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STUDY ON INCIDENCE OF GENETICALLY MODIFIED PLANTS LIKE RAW MATERIALS, IN ROMANIA, DURING 2012 - 2017

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Abstract

The paper aimed to present the distribution of the incidence of genetically modified vegetable like raw materials in Romania, during the period 2012 - 2017, including Calarasi, Iasi, Salaj, Satu-Mare, Suceava, Tulcea, Constanta, Bucuresti counties. It is based on the statistical data provided by National Sanitary Veterinary and Food Safety Authority, submitted by specialized molecular biology laboratories. The data have been processed into the following indicators: species, type of material or product, type of detection and identification method, GMO's specific genetic structures and transformation events identified, degree of transformation compared to the European Union rate of 0,9% for necessary labeling. During the analyzed period, a number of samples ranging from 200 to 300 were analyzed annually. The raw material samples were grains, groats, flours from genetically modified plants, as well as finished products of food or feed, like biscuits, chocolate, bakery products. For the analyzed samples, genetic transformation processes were detected, but without exceeding the limit of 0.9% and not labeled as GMO. As a conclusion, on the Romanian market are safe genetically modified foods and feeds and the traceability and labeling of GMOs are respected.

Key words: *distribution, GMO, raw materials, method, Romania.*

INTRODUCTION

It is well known that the genetically modified food or feed contains as whole or in part genetically modified organisms (GMO) and for this reason special regulation need to be respected. GMO means an organism that possesses foreign genes obtained through the use of modern biotechnology (i.e. genetic engineering) (Cornea, 2010).

European Union legislation lists the conditions for the commercialization of such products through intra-Community trade. Well established traceability and labelling procedures for all genetically modified organisms must be complied in order to provide to consumers the right information and free choice.

The following regulations are the basic legislative framework adopted at European Union level in the field of Genetically Modified Organisms:

• Council Directive no. 2001/18 / EC on the deliberate introduction into the environment of genetically modified organisms;

• Regulation of the European Parliament and of the Council no. 1829/2003 on genetically modified food and feed;

• Regulation of the European Parliament and of the Council no. 1830/2003 on the traceability and labelling of genetically modified organisms and the traceability and labelling of food and feed products, produced from genetically modified organisms.

At European level, the leading role is given to the scientific opinions of the European Food Safety Authority (EFSA) on genetically modified organisms that are issued as a result of the risk assessment which genetically modified organisms may have for public health, animal health and environmental protection. The risk assessment show that for certain conditions of use initially established, the genetically modified product is safe. Procedures of evaluation and authorization of genetically modified organisms are transparent and not have the time limit. (https://www.ansvsa.ro, November 2017)

The risk assessment is based on scientific information at international level, based on well-defined criteria.

(https://www.efsa.europa.eu/en/science/scientific-committee-and-panels, November 2017)
The scientific opinions of the EFSA detail the scientific information analysed, which led to the approval of the marketing of genetically modified products on the European market. 58 genetically modified plants (containing GM events) are authorized for marketing in the member states of European Union. (https://www.efsa.europa.eu/en/science/gmo, November 2017)
Generally, the methods to obtain the transgenic plants are based on the use of *Agrobacterium* system (Cornea, 2010; Kado, 2015; Parmar et al., 2017).
The scientific publications with a major impact on the safety of GMOs authorized by EFSA, are permanently monitored. Until now, the conclusions of the approved opinions have not been changed.
Depending on the transformation event, validated methods for detection and quantification of GMO content of food and feed are available (http://gmo-crl.jrc.ec.europa.eu/gmomethods/, January 2018). Such reference methods are based mainly on molecular techniques (PCR, qPCR) (Levy et al., 2014) and could be applied to a various plant species (maize, soybean, rice, tomato, papaya, carnation, cotton) for specific transformation event or sequences (Bonfini et al., 2012).
At national level, control of the correct traceability and labelling of genetically modified foods and feeds is the responsibility of the National Sanitary Veterinary and Food Safety Authority(NSVFSA) (www.ansvsa.ro).
The aim of the present study is to analyse the distribution of the incidence of genetically modified plant raw materials in Romania and includes data provided by the molecular biology and genetically modified organisms laboratories for the period 2012-2017.

