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# THE PRESENCE OF GMOs IN SOYBEAN SAMPLES AND SOYBEAN PRODUCTS EXAMINED IN SĂLAJ COUNTY

**Nicolae Mocuța[1], Gheorghe Ştețca[2], Ancuța-Maria Puşcaş[3*]**

[1]Food Control Laboratory Zalau, 31/d M. Gorki Street, 450054 Zalău, Sălaj County, Romania
E-mail: nicolaemocuta@hotmail.com

[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture.
3-5 Mănăştur Street, 400372 Cluj-Napoca, Romania

[3]Research Institute for Analytical Instrumentation Cluj-Napoca, 67 Donath Street, 400293 Cluj-Napoca, Romania.
*Corresponding author. E-mail: ancutapuscas@yahoo.com

## ABSTRACT

**The output, trade and cultivation of Genetically Modified Organisms (GMOs) represent highly controversial issues with implications in several domains. The detection of GMOs will become a future necessity due to the legislative requirements that are rather severe in terms of trading these products. In this context, the approach of several methodologies for laboratory diagnosis is needed in order to detect GMOs in food, fodder, seeds etc. Within the Sălaj Sanitary-Veterinary and Food Safety Department Laboratory, the detection activity for GMOs was initiated, by means of the PCR-qualitative method. 54 soybean samples and soybean-derived-products were examined during the year 2008. Positive results were obtained for 11 samples. Although these results are not significant in statistical terms, one may notice that there are genetic modified organisms whose presence must be determined in specialized laboratories.**

**Key words:** Genetic Modified Organisms (GMOs), food, detection, PCR.

## INTRODUCTION

The output, trade and cultivation of Genetically Modified Organisms (GMOs) arouse many controversies, debates and discussions, considering that GMOs are organisms whose DNA was unnaturally altered.

Genetic changes were made in order to alter certain features into more favourable features such as: higher resistance to herbicides, higher yielding capacity, higher nutrient content, higher resistance to unfavourable factors etc., and also better food quality (Nikolić et al., 2009a).

Fears, concerns and protests expressed by civil society regarding the risks induced to human and animal health, as well as to the environment, forced the authorities from many countries to specifically label the foods and other GMOs products (Deng et al., 2008; Holst-Jensen, 2007).

Consequently, highly severe legislative requirements were promoted, regarding the approval of GMOs cultivation, trading and compulsory labelling, which led to the need to develop several laboratory diagnosis methods in order to identify the presence of GMOs in various products: food, fodder, seeds etc. Nowadays, strict laws regulate the safety level of food derived from genetically modified organisms, their impact on the environment, and the effects on human health (BIOPOP Project, 2010).

Worldwide, legislation exists for the authorization and labeling of genetically modified organisms (GMOs) in food products, and many countries have established threshold levels for the unintentional presence of GMOs (Branquinho et al., 2010; Burachik et al., 2010).

Genetic modification of plants involves three factors: the genes or the newly introduced gene in the DNA, the promoter which plays the role of a “switch" and the terminator which provides the completion of the transfer process in due time (IdentiGEN, 2006).

Generally, a virus is used as a promoter, such as cauliflower mosaic virus (CaMV 35S), while a derivative from the synthesis gene of

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NOS, belonging to *Agrobacterium tumefaciens* is used as terminator.

These two elements are present in more than 95% of the genetically modified cultures approved to be merchandized in the EU and the ability to identify these elements (the promoter and/or the terminator) enable the detection of a large majority of genetically modified plants (Bonfini et al., 2001).

GMOs are analyzed based on both the detection of new proteins resulting from genetic modification and DNA sequences. Although specific DNA sequences can be detected by hybridization, PCR (qualitative PCR, end-point quantitative PCR and quantitative realtime PCR) has been generally accepted by the regulatory authorities (Marmiroli et al., 2008; Branquinho et al., 2010). Other methods of analysis, such as biosensors, micro-arrays and visible/near infrared spectroscopy have also been reported (Michelini et al., 2008; Branquinho et al., 2010).

The most frequently used method is the PCR technique consisting in the isolation of DNA from the examined sample, the amplification of specific sequence for the two mentioned elements and then the identification of the PCR product (Gachet et al., 1998; Nikolić et al., 2009b).

## MATERIAL AND METHODS

The extraction and DNA purification from the examined samples was performed using a kit produced by Promega Comany, and Wizard Magnetic DNA Purification System for Food, according to the recommendations in the producer's manual.

50 µl MagneSil PMPs (paramagnetic micro sphere on whose surface DNA is fixed) were added and then isopropanol, with 0.8/1 ml supernatant ratio. This was incubated for 5 minutes at room temperature and the tube was introduced in the magnetic shelf for magnetic separation of the paramagnetic spheres with DNA fixed on their surface with the help of the reaction antigene-antibody. The liquid phase was removed and the tube was taken off the shelf, and 250 µl of buffer B for liza were added. After mixing, the tube was introduced again in the magnetic shelf for one minute. The liquid phase was removed and 1 ml ethanol 70% was added. The tube was placed again in the magnetic shelf and the liquid phase was disposed off, repeating twice the washing action using ethanol.

