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Detection of the genetically modified organisms from food products

Detecția organismelor modificate genetic din produse alimentare

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Abstract

Since the release of the first genetically modified crop, scientists have pro and con opinion on cultivation and their use, because of potential health, and environmental risks. Current EU legislation (Directive 2003/18/EC) provides for public information, monitoring long-term effects, labelling and traceability at all stages of the placing on the market of GMOs. The aim of this study was to evaluate the quality of the food products on the market, in which the detection of presence / absence of GMO. In this regard, two types of maize flour and four types of soy products were analysed during 2013. The kit used for detection of the presence / absence of GMO in the samples tested, comprising the step of DNA extraction, DNA amplification by PCR and agarose gel electrophoresis of amplified products and uses two GM associated sequences - promoter 35S and NOS terminator from Agrobacterium tumefaciens. The presence of GMO was found in a corn sample, also in a soy sample the results illustrate the need for further analysis to identify the exact type and quantity of GMO (the limiting value imposed by European legislation being 0.9% at ingredient).

Keywords: *screening, GMO testing, PCR, food products*

Rezumat

Încă de la apariția primelor culturi modificate genetic, oamenii de știință au avut păreri pro și contra asupra cultivării si utilizării lor, datorită potențialelor riscuri pe care le pot avea asupra sănătății și mediului înconjurător. Legislația europeană actuală (Directiva 2003/18/CE) prevede obligativitatea informării publicului, a monitorizării efectelor pe termen lung, a etichetării și trasabilității în toate stadiile introducerii pe piață a OMG. Scopul acestui studiu a fost evaluarea calitativă a produselor alimentare existente pe piață, în ceea ce privește detecția prezenței/absenței OMG. În acest sens au fost analizate două tipuri de făină de porumb și patru tipuri de produse din soia, în perioada 2013. Kit-ul utilizat pentru detecția prezenței/absenței OMG în probele testate, cuprinde etape de izolare ADN, amplificare ADN prin PCR și electroforeza în gel de agaroză a produșilor amplificați și folosește două secvențe asociate OMG - promotorul 35S și terminatorul NOS de la Agrobacterium tumefaciens. În urma studiului, au fost pozitive în ceea ce privește prezența OMG, o probă de mălai extra și o probă de soia. Rezultatele obținute ilustrează necesitatea efectuării de analize suplimentare pentru identificarea tipului exact de OMG și pentru stabilirea cantității de OMG (pragul limită impus de legislația europeană fiind de 0,9% la nivel de ingredient).

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Introduction

Plant genetic transformation has experienced a spectacular progress from getting first chimeric genes (in the seventies of the last century) to regenerate the first genetically transformed plants, which carry foreign genes [1].

GM foods are foods that contain GM crops components by the insertion of foreign genetic material that may have come not only from another plant but also possibly from a species of another kingdom: animal, fungal, bacterial. Foreign genetic material is typically a gene (transgene) which encodes a protein and confers a benefit comparative with other analogous plant crops. Thus, transgenic organism is an organism whose genetic patrimony has been modified to achieve certain characters, such as: insect resistance, herbicide tolerance, disease, and frost resistance, fruit ripening delay, enhanced fruit production, and augmenting the nutrient content [2,3].

Commercialization of genetically modified plants started in the U.S. in 1994. In 1996, GM commercial crops occupied about 60 million hectares, being present mostly in the U.S., China, Canada, Argentina, Australia, and Mexico. Subsequently, transgenic plants spread rapidly, and are currently used on all continents of the globe [3,4].

The global market of genetically modified plants is entirely provided by four species: soybeans, cotton, corn, and rape, the rest of transgenic plants (potato, papaya, tobacco, pumpkin, etc.) occupying small areas worldwide [3]. The fastest augments have been genetically modified soybeans (GMS), followed by GM maize (GMC) [3].

In the European Union (EU), only a small part from GMC grew commercially is used for food, mostly used as animal feed and as raw material for starch industry that also represented a basic form of foods or food additives [5].

