Romanian Biotechnological Letters *Vol. 14, No. 5, 2009, pp. 4733-4746*

ORIGINAL PAPER

Detection and quantification of GMO and sequencing of the DNA amplified products

Received for publication, March 20, 2009
Accepted, October 5, 2009

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Abstract

As a member of the European Union since 2007, Romania must comply with the rules for placing on the market, traceability and labeling of GMOs as laid down in EU legislation. For this reason, beginning with 2007, Roundup Ready soybean cultivation was banned and the only crop approved for import and cultivation in EU, MON810 maize, was grown on about 300ha. In 2009, MON810 hybrids were cultivated on about 3000 ha. The objective of this work was to assess, in 2009, the soy derived food products on the Romanian market for compliance with labeling legislation using a conventional qualitative Polymerase Chain Reaction (PCR) assay to detect the presence of GM soy and a real-time PCR to quantify the amount of RR soy present in positive samples. Additionally, DNA amplified fragments present in RR soybean and in MON 810 hybrids were sequenced for assessment the nucleotide sequences homology with nucleotide sequences of the same fragments existing in databases. Based on our results, the transgenic Roundup Ready® soybeans are present in a few foods products commercialized in the country. Results of real time PCR quantification evidenced that only five of the fifty quantified samples presented more than 1% of GM DNA in its composition. Since the year 2007, the frequency of food and feed products containing GM soybean in Romania is continuously decreasing. Results of the search in the GenBank for nucleodide sequences homology between DNA amplified fragments (amplicons) from reference lectin gene and for 35S promoter and nos terminator and nucleotide sequences of the same fragments existing in database revealed 100% similarity. For MON810 maize hybrids, the nucleotide sequences homology between the cryIAb and invertase gene amplicons and nucleotide sequences of the same fragments existing in the GanBank were 97.34% and, respectively, 93.8%. Results of our analysis revealed point mutations produced at the level of the amplified regions.

Keywords: food products, seeds, RR soybean, Bt maize, polymerase chain reaction (PCR), real time PCR, sequencing.

1. Introduction

Romania adopted its initial legislation on bio-engineered products in 2000. Based on the new legal framework, the biotech industry was encouraged to enter the Romanian market with products already approved elsewhere. Among the first were the applications submitted by Monsanto, for Roundup Ready soybean cultivation. This biotech crop developed to allow

for the use of glyphosate, the active ingredient of the herbicide Roundup, as a weed control agent, was approved for commercial cultivation at national level.
Roundup Ready soybean (event 40-3-2) was approved for marketing in EU. The decision allowed for the importation of seed into EU for industrial processing into non-viable products including animal feeds, food and any other products in which soybean fraction are used, only. In Romania, herbicide tolerant soybeans (Roundup Ready, RR) were grown commercially beginning with 2000 and accounted for 68% (or, in absolute figures, 137 thousand hectares) of all soybeans planted in 2006 (OTIMAN et al., 2008[1]). In that time, Romania represented one of the nine countries in the world that cultivated RR soybean (JAMES et al., 2007[2]). RR soybean cultivation was banned beginning with January 2007 because as Member State, Romania can commercially grow genetically modified plants approved for market release in EU. With no access to the RR technology, the soybean acreage had started to decline in 2007, reaching 133,200 ha, while in 2009 only 68,200 ha were planted with this crop. Romania is currently increasingly dependent of soybean imports (site of Ministry of Agriculture accessed at 8 August 2009)

MON810 is a genetically modified maize line which is resistant to the European Corn Borer. The event was authorized by the United States in 1996 and has been approved for import and cultivation in Europe since 1998. In 2008 this maize event was cultivated in seven EU countries (Spain, Germany, Czech Republic, Portugal, Slovakia, Poland, Romania) (JAMES et al., 2007[2]). As EU Member State, Romania can commercially grow genetically modified plants approved for market release in EU. In 2007, MON810 was cultivated on about 300ha and in 2009, on about 3000 ha.

In many countries, the labeling of grains and feed - and foodstuffs is mandatory if the genetically modified organism (GMO) content exceeds a certain level of approved GM varieties. For instance, the European Union (EU), have set threshold values at 0.9% (EU Regulation No. 1830/2003), of GMO material in a non-GM background as the basis for labeling. The enforcement of these threshold values has created a demand for the development of reliable GMO analysis methods of a rapid and inexpensive character. Most of the established analytical methods for detecting the GMO identification and quantification in foods are based on the polymerase chain reaction (PCR), due to its sensitivity, specificity, and applicability to the analysis of complex food matrices.

The objective of this work was to determine the compliance with labelling legislation of commercially available soybean derived products in Romania using a conventional qualitative Polymerase Chain Reaction (PCR) assay to detect the presence of RR soy and a real-time PCR to quantify the amount of RR soy present in positive samples. We also verified the stability of the nucleotide sequences targeted by pairs of primers used for detection and quantification both for RR soybean and MON810 events.

2. Materials and Methods

2.1. Samples and reference material

Samples of the major brands of soy-derived food products sold commercially on Romania market were collected. Fifty unlabelled products containing soy such as baby food and diet products, soy drinks and desserts, tofu and tofu products, soy based meat substitutes (fibber, granule, schnitzel) soy protein, flour as well as yogurt were collected and analyzed. All soy-based food products analyzed were made by Romanian companies.

Certified reference material (CRM), soybean powder containing 0.1 to 5.0% Roundup Ready® soybean (IRMM), was used for qualitative and quantitative analyses. RR soybean grains were also used as a control sample. RR soy seeds were kindly provided by the Department of Phytotechnics from Agricultural University Timişoara.

Preparation of test samples. To prepare GM mixed test samples, soybeans (GM seeds and solid soy-derived products) were separately milled to a fine powder using grinders (Retsch, Haan, Germany).

Maize seeds were sampled from a Bt field at harvesting.

2.2. DNA extraction

DNA extraction and purification from raw material and from food products of plant origin was carried out using the High Pure GMO Sample Preparation Kit (Roche) according to the manufacturer's manual.