MATERIALS AND METHODS

The data were collected from laboratories that can perform analyses to identify or quantify foods and feeds that represent or may contain genetically modified organisms (Sanitary Veterinary and Food Safety Laboratory (SVFSL) Calarasi, SVFSL Constanta, SVFSL Iasi, SVFSL Salaj, SVFSL Satu-Mare, SVFSL Suceava, SVFSL Tulcea and the National Reference Laboratory for GMOs from the Institute of Diagnosis and Animal Health were processed and statistically interpreted in order to develop an distribution of the results of the analysed samples.
The period evaluated of study is 2012-2017.
The analyses performed for the GMO detection in the Romanian laboratories through standardized methods are:
• Detection by PCR techniques of genetic GMO-specific genetic structures in food and feed (Screening real time PCR for the identification of GMOs in food and feed of plant origin);
• The identification and quantification of GMOs in foods and feed containing corn;
• Identification of DNA specific for GTS 40-3-2 line (Roundup Ready) from food and feed containing soy;
• Quantification of DNA specific GTS 40-3-2 line (Roundup Ready) from food and feed containing soy;
• Detection of p35S and tNOS elements in food and feed.
The identification and quantification methods used are as follows:
• ISO 21569 - Methods of analysis for GMO detection - qualitative methods
• ISO 21570 - Methods of analysis for the quantification of GMOs - quantitative methods
• ISO 21571 - Methods for the extraction of nucleic acid.
However, currently, the most used methods for the detection and quantification of GMOs in food and feed are:
• Identification of genetically modified soybeans or corn (RENAR accredited method), involving the detection of p35S and tNOS genetic elements by PCR;
• Genetically modified DNA quantification in foods and feed containing soybeans specific to the GTS 40-3-2 (Roundup Ready) line by Real-Time PCR (RENAR accredited method).
The following indicators have been used in order to characterize the incidence of GMOs: plant species, type of material or product, type of detection and identification method, GMO's specific genetic structures and transformation events.
The research methodology used in this study has the following main aspects:

• *bibliographic study of the internal literature;*
• *collecting the concrete information within the researched area;*
• *ordering, processing and presentation of results in synthetic form;*
• *analysis and interpretation of results and formulation of conclusions.*

RESULTS AND DISCUSSIONS

In accordance with the Report on the Assessment of the Economic Performance of Genetically Modified Genetic Plants, published on the European Commission's website(https://ec.europa.eu/food/sites/food/files/plant/docs/gmo rep-stud 2011 report econ-perf.pdf, September 2015), the global area seeded with genetically modified plants has grown significantly annually since 1996, when it was first grown for commercial purposes on an area of 2.8 million hectares. In 2016 this area grew to 185 million hectares (ISAAA, 2016 https://www.isaaa.org/resources/publications/briefs/52/download/isaaa-brief-52-2016.pdf, December 2017). Soy, maize, rapeseed and cotton were mainly grown.
Regarding soybean, it is known that more than 36 million tonnes are needed for animal feed in Europe. Annually, the Member State cultures produce 1.4 million tonnes of non-genetically modified soybean. Genetically modified soybeans are not authorized for cultivation in the European Union but are imported very large quantities soybean (18.5 million tonnes of soybean flour and 13.5 million tonnes of soybeans in 2013). (https://www.ansvsa.ro, November 2017).
In Romania, the National Sanitary Veterinary and Food Safety Authority, as regulatory and controlling authority in the field of genetically modified food and feed, has the role of ensuring that only genetically modified food and feed authorized is introduced on the national market and the provisions traceability and labelling are respected by food and feed business operators. Within the eight laboratories specialized in molecular biology are analysed both the samples taken in the GMO official control program and the samples taken by the food and feed industry operators in the self-control process.
In the last 6 years (Table 1) were analysed a total of 5184 samples of food or feed that may contain parts of genetically modified organisms of plant origin (used as raw material in the production process).
According to the data provided by the County Laboratories and the Reference Laboratory of the Institute of Diagnosis and Animal Health (IDSA), the number of samples analysed varied from 1070 in 2013 to 756 in 2016.