Soy that is known by the Chinese as a holy plant was subject to genetic modification research, attributable its importance. Resistance to diseases and herbicides has become one of the main targets for improvement, especially for certain areas where soybean pathogens have become limiting factors of production and seed quality [6,7]. GMS is designed primarily as feed for cattle and chickens, but also to produce certain food additives and food ingredients or to extract soybean oil [5].

Our country has received authorization for cultivation for MON810 maize, soybean GTS 40-3-2 (until 2006) and BASF Starch-potato (in 1999-2001), so many GMOs existing on the Romanian market is due to imports [5].

Since the tested food samples and detection of GMOs are different forms, for this purpose may use methods based on protein analysis (enzymatic methods - ELISA), methods based on DNA analysis (PCR, RT-PCR, microarrays), also chromatographic and spectral methods [4].

Currently, the main legal regulation instrument for biotechnology domain, in the EU is 2003/18/EC Directive about the deliberate release into the environment of GMOs. The purpose of the Directive is to protect human health and the environment, and ensure coexistence at all levels of products derived from biotechnology and conventionally. Also, has been set to draw up a single register of GMO molecular evidence, into a database, for collecting and interpreting information on GMO screening [8,9].

Regulation 1829/2003 sets a new threshold for GMO labelling approved ingredient 0.9% level. The Annex I from the Regulation 641/2004, stipulates the obligation of the applicant to provide in the dossier for authorization, a methodology for quantifying and corresponding reference transformation event [10,11].

Materials and methods

Food samples

The study was performed on two types of maize flour and four types of soy products; their selection being random among different food manufacturers knew on Romanian market. The corn samples had the same texture (maize flour) and were encoded P1 and P2. The soybean samples had different texture (granules, blocks, grains) and were named S1, S2, S3, and S4. Samples S1, S2 and S3 were labelled „non-GMO product". All samples were analysed using the Biotechnology Explorer GMO Investigator kit (Bio-Rad) and compared with a positive and negative control sample (Bio-Rad Certified) contained in the kit.

DNA extraction

Biotechnology Explorer GMO Investigator kit contains a standard protocol for GMO extraction, amplification, and identification, and uses two different GMO-associate sequences - the 35 S promoter of the cauliflower mosaic virus (CaMV 35S) and the terminator of the nopaline synthase (NOS) gene of *Agrobacterium tumefaciens* that is present in ≈ 85% of all GM crops currently approved around the world.

The integrity of the plant DNA extracted from food was tested using PCR to identify a third sequence of DNA, the photosystem II (PSII) chloroplast gene, which is common to most plants [2].

The first sample processed it was one GMO-negative, to avoid contamination. There were weighed (Kern analytical balance) 2 g of each sample (GMO-negative, GMO-positive, P1, P2, S1, S2, S3 and S4), were smashed into the mortar and 10 mL distilled water was added for each sample. DNA extraction was made with Biotechnology Explorer GMO Investigator kit, following the kit instructions. For each PCR reaction, 20 μL of extracted DNA were used.

PCR reactions

For PCR reactions we used the same kit which contains: master mix (dNTPs, buffer and Taq DNA-polymerase enzyme); GMO primers (red); Plant PSII primers (green). For each sample, there were used two 0.2 mL PCR tubes (one of them contained 20 μL master mix and GMO primers and the others 20 μL master mix and Plant PSII primers). PCR reactions were made with Biotechnology Explorer GMO Investigator kit, following the kit instructions. Amplification reactions were performed in the Bio-Rad IQ^5 PCR termocycler (base, without optical module) and the amplification conditions were as follows: 2 minutes at 94^{o}C for DNA denaturation (1 cycle), followed by 40 cycles for PCR amplification: 1 minute at 94^{o}C (denature), 1 minute at 59^{o}C (anneal), 2 minutes at 72^{o}C (extend); and 10 minutes at 72^{o}C (1 minute) for final extension.

Electrophoresis of PCR Products

The DNA fragments amplified from the 35S promoter and NOS terminator are 203 and 225 base pairs (bp) respectively. The PCR product generated from the photosystem II gene is 455 bp. Resolving bands in this size range requires 3% agarose gel that allows separation of the similar-sized DNA bands generated from a test food that contains both the CaMV 35 S promoter and NOS terminator [2].