2.3 Yield and quality of nucleic acid extracts

Extracts were assessed for total DNA yield and purity using spectrophotometer. Further, gel electrophoresis was used to assess genomic DNA quality. The presence of inhibitors in the PCR reactions was determined by amplifying a portion of the soybean lectin gene used as an endogenous reference using the plant-specific primer pair GMO3/GMO4 (MEYER et al., 1996[3]; HUBNER, et al., 1999 [4]).

For maize MON810, the primers Zein3/Zein4, for zein gene (MATSUOKA et al., 2000 [5]), and the primers Ivr1/ Ivr2, for the invertase gene**,** which encodes acid-soluble isoforms, were used (Xu JM at al., 1996 [6]).

2.4 *Qualitative PCR analysis of transgenic sequences*

Screening targets commonly used for RR soybean are promoter and leader for the cauliflower mosaic virus (CaMV) *35S* RNA containing the duplicated enhancer region (p35S) and 3' transcript termination sequence of the nopaline synthase (*nos*) coding sequence from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation (t nos). For detection RR soy GMO specific primers for P-e35S promoter & T-nos terminator sequences were used.

MON810 maize was detected using the gene- and event- specific primers: for cry1Ab gene sequence and, respectively, for P-e35S promoter (ZIMMERMANN et al., 1998 [7]).

Primers were synthesized and purified by TibMolBiol and the sequences are listed in Table 1.

Table 1. Sequences of primers used and the size of amplicons obtained

Primer	Sequence (5'−3')	Target or Reference Gene	Length of Amplified Fragment
Roundup Ready Lectin			
GMO3	GCCCTCTACTCCACCCCCATCC	Soya lectin gene	118bp
GMO4	GCCCATCTGCAAGCCTTTTTGTG		
35S promoter			
35S1	GCTCCTACAAATGCCATCA	CaMV (Soya specific)	195bp
35S2	GATAGTGGGATTGTGCGTCA		
***nos*-terminator**			
HA-nos 118-f	GCATGACGTTATTTATGAGATGGG	*NOS* terminator	118bp
HA-nos 118-r	GACACCGCGCGCGATAATTTATCC		
Maize MON810			
ZEIN3	AGTGCGACCCATATTCCAG	Zein maize gene	277bp

ZEIN4	GACATTGTGGCATCATCATTT		
CRYIA1	CGGCCCCGAGTTCACCTT	cryIA(b)	189bp
CRYIA2	CTGCTGGGGATGATGTTGTTG	Bt-Maize specific	
CRYIA3	CCGCACCCTGAGCAGCAC		
CRYIA4	GGTGGCACGTTGTTGTTCTGA		
Ivr1F	CCGCTGTATCACAAGGGCTGGTACC	Ivr-(Maize invertase)	226bp
Ivr1R	GGAGCCCGTGTAGAGCATGACGATC		
Vw01	TCGAAGGACGAAGGACTCTAACG	Zea Mays (Maize)	170bp
Vw03	TCCATCTTTGGGACCACTGTCG	insect resistant	

Amplification reactions were performed in a thermocycler model ICycler BIO-RAD, in a final volume of 20 µL 20µl with 5 µl DNA solution. Concetration of reagents/ reaction: 0.0025 U Ampli GoTaq Polymerase- Promega, 2.5mM $MgCl_2$, 0.2mM dNTP mix, buffer 10x for polymerase and 0.5µM of each primer (Van DUIJN, G., et al., 2002[8]).Conditions for all the amplification are given in Table 2.

Table 2. PCR conditions used for amplification

Primers	Denaturation	Amplification	No. of cycles	Final extension
35S1/ 35S2 HA-nos118- f/HAnos118-r	3 min - 95^{0}C	25s -95^{0}C; 30s -62^{0}C; 45s - 72^{0}C	50	7 min - 72^{0}C
GMO3/GMO4	3 min - 95^{0}C	30s - 95^{0}C; 30s - 63^{0}C; 30s - 72^{0}C	40	3 min - 72^{0}C
ZEIN3/ZEIN4	3 min - 95^{0}C	1min - 96^{0}C; 1min - 60^{0}C; 3min - 72^{0}C	40	3 min - 72^{0}C
IVR1/IVR2	3 min - 94^{0}C	45s - 94^{0}C; 45s - 60^{0}C; 35s - 72^{0}C	45	7 min - 72^{0}C
VW01/VW03	12 min - 95^{0}C	30s - 95^{0}C; 30s - 64^{0}C; 30s - 72^{0}C	40	10 min - 72^{0}C
cryIA1/cryIA2 cryIA3/cryIA4	3 min - 95^{0}C	40s - 95^{0}C; 40s - 60^{0}C; 40s - 72^{0}C	25	3 min - 72^{0}C

The amplified products were analyzed by electrophoresis on on a 2.5% agarose gel (100V for 60min) containing ethidium bromide in TBE buffer (10mM Tris, 2.75g boric acid/l, 1mM Na_2 EDTA).

2.5. Real-time PCR

Real-time PCR technique allows us to monitor the amplification reaction (in real-time) in a closed environment without interfering with the reaction. Fluorescence signal corresponding to increased amount of amplification product can be measured and visualized on a computer screen. By software the signal can be immediately convert into quantitative estimation. With the fastest available alternative quantitative results can be made available within approximately 30 min from the beginning of the first PCR cycle. In comparison, competitive PCR would require at least 3h, usually more.

For quantitative PCR we used foodproof GMO soy Quantification Kit from BIOTECON Diagnostics and LightCycler 2.0 Real Time equipment.

2.6. Sequencing

The products that were positive after conventional PCR analysis were subject to sequencing, after purification with Wizard PCR Preps DNA Purification System (Promega). The same primers used for PCR amplification were labelled with BigDye (ABI PRISM ® BigDye ™ Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems). The products were separated by gel electrophoresis and analyzed at ABI PRISM 3130 DNA amplification system (Applied Biosystems). The sequences were aligned with the once from GenBank using the CLUSTAL W multiple alignment application software (BioEDit software), that was used for designed primers.