Table 1. Number of samples analysed annually for GMO detection, during 2012-2017

	2012	**2013**	**2014**	**2015**	**2016**	**2017**	**Total**
Calarasi	46	53	22	30	32	40	**223**
Constanta	31	32	23	34	38	29	**187**
Iasi	41	141	150	124	105	109	**670**
Satu Mare	40	38	29	9	12	13	**141**
Salaj	216	249	208	251	268	313	**1505**
Suceava	59	64	50	37	25	11	**246**
Tulcea	29	57	21	26	23	26	**182**
IDSA	509	436	313	278	253	241	**2030**
Total	**971**	**1070**	**816**	**789**	**756**	**782**	**5184**

The detailed evidence of the number of samples transmitted from the 7 County Laboratories of Molecular Biology and GMO and the Reference Laboratory of IDSA showed a total of samples processed in each laboratory (Figure 1), as follows: for Calarasi County 223 analysed samples for identification of genetic modification events, for Constanta County 187 samples, for Iasi County 670 samples, for Satu Mare county 141 samples, for Salaj county 1505 samples, for Suceava county 246 samples, for Tulcea county 182 samples and for IDSA 2030 samples.

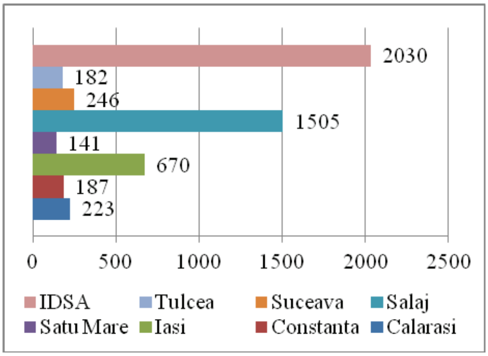

Figure 1. The total number of analysed samples (by counties) for the identification of GMOs (2012-2017)

The largest number of samples were analysed in the laboratories of IDSA, but also in Salaj and Iasi counties.
The total number of samples processed in all the 8 laboratories is close and has an average of 864 samples per year, except 2013 (Figure 2). The interlaboratory difference is approximately ± 100 samples per year.

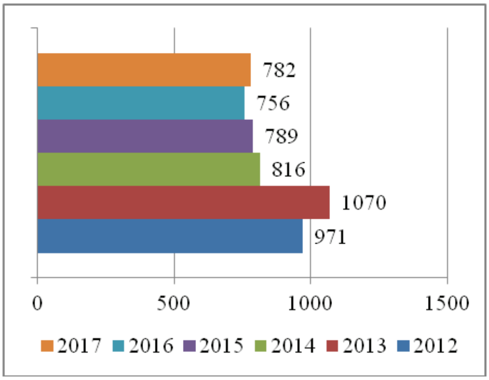

Figure 2. The total number of samples analyzed (annually) for the identification of GMOs

Regarding the identification of the sequences of the promoter P-35S and the T-NOS terminator specific to the genetically modified plants, the distribution of the number of samples processed annually in each of the 8 laboratories in Romania is shown in Figure 3.

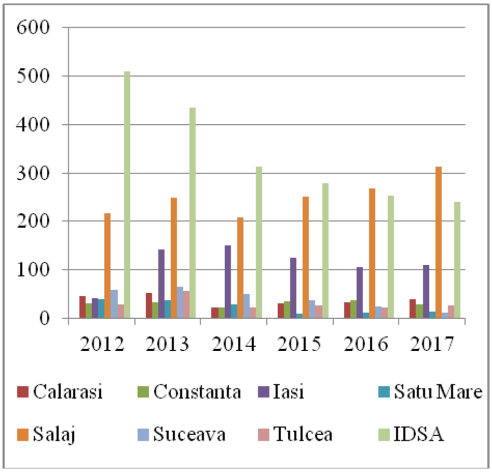

Figure 3. Annually distribution of the number of samples analysed for GMO identification