The PCR tubes were briefly centrifuged (5000 x g, 3 seconds). Subsequent to thaw, Orange G loading dye and PCR molecular weight

ruler was briefly centrifuged (5000 x g, 3 seconds). In each amplified sample, were added 10 µL Orange G and the tubes were mixed well to the vortex.

The Consort M.V. 265 gel electrophoresis apparatus it was set up (gel casting, gel tray, combs, power supply, UV transilluminator and image analyser).

Two gels: the one for maize samples and the other one for soybean samples, were prepared and analysed in the same conditions.

There it was prepared a running buffer TAE 1x (Tris-acetate-EDTA), with pH = 8.0 and agarose gel 3% (with the same buffer - 3 g agarose to 100 mL TAE 1x); for the gel staining, to the agarose solution were pipetted 20 µL EtBr 1%. Migration parameters were: 100 V, 30 minutes. The gel was red using UV transilluminator after migration and separation, and the gel image was saved in the recorder.

Results

The DNA fragments amplified which illustrated GMO presence (35S promotor and NOS terminator) are 203 and 225 bp respectively; thus, it must be appearing on the gel nearby 200 bp band. This type of protocol cannot distinguish the two types of fragments, but their presence is shown by the appearance of a band of 200 bp. The PCR product generated from the photosystem II gene is 455 bp; thus, it must be appearing on the gel nearby 500 bp band.

In Figure 1 (for corn) and Figure 2 (for soybeans) images of agarose gel observed after UV gel electrophoresis analysis, are shown. Unknown samples (P1 and P2, respectively S1, S2, S3 and S4) are compared with a negative and a positive control.

For corn samples, in Figure 1, on the agarose gel the following has been observed: a band near

