zea 8 m using primers Zea8m130f/Zea8m130r (A) and Zein using primers Zein102f/Zein102r (B).
Samples: lane 1. corn flour; lane 2. nachos round chips; lane 3. tortilla chips; lane 4. sweet puffcorn; lane 5. popcorn kernels; lane 6. fire roasted popcorn; lane 7. crunchy puffcorn snack; lane 8. crispbread; lane 9. water. M. GelPilot 100 bp ladder (Qiagen).

Agarose gel electrophoresis of the PCR products showed that one amplicon of the expected

length, 130 bp (Fig. 2A) and 102 bp (Fig. 2B), was obtained for primer pairs Zea8m130f/Zea8m130r and ZEINf/ZEINr, respectively. However, the comparison of foodstuffs revealed different intensities of Zea8m130 bp PCR products, in particular, the bands produced by sweet puffcorn and fire roasted popcorn have the lowest intensity (Fig. 2A, lanes 4, 6). This indicates a strong degradation of the chitinase gene during the processing of these food products. However, zein102 amplicons showed almost similar intensities for all foodstuffs (Fig. 2 B, lanes 1–8). This indicates a similar degradation of the zein gene during the production of these food products. In addition, the absence of PCR product in the water control (Fig. 2 A, B, lane 9) confirmed the purity of the experiments.

Fig. 3. PCR amplification of cry1Ab gene using primers Cry102f/Cry102r.
Samples: lane 1. 10% MON 810; lane 2. 2% MON 810; lane 3. corn flour; lane 4. nachos round chips; lane 5. tortilla chips; lane 6. sweet puffcorn with vanilla; lane 7. popcorn kernels; lane 8. fire roasted popcorn; lane 9. crunchy puffcorn snack; lane 10. crispbread; lane 11. water. M. GelPilot 100 bp ladder (Qiagen).

The insect resistant event MON810 maize was selected as the GMO reference material because it is frequently utilized in food production. Correspondingly, the gene for the potential allergenic protein Cry1Ab delta-endotoxin was investigated, since it has been cloned into transgenic maize MON810. Fig. 3 shows a gel electrophoresis image of PCR products generated with Cry102f/Cry102r primers targeting the Cry1Ab gene. A 102 bp PCR product was produced for the 2% and 10% MON810 templates (Fig. 3. lanes 1–2) as expected.

It is worth noting the increased intensity of the PCR bands in accordance with the increased amount of transgenic material in the samples (Fig. 3. lanes 1-2). This indicates sufficient sensitivity of the PCR method to detect 2% Bt maize. However, no visible bands were detected in food samples and water (Fig. 3. Lanes 3-12), indicating the absence of GMO-specific Cry1Ab delta-endotoxin in the analyzed food.

Conclusion

The outcomes of the present study clearly demonstrate that food processing induces genomic DNA degradation, while the level of degradation depends on the form and strength of food treatment. A cetyltrimethyl ammonium bromide (CTAB)-based method is suitable for extracting amplifiable genomic DNA from processed foods. PCR methods using primers Zea8m130f/Zea8m130r and ZEINf/ZEINr can be applied for reliable detection of corn allergens Zea m 8 and zein in food products. In addition, the PCR method with primers Cry102f/Cry102r can be used for the analysis of the GMO-specific putative allergen Cry1Ab delta-endotoxin. Analysis of various processed products showed that the PCR methods applied in this study can be successfully used to test for genetically modified corn allergens in food.

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ბიოტექნოლოგია

გენეტიკურად მოდიფიცირებული სიმინდის ალერგენების გამოვლენა საკვებ პროდუქტებში პჯრ-მეთოდებით

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სიმინდი (*Zea mays* L.) არის ერთ-ერთი ყველაზე მნიშვნელოვანი მარცვლეული კულტურა, რომელიც გამოიყენება ადამიანებისა და ცხოველების საკვებად მთელ მსოფლიოში. სიმინდის პროდუქტების ფართო გამოყენების გამო სულ უფრო და უფრო რთული ხდება სიმინდის ალერგიის პრევენცია. გარდა ამისა, სიმინდი ერთ-ერთი ყველაზე გავრცელებული ტრანსგენური კულტურაა. სიმინდის ალერგენების გამოვლენა მნიშვნელოვანია სურსათის უვნებლობისა და ჯანმრთელობის დაცვისათვის, ზუსტი ეტიკეტირებისა და მომხმარებლის ინფორმაციისთვის. ეს კვლევა მიზნად ისახავდა გენმოდიფიცირებული (გმ) სიმინდის ალერგენების შეფასებას საკვებში პოლიმერაზულ ჯაჭვურ რეაქციაზე (პჯრ) დაფუძნებული მეთოდებით. გამოკვლეული იყო სამი მნიშვნელოვანი ალერგენი, კერძოდ, სიმინდის ზეინი, *Zea m* 8 (ჩიტინაზა) და გმო-სპეციფიკური Cry1Ab დელტა-ენდოტოქსინი. შემოწმდა სიმინდის სხვადასხვა საკვები, როგორიცაა ფქვილი, ჩიფსები, ბურბუშელა, ბატიბუტის მარცვლები, მოხალული ბატიბუტი და ორცხობილა. პჯრ-პროდუქტების აგაროზას გელზე ელექტროფორეზმა გამოავლინა ალერგენების გენები ყველა შემოწმებულ პროდუქტში. თუმცა, ამპლიფიცირებული ფრაგმენტები დეგრადირებული იყო საკვების გადამუშავების ფორმებისა და სიძლიერის მიხედვით. მიღებული შედეგები მიუთითებს, რომ ამ კვლევაში აღწერილი პჯრ-მეთოდებით შესაძლებელია საკვებში გმ სიმინდის ალერგენების საიმედო გამოვლენა.

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