The paramagnetic micro spheres were dried at 65°C for 10 minutes, and 100 µl were added. The nuclease free water was mixed by means of the vortex and incubated at 65°C for five minutes. There were inserted again in the magnetic shelf and the liquid phase was harvested (the DNA) in a PCR reaction tube. It was completed with nuclease free water at 100 µl. It is kept in fridge for a short period or frozen (-24°C) for a longer period. The DNA extracted and purified was used to achieve the PCR in order to detect the GMOs.

The 50 µl of MagneSil PMPs added in the sample enable the collection in pure phase of 5 µg DNA in 100 µl water, respectively 50 ng DNA/1 µl water. The detection of GMOs by means of the PCR technique was performed using a kit produced by BIOTOOLS (BIOGENICS Kits, 2009).

The kit contains master mixes (PCR buffer, dNTP-s and primers) and magnesium chloride, polymerase and DNA purified from the sample will be added later.

The preparation of the compounds for PCR was performed in the PCR room beneath the laminar flow hood and observing precisecely the reagents quantities indicated by the producer company.

*Table 1.* Reagents quantities indicated by the producer company

| Reagent | P35S | NOS | Corn | Soybean | Plants |
|---|---|---|---|---|---|
| MgCl - 2 solution | 2.5 µl | 2.5 µl | 2.0 µl | 2.0 µl | 2.0 µl |
| Master Mix | 15 µl | 15 µl | 15 µl | 15 µl | 15 µl |
| Polymerase | 1.4 µl | 1.4 µl | 1.2 µl | 1.0 µl | 1.0 µl |
| Tap water | 21.1 µl | 21.1 µl | 21.8 µl | 22 µl | 22 µl |
| TOTAL | 40 µl | 40 µl | 40 µl | 40 µl | 40 µl |

The tubes prepared for PCR were transferred in the room for the extraction of DNA (pre-amplification) where, also under the laminar flow hood 1-2 µl of the DNA

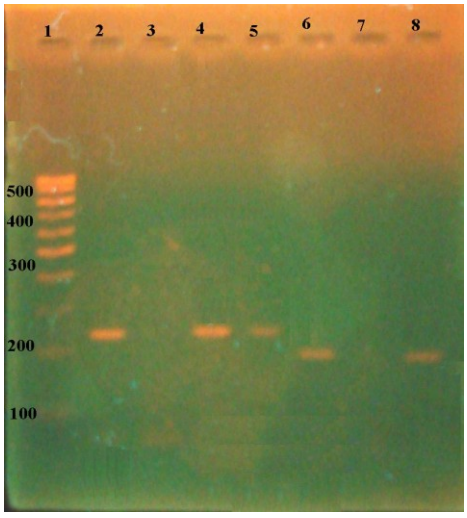
extracted from the samples were added. 10 µl of the control DNA (positive control variant) and 10 µl PCR nuclease free water, negative control (blank) were added in the two tubes with control variants. The overall volume of 50 µl was completed with PCR water and one mineral oil drop was put in each PCR tube. After that, the tubes were placed in the thermacycler for amplification.

The timetable for the thermalcycler was established according to the indications in the kit usage instructions.

*Table 2.* The timetable for the thermalcycler following the indications in the kit usage instructions

| Initial denaturation | Plants, 35S, NOS<br>94°C x 3 min. | Corn<br>94°C x 10 min. | Soybean<br>94°C x 10min. |
|---|---|---|---|
| The amplification cycle | 94°C x 30 sec.<br>55°C x 40 sec.<br>72°C x 1 min. | 94°C x 30 sec.<br>70°C x 30 sec<br>72°C x 30 sec. | 94°C x 30 sec.<br>60°C x 30 sec.<br>72°C x 1 min. |
| Number of cycles | 45 | 40 | 40 |
| Final elongation | 72°C x 3 min. | 72°C x 10 min. | 72°C x 3 min. |

After amplification, the PCR evaluation was performed by means of the electrophoresis. Agarose gels 2.5% (agarose LE-AG) were prepared in TAE buffer including the ethidium bromide in gel, for 5µl bromide solution 10 mg/ml for 100 ml gel ratio. The migration was made in TAE buffer IX at 100 V in Mupid equipment – 2. The results were interpreted according to the migration distance and position towards the weight marker of 100 bp, as can be seen in figure 1.




GEL II – P-35S, NOS
1. Molecular weight marker 100 bp
2. P-35S Amplification Control
3. Blank
4. Sample no. 25
5. Sample no. 30
6. NOS Amplification Control
7. NOS Blank
8. NOS Sample no. 25

Plant Master Mix – 190 bp
35S Master Mix – 226 bp
NOS Master Mix – 180 bp
Maize Master Mix – 225 bp
Soya Master Mix – 118 bp

*Figure 1.* The results of the electrophoresis performed with PCR for two samples

All the positive response samples, respectively with the presence of P-35S and/or NOS were sent in order to be confirmed and quantified by means of Real-Time PCR to the Molecular Biology unit within IDSA Bucharest.