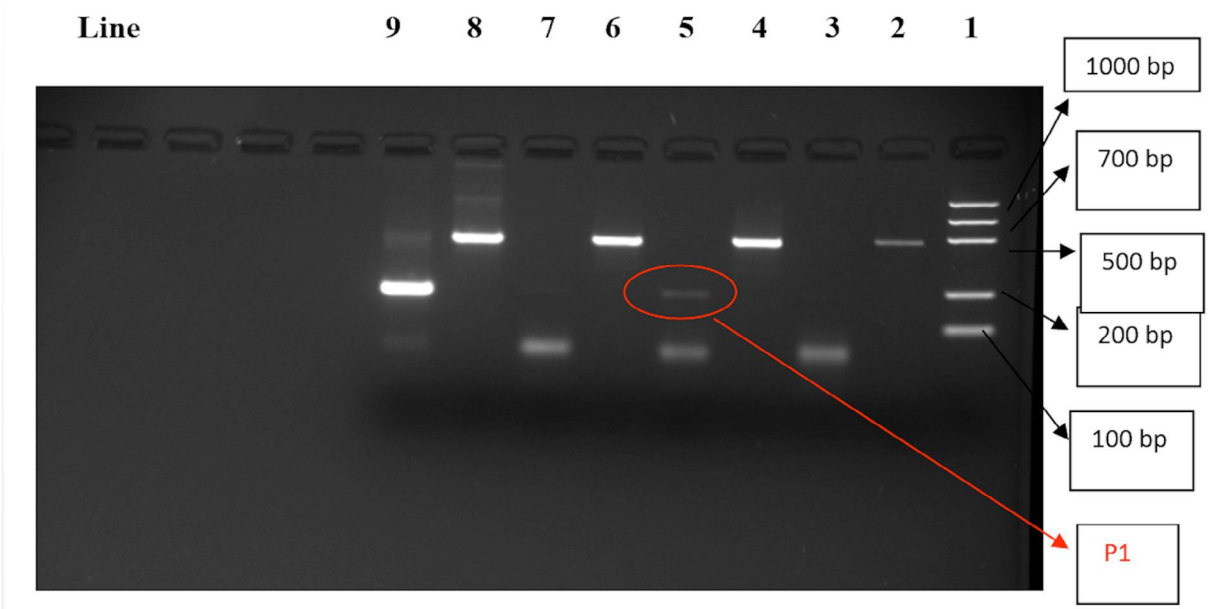

Figure 1. Qualitatively evaluation through agarose gel electrophoresis of the DNA obtained from corn samples. Samples: line 1-DNA weight ruler (100-1000 bp); line 2- DNA negative control with plant primers; line 3- DNA negative control with GMO primers; line 4-P1 with plant primers; line 5-P1 with GMO primers; line 6-P2 with plant primers; line 7-P2 with GMO primers; line 8-GMO positive control with plant primers; line 9-GMO positive control with GMO primers.

500 bp not very intense (line 2 - GMO negative control with plant primers); no band near 200 bp (line 3 - GMO negative control with GMO primers); a band near 500 bp intense enough (line 4 - P1 with plant primers); a band near 200 bp not very intense (line 5 -P1 with GMO primers); a band near 500 bp intense enough (line 6 - P2 with plant primers); no band near 200 bp (line 7 - P2 with GMO primers); an intense band near 500 bp (line 8 - positive control with plant primers); a very intense band near 200 bp (line 9 - positive control with GMO primers).

On the gel electrophoresis image from Figure 2, for soybeans samples the following has been observed: a band near 500 bp not very intense (line 2 - GMO positive control with plant primers); an intense band near 200 bp (line 3 - positive control with GMO primers); an intense band near 500 bp (line 4 - S1 with plant primers); no band near 200 bp (line 5 - S1 with GMO primers); an intense band near 500 bp (line 6 - S2 with plant primers); no band near 200 bp (line 7 - S2 with GMO primers); an intense band near 500 bp (line 8 - S3 with plant primers); no band near 200 bp (line 9 - S3 with GMO primers); a band near 500 bp not very intense (line 10 - S4 with plant primers); a band near 200 bp not very intense (line 11 - S4 with GMO primers); a band near 500 bp not very intense (line 12 - negative control with plant primers); no band near 200 bp (line 13 – negative control with GMO primers).

Discussions

The presence of bands near 500 bp in all analysed samples shown a good isolation of DNA, and that the samples were well prepared, isolated DNA being undivided. Also, according to the