The largest number of samples (509) was processed in 2012 by the reference laboratory for molecular biology and GMO at the Institute of Diagnosis and Animal Health.
Regarding the plant species examined for transformation events analyses have been carried out to identify GMOs, raw material of plant origin, food or feed containing genetically modified soybeans (2678 samples) and genetically modified maize (2286 samples).
The types of vegetal material analysed were:
• for soy: beans, wheat flour, protein isolate, textured, milk, fibres, granules, cubes, snuff, slices, bread, chocolate, pate, sauce, salami, sausages, bakery brewers, cakes, waffles, biscuits, pudding, lecithin, canned meat, pastry premix and cream;
• for corn: grains, malt, popcorn, puff, chips, oil, flakes, sweet corn, flour, starch, fodder yeast, compound feed.
The total number of samples processed by each of the 8 laboratories (Table 2) shows that in Romania soy is more used in animal feed and food production, compared to corn.
This idea is sustained by the fact that in the laboratories from Calarasi, Satu Mare and Suceava were processed only samples to identify the DNA sequence specific to genetically modified soybean GTS 40-3-2 (Roundup Ready).

Table 2. Total number of samples of raw materials of soy and corn processed during 2012 - 2017

	Species	Total
Calarasi	soy	223
Constanta	soy	142
	corn	45
Iasi	soy	246
	corn	424
Satu Mare	soy	141
Salaj	soy	952
	corn	553
Suceava	soy	246
Tulcea	soy	176
	corn	6
IDSA	soy	772
	corn	1258

Moreover, the highest number of samples examined for genetically modified soybeans was in the Salaj laboratory (952 samples) and at IDSA laboratory (772 samples) (Figure 4.)

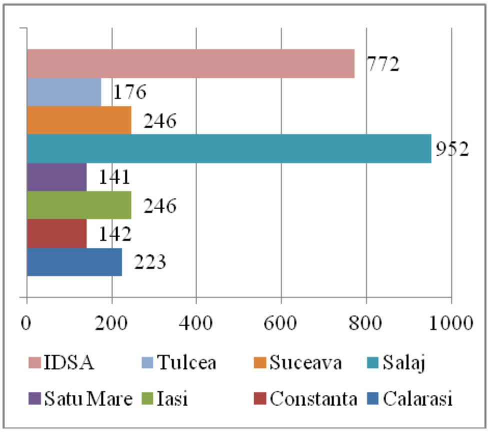

Figure 4. The total number of samples analysed for the identification the specific modified DNA of line GTS 40-3-2 (Roundup Ready) during 2012-2017

The samples processed to identify genetically modified corn (Figure 5) are shared between three laboratories that correspond to the historical geographic areas. Half of the samples were analysed by IDSA (1258 samples), and the other half of the samples are split between laboratories from Salaj (553 samples) and Iasi (424 samples).

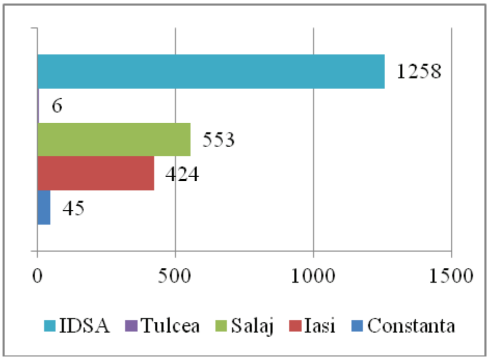

Figure 5. The total number of samples analyzed for P-35 S and T-NOS identification from corn-containing raw materials during 2012-2017

From the analysis of the statistical data regarding the number of samples of raw materials of vegetal origin (Table 3) it is found that each laboratory has processed annually a relatively constant number of samples, but their averages are quite different from one county to another, ranging from dozens of samples. The working capacity is very different between the 8 laboratories.

Table 3. Number of samples raw materials of vegetable origin (soy and corn) analysed (P-35 S, T-NOS)

	Species	2012	2013	2014	2015	2016	2017
Calarasi	soy	46	53	22	30	32	40
Constanta	soy	31	32	23	18	20	18
	corn	-	-	-	16	18	11
Iasi	soy	40	47	44	41	40	34
	corn	1	94	106	83	65	75
Satu Mare	soy	40	38	29	9	12	13
Salaj	soy	121	138	126	180	168	219
	corn	95	111	82	71	100	94
Suceava	soy	59	64	50	37	25	11
	corn						
Tulcea	soy	27	57	21	26	19	26
	corn	2	-	-	-	4	-
IDSA	soy	168	131	142	117	117	97
	corn	341	305	171	161	136	144

In 2012, for the identification of genetically modified corn a maximum of 341 samples were processed at the IDSA and a minimum of 1 sample were processed by the Iasi laboratory.