2.7 Data Collection and Analysis

The percentage of GM in different samples was determined by relative quantification using standard curve method plotted Ct (cycle threshold) or delta Ct values as a logarithm function of the GM percentage or copy numbers.

3. Results and Discussions

DNA extraction from soybean food products

The samples analyzed included a range of soybean products, from the very low processed such as the flour, to those highly processed such as tofu, soy milk, soy drinks and desserts. DNA isolated from all soy products by High Pure GMO Sample Preparation Kit (Roche), with slight modifications, was of good quality for PCR amplification. Heating and fermentation processes does damage DNA template for PCR reaction, but PCR generating the smaller DNA fragment still can detect the presence of DNA (Jeng et al., 2003)

The results of amplification of an endogenous gene for lectin confirms that all samples contained soybean (Figure 1)

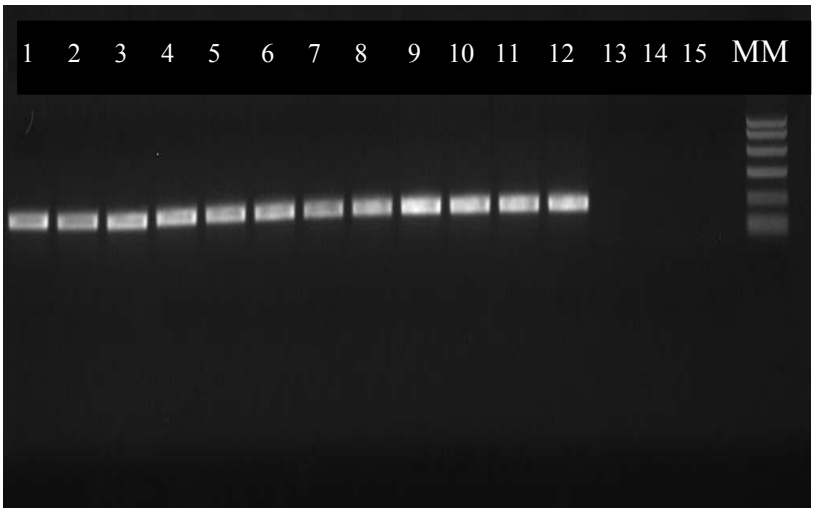

Figure 1 Agarose gel electrophoresis for lectin PCR products from soy based meat substitutes: 1-10; 10 different brands of meat substitute products; 11- GM soybean seed; 12 - 5% CRM; 13 - EB (extraction buffer); 14 –EC (environmental control); 15 – NTC (PCR control); 16- Marker PCR

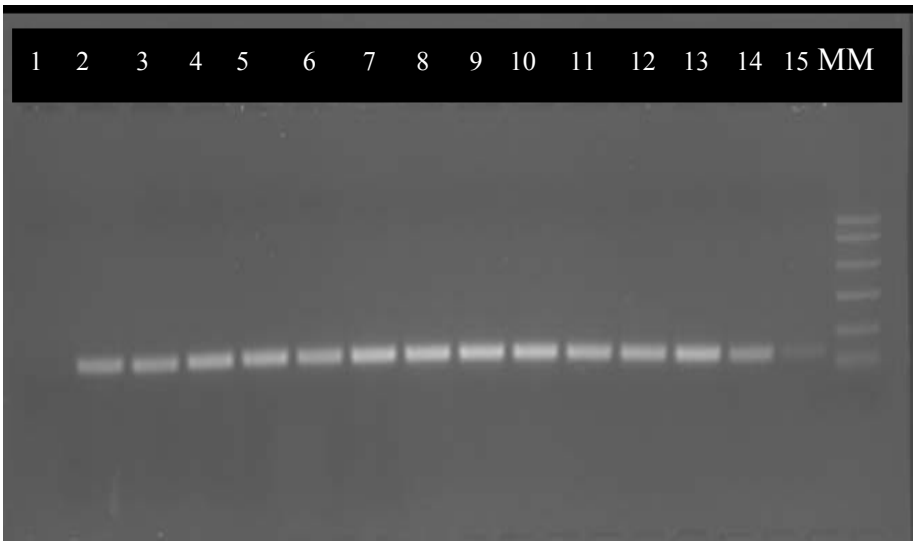

Figura 2 Agarose gel electrophoresis of the PCR products for lectin gene, from soy based products: 1-2 soy mayonnaise; 3-4 soy dessert; 5- 6 soy yogurt; 7- 10 soy beverages; 11-12 – smoky tofu; 13- 5% CRM ; 13 -EB (extraction buffer); 14 – Negative control; 15 –. Marker PCR