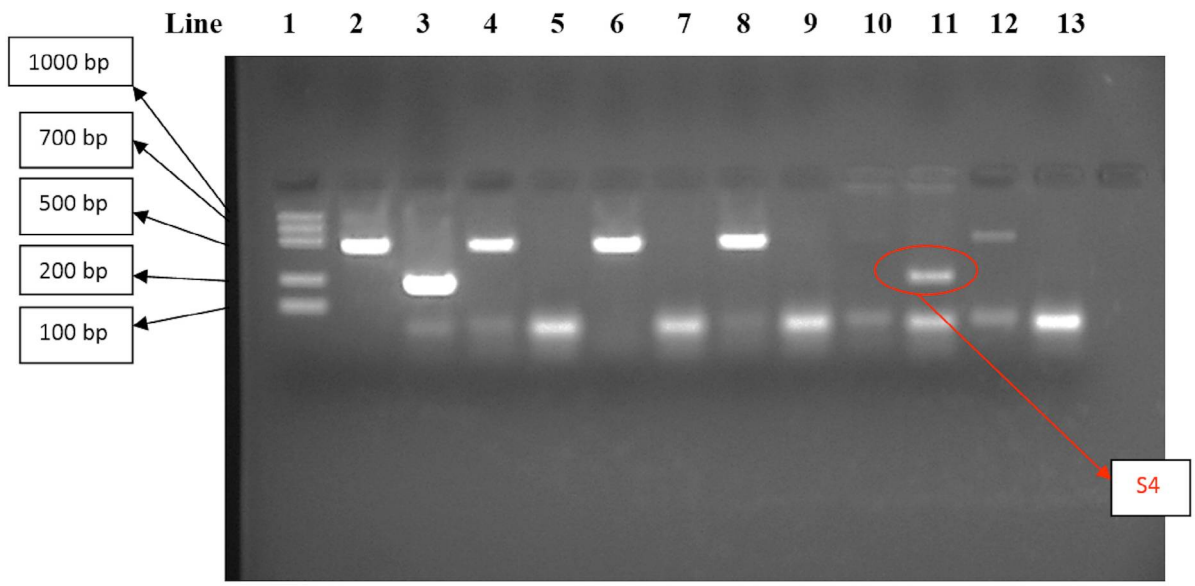

Figure 2. Qualitatively evaluation through agarose gel electrophoresis of the DNA obtained from soy samples. Samples: line 1-DNA weight ruler (100-1000 bp); line 2-GMO positive control DNA with plant primers; line 3- GMO positive control DNA with GMO primers; line 4-S1 with plant primers; line 5-S1 with GMO primers; line 6-S2 with plant primers; line 7-S2 with GMO primers; line 8-S3 with plant primers; line 9-S3 with GMO primers; line 10-S4 with plant primers; line 11-S4 with GMO primers; line 12- DNA negative control with plant primers; line 13- DNA negative control with GMO primers.

principle of the gel electrophoretic, it could be seen that the smaller fragments migrated faster than the larger ones (Figures 1 and 2). Check contamination of samples was performed by using a negative control sample. Absence of 200 bp band in lane 3 for maize (Figure 1) and in line 13 for soybean (Figure 2), which represents a negative control sample for GMO, proves that technique was correct for both isolation and amplification and the samples were not contaminated; if there were contamination during processing of samples, these bands would have been visible on the gel. Also, the presence of the 200 bp band in line 9 for maize and in line 3 for soybean, which represents a positive control sample for GMO, demonstrates a proper handling and preparation of samples.

Regarding the maize test samples, any P1 sample showed the band of 200 bp (line 5), which indicated the presence of GMO (Figure 1). Of the four types of soybean samples, only S4 sample showed a 200 bp band (line 11), which showed the presence of the GMO (Figure 2). Although the intensity of these bands was low, the samples can be regarded as positive.

The protocol used in this paper enables to achieve a qualitative screening of food, regarding of the presence or absence of GMOs [2]. The sample S4 found positive in terms of the presence of GMOs, was the only one that had no specification about being genetically modified.

For quantification of GMO content in samples considered positive are needed more complex studies that include quantitative methods, such as RT-PCR or microarray. Also required studies regarding precisely identify the types of GMC and GMS existing in these samples of corn and soybeans [12], taking into account that the products on the Romanian market are not adequately labelled and there is no specification in regarding the presence of GMOs in the composition and amount (in percentage) thereof.

A recent study on the detection of this type of GMS Roundup Ready (GTS 40-3-2) in various foods [12], showed the need for qualitative tests (presence/absence), quantitative (RT-PCR) to identify, and the small amounts of genetically modified material. In Romania, the monitoring of this type of GMS is very important, because although in EU its cultivation is banned, in our country was cultivated until 2007. The study results were positive for raw soybean seeds [12].

Some researchers affirm that eventually, the picture regarding GMOs will be more complex. Thus, traditional approaches, mostly based on sequential detection of a single target in a given time, should be replaced with new analytical approaches and technologies developed through the use of fast and accurate selection strategies. These involve the use of detection systems with multiple targets, as well as algorithms that allow the conversion of analytical results through indicate the presence of a GMO in an unknown sample [13].

Thus, in order to achieve fast protocols and with low cost for monitoring of the existing products on the market, and to detect the unauthorized, researchers have developed a number of new techniques for qualitative and quantitative detection. Using current quantitative techniques, especially RT-PCR, qPCR, and duplex or multiplex PCR, there were detected different types of unauthorized GMO from maize food (application on 35S promoter) (Bt11, Bt176, MON810 and T25) [14,15] as well as other types of food [16,17]. These methods allow the amplification of multiple target sequences simultaneously, in order to shorten the analysis and monitoring time of food products and to reduce costs of analysis. Studies have shown the effectiveness of the PCR technique (91-100%), and its specificity by determining the limit of detection [18].