Figure 6 and 7 reveal the distribution of the number of samples processed to detect genetically modified soybeans is much higher than the number of samples processed to identify genetically modified corn.

The average is relatively uniform for genetically modified soy in all the laboratories (Figure 6).

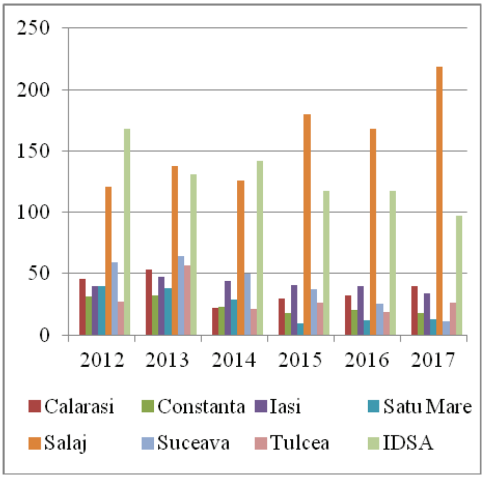

Figure 6. Counties distribution of the number of samples of raw materials containing soybean

Differences were recorded for the identification of genetically modified corn: in at least two laboratories no corn samples were examined (Figure 7).

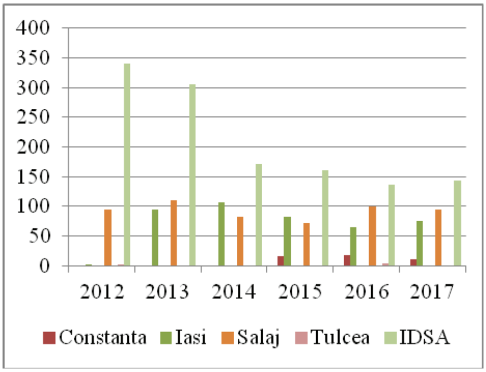

Figure 7. Counties distribution of the number of samples of raw materials containing corn

In Romania is approved for cultivation only maize MON 810, but the introduction on the national market and the use of GMO as raw materials in various forms, in feed and food industry is allowed, according to current legislation.

EU legislation obliges food and feed business operators to label all genetically modified products if their presence exceeds 0.9%. [3] For this reason the possible presence of GM events have to be identified throughout the food chain.

During the analysed period (2012-2017) it was shown that all the samples processed to identify genetically modified corn had a negative result (under the 0.9% limit). However, positive results were recorded in several samples examined for the identification of genetically modified soybean (Table 4). The highest positive number of samples was detected in Salaj laboratory (29 samples).

Table 4. Total number by counties of samples with positive result on identification for GMO

	Species	Total
Calarasi	soy	10
Constanta	soy	10
	corn	-
Iasi	soy	14
	corn	-
Satu Mare	soy	-
Salaj	soy	29
	corn	-
Suceava	soy	20
Tulcea	soy	25
	corn	-

The representation of the distribution on each laboratory that had positive results for food or feed containing genetically modified soybean (Figure 8) shows an approximately uniform distribution for the historical-geographic areas in Romania. Of the 2898 samples analysed, 108 of them had a positive result, which represents only 3%, which means that a small amount of genetically modified soybeans has been imported into our country through intra-community trade or import.

The proportion of positive results for GM soybean from the total of samples processed at County Laboratories of Molecular Biology and GMO is low, ranging from 3% for Salaj laboratory to 14.2% for Tulcea laboratory. At Calarasi laboratory this proportion is 4.5%, at Constanta laboratory is 7%, at Iasi laboratory is 5.7%, at Suceava laboratory is 8.1%.