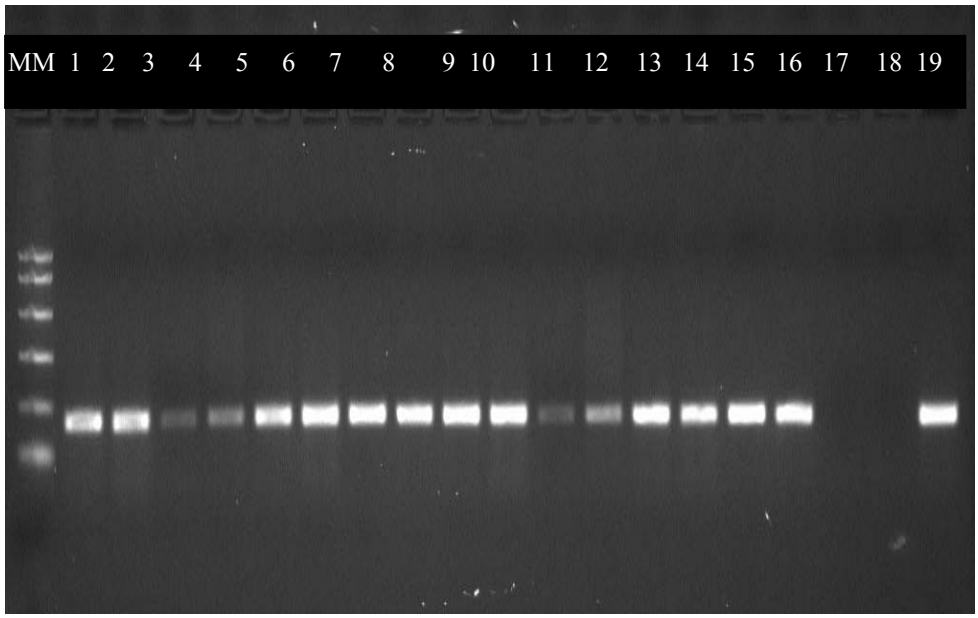

Figure 3. Agarose gel electrophoresis of the PCR products for lectin gene, from soy based products (118 bp). 1- Marker PCR; 2-15 different brands of vegetal pate; 16-17 GM soybean seeds; 18 -EB (extraction buffer); 19 – EC (environmental control); 20 - 5% CRM.

Qualitative and quantitative PCR analysis of transgenic sequences

Amplification with primers for the 35S promoter produced the DNA fragment of 123 bp confirming the presence of RR soy in some samples, as illustrated in Fig. 3a, (lines 2, 3, 4 and 6). Additionally, amplification of *nos* terminator produced the DNA fragments of 118 bp in the same samples (Figure 3b, lanes 1, 2, 3, 5 and 6).

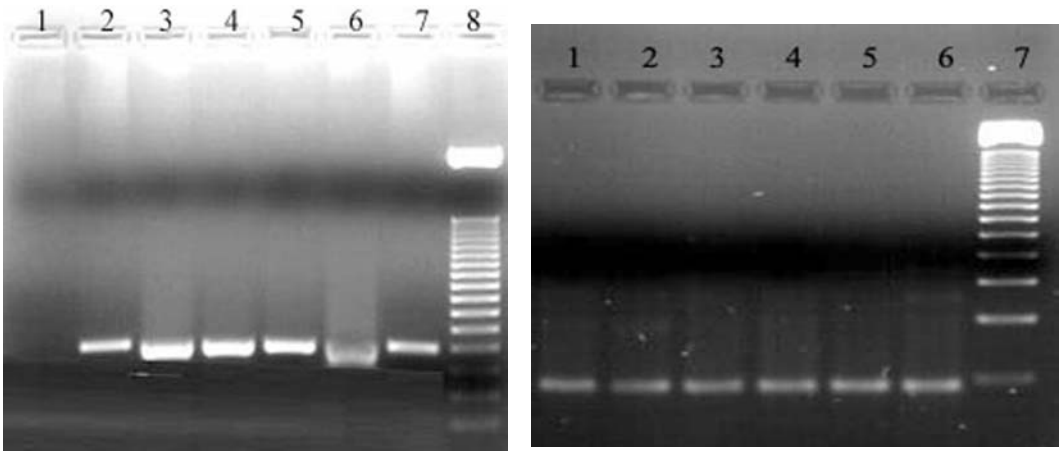

Figure 4. a. PCR products obtained for the ***promoter p35S*** -123 bp, from soy samples: lane 1- negative control; lane 2 - fibre; lane 3 - granule; lane 4- schnitzel; lane 5- seeds; lane 6- flour; lane 7- granule from another producer; lane 8-MM 50bp; **b.** PCR products obtained for ***terminator (tnos)*** - 118 bp, from soy samples: lane1- fibre; lane 2- granule; lane 3- schnitzel; lane 4- seeds; lane 5- flour; lane 6- granule from another producer; lane 7- MM 100bp.

The results for the fifty analyzed samples are presented in Table 3. The presence of the GMO was demonstrated in 5 cases. The positive samples were soy based meat substitutes: fiber (1), granule (1), flour (1), Schnitzel (1) and flour (1).

Table 3. Results of GMO analysis of different soy - derived food product sold on Romanian market

Products	Number of samples analyzed	Number of samples positive for lectin gene	Number of samples with GM content < 1%	Number of samples with GM content > 1%
Soy beverages	5	5	5	0
Soy milk	10	10	10	0
Soy flour	4	4	5	0
Vegetal pate	7	7	7	0
Feed for chicken	1	1	1	0
Texturized soy protein	8	8	8	0
Soy desserts	5	5	5	0
Tofu	5	5	5	0
Fiber soy	1	1	0	1
Soy granule	1	1	0	1
Soy flour	2	2	0	2
Soy Schnitzel	1	1	0	1
Total	**50**	**50**	**45**	**5**

GM positive soy-containing samples were analyzed by RR soy event-specific real-time PCR.

The actual calculation is performed by the LightCycler Relative Quantification Software, applying the following basic calculation steps: determination of the relative ratio of Roundup; the amount of Roundup Ready soya specific target DNA is expressed as a relative ratio to a soya reference gene, which is normalized to a calibrator.

This procedure provides maximum reproducibility and controls for factors influencing quantification. The calculation of the relative GMO content is based on the resulting crossing points of one particular sample, and the efficiency of the PCR. The amount of Roundup Ready soya is calculated as a ratio of Roundup Ready soya DNA to total soya DNA. The ratio of Roundup Ready soya DNA to total soya DNA of the sample is divided by the GMO: reference ratio of the Calibrator DNA which is run in parallel with each sample reaction.

This procedure compensates for constant differences between the GMO and reference gene during detection in the LightCycler Instrument (e.g., Probe annealing, extinction coefficients of dyes, efficiency of FRET process), and provides a constant calibration point between PCR runs. The final result is expressed as a ratio of GMO:reference in the sample, relative to the ratio of GMO:reference in the Calibrator DNA. The ratio of GMO: reference in the foodproof GMO Soya Calibrator DNA provided with the kit has a value of 1.00

Five sample contained RR soy over the 0.9% threshold limit. The results demonstrated the presence of RR soybean in a few processed soy derived products commercially available in Romania (Figure 5).