In order to increase the efficiency and reduce the costs of analysis for food products which are based on GMC, researchers have developed

other modern methods that allow detection of multiple types of GMC. One of them is multiplex PCR combined with capillary gel electrophoresis with fluorescence detection, the limit of detection being 0.1% per each GMO, in this specific case [19]. Another method developed for the parallel identification of multiple types of GMC, requires a modular detection system The system is based on an RT-PCR method and a modular microplate that can simultaneously identify 15 types of GMC in a single analytical application lasting for about 2 hours. With this type of module, 23 food samples, 14 feed samples, and 8 samples of seeds were successfully analysed [20]. In addition, by using PCR it was performed a screening on 10 targets (promoters and terminators) that can be found in different types of GMOs. In this study there were analysed: six promoter sequences (pFMV, pNOS, pSSuAra, pTA29, pUbi, pRice, actin) and four terminator sequences (t35S, tE9, tOCS, tg7) to identify unauthorized GMOs and those that do not use p35S and NOS [21].

Another method used to detect both authorized and unauthorized GMOs is Scorpion PCR (using Scorpion primers) comparative with HRM analyses (high resolution melting). The scorpion primer is used as a method of analysing the type RR of soybean (RR 40-3-2) from large number of samples. Both methods can differentiate between the two alleles, homozygote, and heterozygote. For a better discriminate of the potentially unstable samples regarding the detection interference and specific identification, researchers suggest the use of both methods [22].

Because pro and contra researches points of view regarding GMOs consuming impact on human health, studies of GMOs specific types should be extended on laboratory animals, in order to evaluate a possible negative effect or a certain degree of toxicity on animal health and more.

The current bibliographic database illustrates existence of studies on the effects of a GMC variety on mammal's health. A comparative analysis it was revealed in some studies, on blood and urine, the biochemical analysis from samples of mice that were fed with three types of GMC (NK 603, MON 810, MON 863); analyses were performed after 5 and 14 weeks of feeding, using a control group of mice fed with not-GM foods. The analysis allowed the investigation of various factors: sex, diet, dose, and group and revealed new effects by consuming three types of GMC, effects that were dependent on sex and dose. Most effects were associated with changes particularly in the kidney and liver (hepatorenal toxicity findings), probably due to the new specific pesticides to each type of GMC, but effects on the heart, the spleen and hematopoietic system were observed [23].

Recent studies for two years in mice have shown toxic effects of Roundup on their health. All results were dependent on sex and hormonal composition, and pathological profiles were comparable. Females developed large mammary tumours, and hormonal balance changed significantly after feeding on GM. Males showed hepatic congestion and necrosis [24].

In addition, current bibliographic database illustrates the existence of studies on the effects of certain GMS types of mice health. In this study, there were evaluated (for 455 days) three groups of 10 mice that were fed with GMS, non-GM soya, and a group of control mice. Finally, it was found that groups fed with soybean (genetically modified or unmodified) have greater weight than the control group not fed with soy. Haematocrit and haemoglobin values were lower in the groups fed soy than those recorded for the control group, blank the other clinically significant changes [25].

Conclusions

The presence on market of food containing GMOs has been and remained a disputed topic. Therefore, a very important first step in their monitoring is screening regarding presence/absence of GMOs.

Applying a qualitative evaluation protocol that uses two sequences associated with the presence of GMOs; their presence was detected in a sample of corn and one soybean.

To increase the effectiveness of monitoring of the authorized products, to identify the presence of some unauthorized GMOs, and to shorten the time and cost of analysis, should be carried out or performed specific and complex molecular analyses, specifically through identification of the types of GMO, their detection and quantification.

Although so far there is no clear evidence to confirm the fears of some researchers regarding the occurrence of possible adverse negative or toxic effects on human health by eating foods containing GMOs, we recommend a circumspect approach, especially in terms of their irrational use.

Abbreviations

GMO-genetically modified organism
PCR-Polymerase chain reaction
GMC-genetically modified corn
GMS-genetically modified soy
UE-European Union
ELISA-Enzyme-linked immunosorbent assay
RT-PCR-Real-time PCR
qPCR-Quantitative PCR
bp-base pairs

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