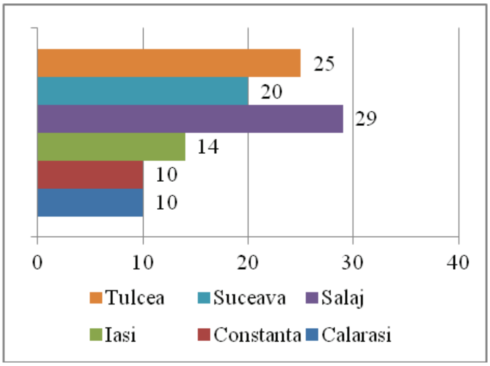

Figure 8. The total number of positive samples for GMO identification in 2012-2017

The annual distribution of positive results (Table 5) shows a maximum of 13 samples in 2017 at Tulcea laboratory and 13 samples in 2015 at Suceava laboratory.

Table 5. Number of samples by counties and annually with positive result on identification for GMO

	Species	2012	2013	2014	2015	2016	2017
Calarasi	soy	2	1	-	3	3	1
Constanta	soy	7	2	1	-	-	-
	corn	-	-	-	-	-	-
Iasi	soy	4	1	4	1	4	-
	corn	-	-	-	-	-	-
Satu Mare	soy	-	-	-	-	-	-
Salaj	soy	4	11	8	4	2	-
	corn	-	-	-	-	-	-
Suceava	soy	-	-	4	13	3	-
Tulcea	soy	-	4	2	2	4	13
	corn	-	-	-	-	-	-

From the analysis of these results, for the period 2012 - 2017, a similar amount of food and feed containing genetically modified soybeans was introduced annually in our country. We note, therefore, that the food industry and livestock activity had a relatively constant activity during this time period.
Distribution of the number of samples that had a positive result for the identification of genetically modified soybean (Figure 9) reported annually and for each laboratory reveals a non-homogeneous pattern.
In 2017, a number of samples with a positive result were detected only for Tulcea laboratory. The same situation was in 2015 at Suceava laboratory and in 2013 at Salaj laboratory. A homogeneous distribution is only in 2016.

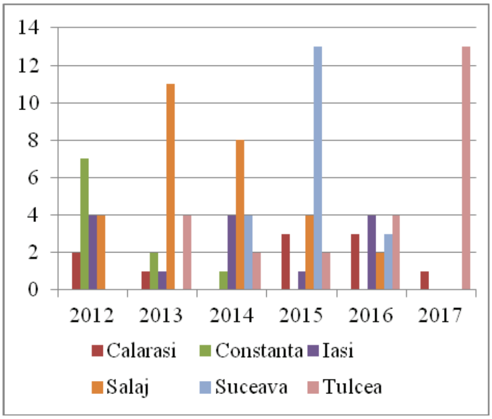

Figure 9. Counties and Annually distribution of the number of samples with positive result on GMO identification

CONCLUSIONS

In Romania is allowed for cultivation only one transformation event (maize MON810), but they are allowed to be placed on the national market and used like animal feed and in the food industry. They can be used to obtain food products and can therefore be found throughout the food chain.
During 2012-2017, eight molecular biology laboratories processed 5184 samples of raw materials, food or feed of plant origin to identify specific genetic modification sequences.
The methods used for the detection and quantification of GMOs (soybean or corn) in food and feed are RENAR accredited.
In Romania, there is a representative laboratory for the number of processed samples for the three historical-geographic areas: Muntenia, Transylvania and Moldova.
The number of samples processed for identifying genetically modified soybeans (2678 samples) is higher than the number of samples processed to identify genetically modified corn (2286 samples).
For the identification of genetically modified soybeans, the Salaj laboratory (952 samples) and IDSA (772 samples) were processed the largest number of samples in comparison with all the other laboratories.
The samples processed to identify genetically modified corn had been processed half of the samples were analysed by IDSA (1258 samples), and the other half of the samples are split between laboratories from Salaj (553 samples) and Iasi (424 samples).
All samples processed to identify genetically modified corn have had a negative result (under the limit of 0.9%), which reveals that this products respected the current legislation (during 2012-2017).
A small amount of genetically modified soybeans has been imported into our country through intra-community trade or import: by the 2898 samples analysed, 108 (3%) had a positive result.
The data presented in this work confirm the compliance of the legislation in GMO domain in Romania, regarding not only the cultivation but also the detection and quantification.

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