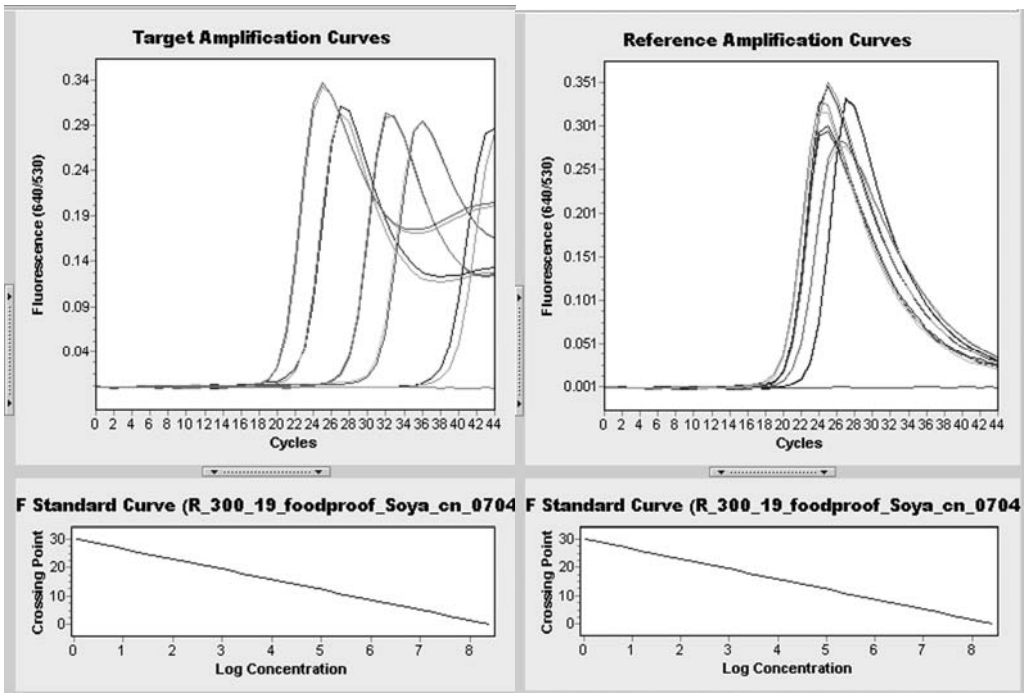

Figure 5. Real Time PCR quantification for Roundup Ready Soybean products.

The ratio of GMO: reference in the foodproof GMO Soya Calibrator DNA provided with the kit has a value of 1.00 (Table 4).

Table 4. Real –Time PCR quantification condition used in our study

Sample number	Sample name	Normalized Concentration ratio	% Normalized concentration
1	Soya Fiber	0.9	90
2	Soya Granule	0.56	56
3	Soya Schnitzel	0.19	19
4	Seeds	1	100
5	Soya flour	0.19	19

Our data confirms the conclusion that on Romanian market RR soy containing food and feed products is continuously decreasing (LEAU F. et al., 2008 [9]). On the other hand, the intensively decreasing rate of non-compliance reveals that food producers have followed the provisions of labeling legislation for genetically modified food.

Sequences analysis of the amplicons derived from RR soy containing food products

The results of the search for nucleotide sequences homology of the amplified fragments from the five genetically modified soybean samples obtained with primers for lectin gene (Figure 6), for *t-nos* (Figure 7) and for p 35S promoter (Figure 8) have revealed 100% homology with the nucleotide sequences of the same genetic elements published in the GenBank. The lectin gene is the reference gene while the CaMV35S promoter and the *t-nos* terminator are the targeted genetic elements. The integrity of the nucleotide sequences of the soybean DNA in samples of raw, partially processed and highly processed food may be explained by the small size of the DNA amplified. However, studies regarding the effect of food matrices on the stability of DNA, revealed that DNA fragments of up to 957 bp in maize products and 714 bp in highly processed soy products are still present (BAUER et al., 2003[10]).

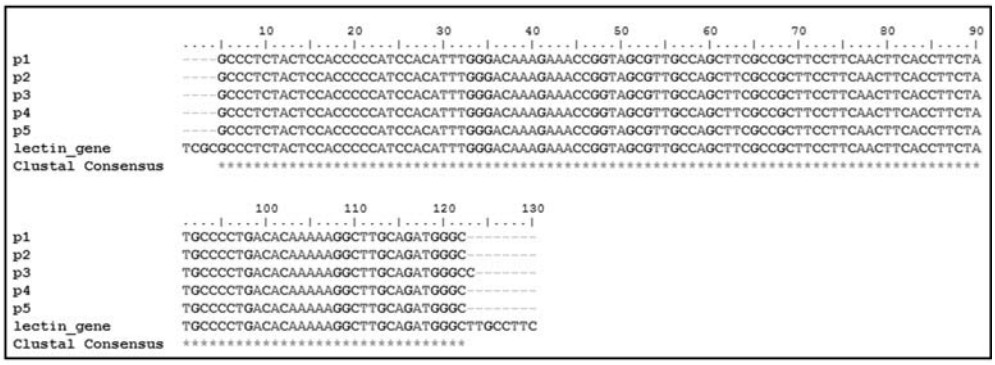

Figure 6. Alignment with the BioEdit software (the CLUSTAL W application) of the sequences for the *lectin* gene (five samples). The analysis was carried out by comparison with the gene sequences in the GenBank data base.