The analyzed samples were collected from 8 counties in the country from the administrative area of the Salaj Sanitary-Veterinary Laboratory, which is certified to examine samples in the 2008 Food Safety Survey and Control Programme. The number of collected samples and the objectives established for the sampling are determined on an yearly basis by the National Sanitary-Veterinary and Food Safety Authority of Romania through the national food safety survey and control Programme.

The sampling was performed by assigned and trained specialists, who operated

according to the provisions of the European Commission in 2004 (Commission Recommendation of 4 October 2004 on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003) and respectively according to the Order no**.** 94/2006 of ANSVSA on the approval of guidelines regarding the sampling and analysis of OMGs, of the products which contain or might contain OMGs**.**

National Sanitary-Veterinary and Food Safety Authority of Romania establishes on a yearly basis by the 2008 Food Safety Survey and Control Programme for each county, the number of samples being collected, their category and the DSV laboratory where the analyses regarding the OMGs detection are performed.

## RESULTS AND DISCUSSION

The results obtained after the examination of the analyzed samples are shown in table 3.

*Table 3.* The results of the GMOs detection in the soybean and soybean-derived products samples examined in the lab

| No. | Sample type | Total examined samples | Of which | |
|---|---|---|---|---|
| | | | Total | % |
| 1 | Textured from granular soybean | **11** | **1** | **9** |
| 2 | Textured from soybean cubes | **5** | **0** | **0** |
| 3 | Textured from soybean slices | **5** | **0** | **0** |
| 4 | Textured from powder soybean | 7 | 1 | 14.2 |
| 5 | Proteinic concentrate of soybean powder | 8 | 1 | 12.5 |
| 6 | Proteinic isolate of soybean powder | 8 | 1 | 12.5 |
| 7 | Proteinic flour deri-ved from soybean | 1 | 0 | 0 |
| 8 | Soybean sauce | 1 | 0 | 0 |
| 9 | Soybean flour | 6 | 6 | 100 |
| 10 | Soybean grains | 2 | 1 | 50 |
| Total of samples | | 54 | 11 | 21.3 |

From the above table, we may conclude that of the 54 analyzed samples, 11, respectively 21.3% have shown a positive result to the qualitative PCR reaction (screening) for the detection of GMOs, respectively of the P35S Promoter and/or of the NOS terminator.

If we consider each positive sample we should mention that for three of the samples (textured from soybean, soybean concentrate and soybean proteinic isolate) the positive result was also confirmed by the Real Time PCR test, but at the quantitative determinations, the GMO concentration in the sample was much below the limit admitted by the legislation, respectively below 0.9%, so that no measures were necessary.

Regarding the soybean meal and soybean beans positive samples, these were collected in the Fodder Production Units (FPU) using almost entirely and permanently the GM soybean imported from Brazil, USA, China etc., legally and with all the documents required by the legislation. However, this product was used exclusively in feeding the animals.

The textured sample from granular soybean is still under debate, which is correctly and tightly sealed-packed in aluminium plastic bag labelled in Romanian language, collected in a general store and of import origin. During the quantitative PCR test on the respective sample, the presence of OMG in a 2% concentration was confirmed. Obviously, the sanctions required by law were applied, respectively the 500 to 2,000 lei (RON) fee, according to the Governmental Decision no. 173 of 9 February 2006 on the traceability and labeling of genetically modified organisms and the traceability of animal food obtained of genetically modified organisms, as the bag was not labeled, according to article 10, point b. Also, the Governmental Decision no. 106 of 07.02.2002 regarding the labelling of the food products and the Regulation (EC) No. 1830/2003 forbade the selling of the unlabelled products.

One cannot question thus the infringement of the Regulation (CE) no.

1829/2003 of the European Parliament and of the Directive 2001/18/EC.

However, we should point out the fact that although a moratorium on the GMOs cultivation was established in Europe, the EU imports yearly tens of tons of soybean (approx. 40-50 tones per year) from the large producing countries of genetically modified soybean and that the OMG products or containing GMOs are allowed in markets without any restrictions unless they exceed the 0.9% OMG threshold value or these are permitted for conditional use, that is if they are labelled. On the other hand, the product on shelf may be consumed before receiving the results of the test, which means that the merchandiser will be fined. Of course, this situation complicates and impedes the efforts against the spreading, cultivation and consume of GMOs in Europe.

## CONCLUSIONS

54 samples of soybean and soybean-derived products were examined in order to detect the presence of GMOs. A qualitative method was used for detection, as only the presence or absence of GMOs was established in the sample.

The minimum detection limit for the kit used is 0.1% (compared to 0.9% which represents the maximum admitted value). Of the total of 54 samples examined, a number of 11 samples proved to be positive, respectively 21.3%. For several categories of samples, their low number does not enable a significant statistical conclusion.

One must notice the high percentage of the genetically modified soybean samples related to the soybean flour and the soybean grains.

Due to the fact that the problem of GMOs is extremely controversial we believe that public debates would be needed to approach the multiple issues involving the use and cultivation of GMOs.

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