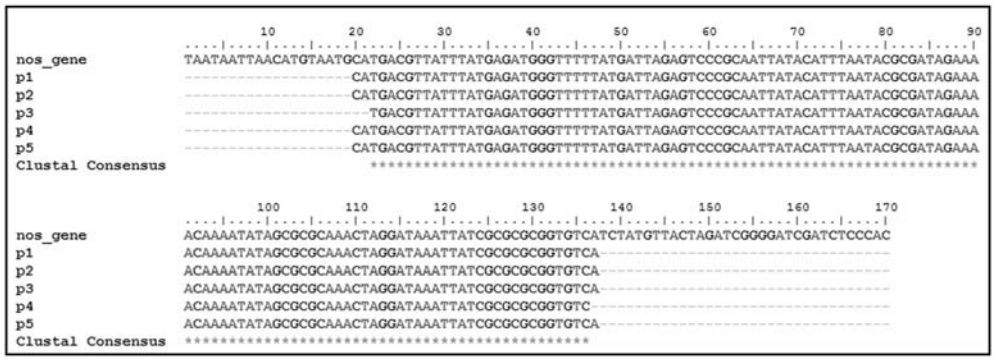

Figure 7. Alignment with the BioEdit software (the CLUSTAL W application) of the sequences for the *t-nos* terminator region (five samples). The analysis was carried out by comparison with the gene sequences in the GenBank data base.

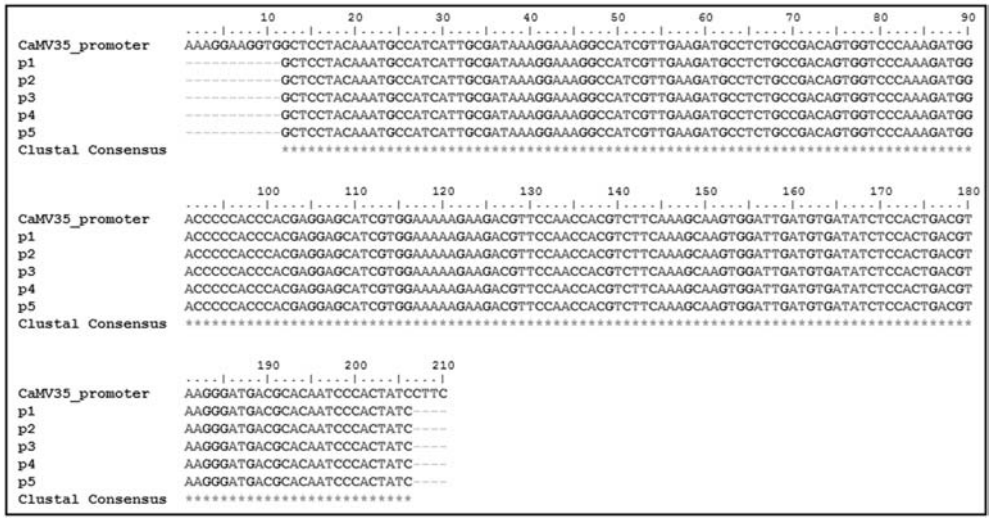

Figure 8. Alignment with the BioEdit software (the CLUSTAL W application) of the sequences for the *CaMV35* promoter region (five samples). The analysis was carried out by comparison with the gene sequences in the GenBank data base.

Analysis of Maize seeds MON810

For maize MON810 the targeted fragments were gene- and event specific, *cryIAb* and, respective, the E35S promoter and the resulted amplicons were of 189 bp and 170bp (Figures 9,10).

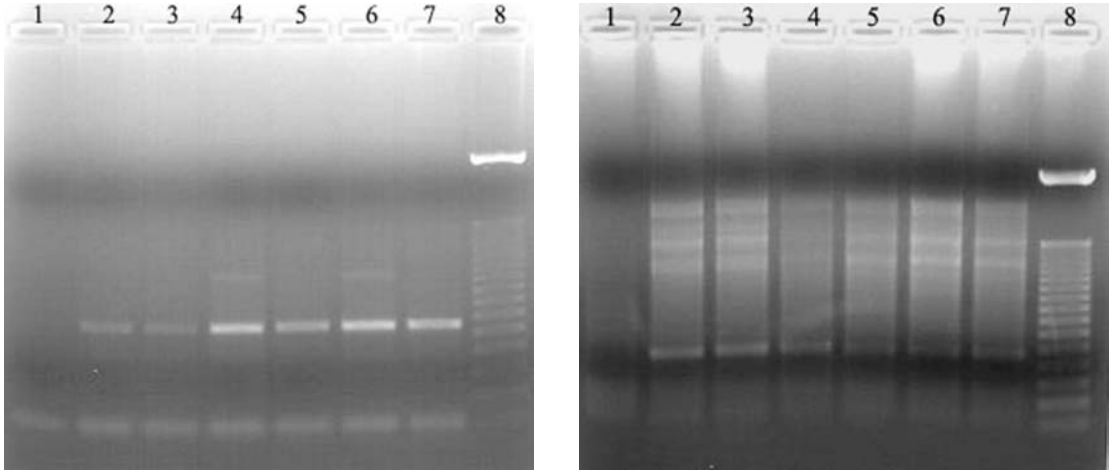

Figure 9 a. PCR products obtained for ***zein*** *gene* (277 bp) from maize seeds samples: 1- negative control; 2-7 maize from different hybrids; 8.MM 50bp; **b.** PCR products obtained for maize ***invertase*** *gene* (226 bp) from maize samples: 1- negative control; 2-7 maize from different hybrids; 8.MM 50bp.

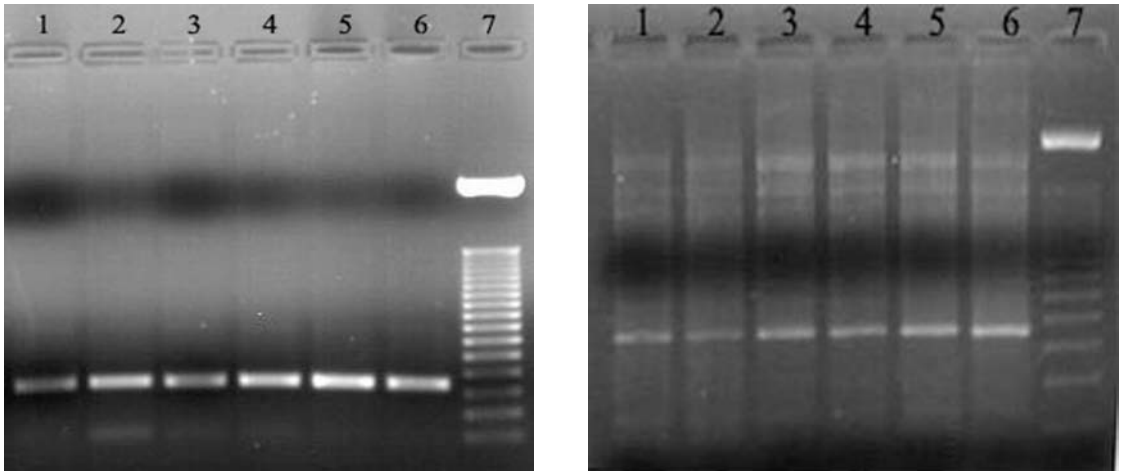

Figure 10. a. PCR products obtained for the ***P-E35S*** *gene* (170 bp) from maize seeds samples: 1-6 maize seeds from different producers; 7.MM 50bp; **b.** PCR products obtained for the ***cryaIA(b)*** *gene* (189 bp): 1-6 maize seeds from different producers; 7.MM 50bp

The zein and invertase fragments were observed for all maize samples (Figure 9a, 9b). Additionally, all maize samples were positive for the presence of MON810 event (Figure 10a, 10b)

The nucleotide sequences obtained for the E35S amplicon (Figure 11) and for the zein gene region (Figure 12) had 100% homology with the sequence published in the GenBank.

The nucleotide sequences for the invertase gene (Figure 13) and for the *cryIAb* amplicon (Figure 14) had 93.8% and, respectively, 97.34% homology with the sequences published in the GenBank.

Sequences analysis of the amplicons obtained from Maize seeds MON810

```
                                10        20        30        40        50        60        70        80        90
                                |         |         |         |         |         |         |         |         |
E35S_promoter                   CAAGGGAAATAATGCTTCGAAGGACGAAGGACTCTAACGTTTAACATCCTTTGCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATA
p1                              ----------------TTCGAAGGACGAAGGACTCTAACGTTTAACATCCTTTGCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATA
p2                              ----------------TTCGAAGGACGAAGGACTCTAACGTTTAACATCCTTTGCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATA
p3                              ------------------CGAAGGACGAAGGACTCTAACGTTTAACATCCTTTGCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATA
p4                              ----------------TTCGAAGGACGAAGGACTCTAACGTTTAACATCCTTTGCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATA
Clustal Consensus                               **********************************************************************

                                100       110       120       130       140       150       160       170       180
                                |         |         |         |         |         |         |         |         |
E35S_promoter                   GTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCC---
p1                              GTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
p2                              GTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
p3                              GTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
p4                              GTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAA
Clustal Consensus               **********************************************************************************

                                ....|..
                                |   |
E35S_promoter                   -------
p1                              GATGGAA
p2                              GATGGAA
p3                              GATGGAA
p4                              GATGGAA
Clustal Consensus
```

Figure 11. Alignment with the BioEdit software (the CLUSTAL W application) of the sequences for the *promoter E35S* (six samples). The analysis was carried out by comparison with the gene sequences in the GenBank data base.

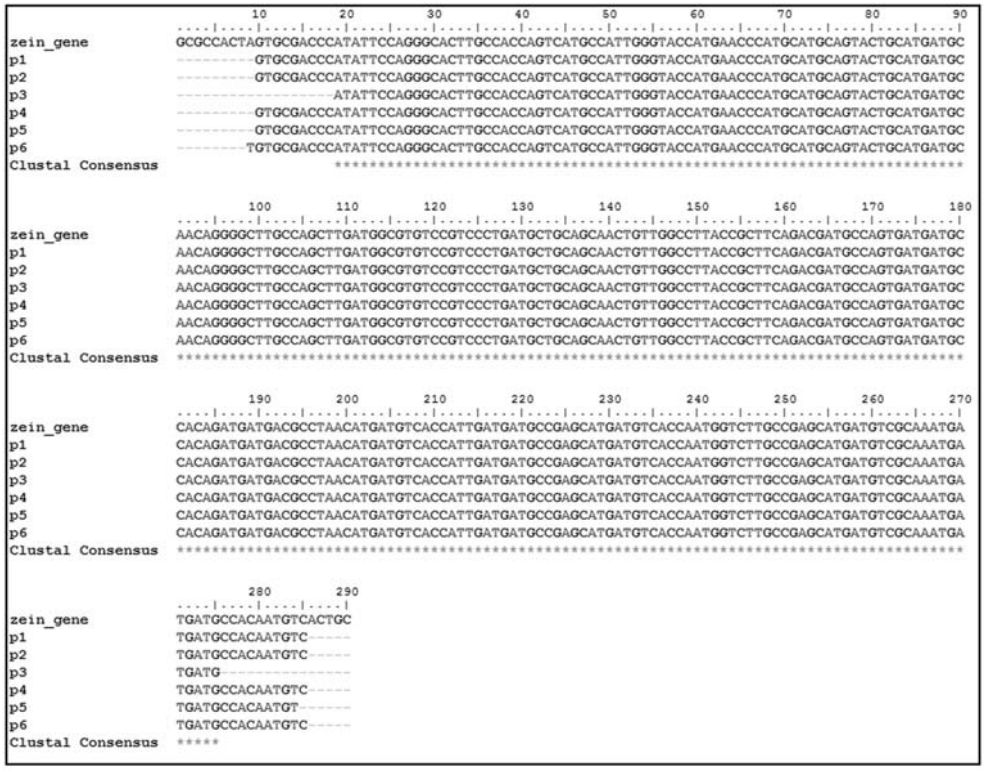

Figure 12. Alignment with the BioEdit software (the CLUSTAL W application) of the sequences for the *zein* gene (six samples). The analysis was carried out by comparison with the gene sequences in the GenBank data base.

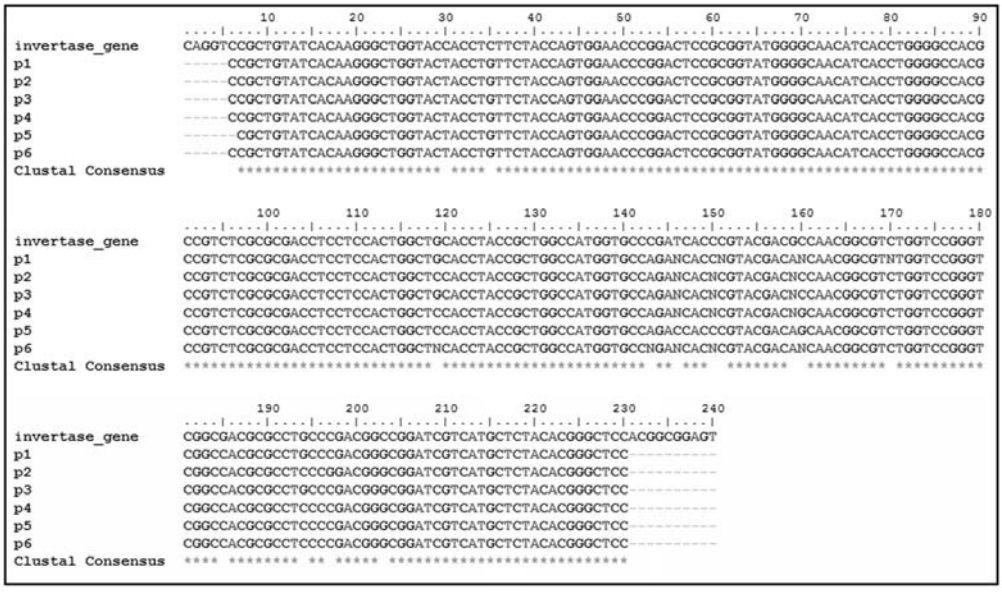

Figure 13. Alignment with the BioEdit software (the CLUSTAL W application) of the sequences for the *invertase* gene (six samples). The analysis was carried out by comparison with the gene sequences in the GenBank data base.

Observation: Heterozygous plants are presented as having nucleotides marked with N. This may refer to any of the nucleotide types located at the level of that particular site. In the alignment, the N nucleotide in the 150, 151 and 170 positions may be either C or G.

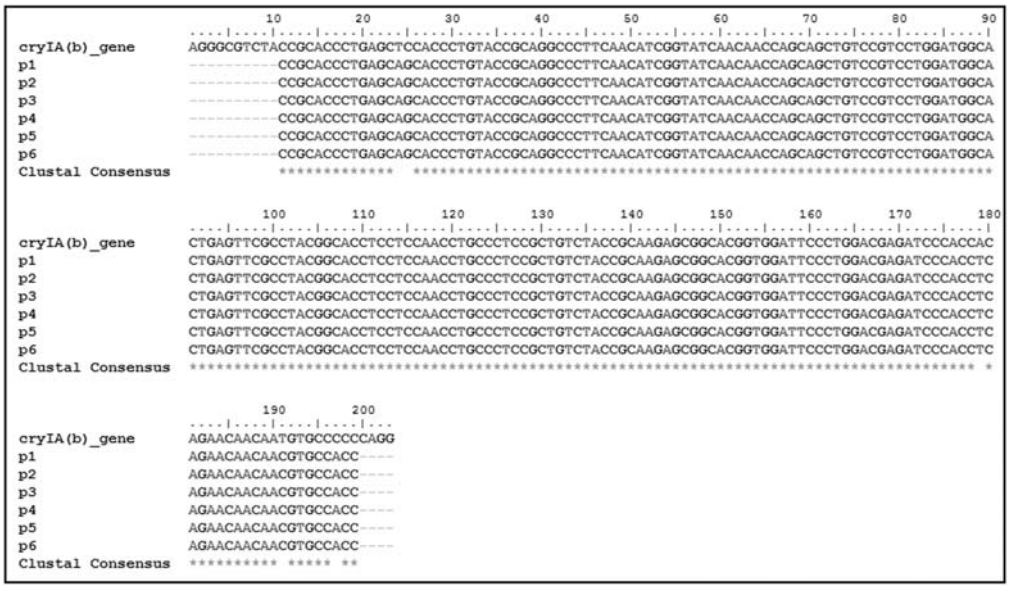

Figure 14. Alignment with the BioEdit software (the CLUSTAL W application) of the sequences for the *cryIA(b)* gene (six samples). The analysis was carried out by comparison with the gene sequences in the GenBank data base.

Results of our analysis revealed point mutations produced at the level of the regions amplified. There are several conflicting reports on transgene stability in MON810 maize. HERNANDEZ et al. [11], (2003) and CHANDRA et al., (2007) [12] evidenced a truncation event MON810 at the 3' end of the *cryI(A)b* gene with the complete loss of the NOS terminator. The authors recommend use of the sensitive detection and quantification tests that would enable monitoring of transgene stability over successive generations and would provide information on the effect of the genomic background on the DNA insert stability.

Single nucleotide polymorphism (SNPs) occurs in the plant genome as a results of plant- environmental interactions and are part of the normal life cycle of the plant (MADLUNG&COMAI, 2004[13]). The effect of a minor modification such as a single base pair mismatch in the primer attachment site on the real time PCR amplification and GMO quantification was studied (GHEDIRA et al., 2008[14]). The presence of a single base pair mismatch between the primer and DNA template resulted in a statistically significant shift of the Ct value.

During transgene inheritance, deletion, duplication, rearrangement and repeated sequence recombination for transgenic loci have been reported (YIN et al., 2004[15]). However, the functional stability of the truncated MON810 transgene, was confirmed by detection of Cry1Ab protein by a ELISA assay (data not shown).

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