

GMO IDENTIFICATION IN FOOD AND FEED FOR 2019 AND 2020 (PANDEMIC YEAR) - A COMPARATIVE STUDY

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Abstract

In this article, we will present a comparative study by the incidence of genetically modified soy and corn, in food and feed, in Romania, during 2019 and 2020. The laboratory analyzes performed on their determination are compared. These data refer to the samples from different food and feed matrices. This paper includes the data provided by the molecular biology laboratories from Braila, Bucharest, Calarasi, Iasi, Satu-Mare, Salaj, Suceava, and Tulcea and the data provided by the County Veterinary Sanitary and Food Safety Directorates. The paper presents the data for each county that has been processed for the indicators: the number of samples of food or feed processed annually and analyzed to identify genetically modified soy and corn. During this period, were analyzed in 2019 between 0 samples by Tulcea laboratory and 158 samples by Salaj laboratory, in 2020 (known as the Covid year) were analyzed fewer samples. The matrix samples were cereals, flour, mixed feed, textured, soy isolate, lecithin, starch, drinks, sauces, pastries, pasta, and popcorn. For the analyzed samples, genetic transformation processes were not detected. In conclusion, for the period 2019-2020 on the Romanian market were marketed safe food and feed, non-genetically modified products, and their traceability was following the European and national legislation.

Key words: GMO, determination, soy, corn, 2019, 2020.

INTRODUCTION

GMO means an organism that possesses foreign genes obtained using modern biotechnology. Generally, methods for obtaining transgenic plants are based on the use of the *Agrobacterium* system. (i.e. genetic engineering) (Cornea, 2010).

Biotechnology has revolutionized crop improvement by producing genetically modified crops with increased availability and the use of important traits (Icoz and Stotzky, 2008).

Researchers are constantly contributing through innovations in biotechnology, creating new GMOs beneficial to farmers around the world. Improving genetically modified crops that are obtained through genetic engineering methods is particularly important, because the issues of environmental protection, maximizing crops to combat or mitigate malnutrition around the world, the needs of a growing population are evident worldwide.

This comparative study presents an analysis of the number of samples by food or feed examined for the identification and incidence of genetically modified events into products

obtained from soy and corn or containing soy and corn. The study includes data provided for the period 2019 – 2020, by the official national laboratories.

Only genetically modified MON 810 corn is allowed to be cultivated in Romania by national and European legislation.

The evidence of genetically modified corn crops is public on the website of the Ministry of Agriculture and Rural Development, accessing the link <https://www.madr.ro/organism-modified-genetic.html>.

The Romanian cultivators can establish crops with genetically modified corn MON 810 Bt but recently have chosen not to establish such cultures.

This type of corn is resistant to lepidopteran insects, identification code MON-ØØ81Ø-6, according to the European Commission Regulation no. 65/2004 establishing a system of elaboration and allocation of unique identifiers for genetically modified organisms.

After 2015 year, the corn line MON 810 Bt was not cultivated in Romania. In Secuieni commune, Neamt county were cultivated 2.5 ha (www.madr.ro, January 2021).

Data were collected and were statistically processed and interpreted from laboratories that can analyze the identification and quantification of genetically modified events in plants. These laboratories are Veterinary Sanitary and Food Safety Laboratory (LSVSA) by Romanian counties: Brăila, Călărași, Iași, Satu Mare, Sălaj, Suceava, Tulcea, and the Institute of Diagnostic and Animal Health (IDSA) from Bucharest.

Genetically modified food and feed are currently limited almost exclusively to agricultural plants.

The indicators used for the characterization of the Genetically Modified Organisms and the possible events necessary for this comparison were the total number of samples during the whole period of study, annually and in each county laboratory of the food and feed samples processed for the determination of genetically modified organisms, species of vegetal material or food and feed product obtained from soy and corn.

Generally, the methods to obtain transgenic plants are based on the use of the *Agrobacterium* system (Cornea, 2010; Kado, 2015; Parmar et al., 2017).

Countries that have developed specific legislation for foods and feed products containing, consisting of, or produced from genetically modified organisms, focus mainly on the analysis of risks to consumers' health, possible environmental problems, as well as the practical control (possibilities for detection, testing, and labeling of these foods and feed products).

The methods used for the efficient transfer of genes of interest to plant organisms have progressed a lot, from indirect methods such as bacterial-mediated transformation to methods of direct DNA shooting into target tissues.

The European Union has been developed laboratory methods to control plants or products containing genetically modified plants, most of which are based on DNA testing by PCR or protein testing by enzyme-linked immunosorbent assay.

The detection international standards are also used in the Romanian county laboratory's activity and this is:

- ISO 21569 – quality standard,
- ISO 21570 – quantitative standard,

- ISO 21571 – extraction of nucleic acid standard.

The methods used in Romanian county laboratories for detecting GMOs in food and feed are:

- PCR identification of p35S and tNOS genetic elements from soy or corn (the RENAR accredited method);
- Real-Time PCR quantification of soy-specific genetically modified line GTS 40-3-2, (the RENAR accredited method).

Methods are based mainly on molecular techniques (PCR, qPCR) (Levy et al., 2014) and could be applied to various plant species (corn, soybean, rice, tomato, papaya, carnation, cotton) for specific transformation event or sequences (Bonfini et al., 2012).

MATERIALS AND METHODS

The research methodology used in this study has the following main aspects:

- Bibliographic study of the national literature;
- Collecting the concrete information within the research area;
- Ranking, processing, and presentation of results in synthetic form;
- Analysis and interpretation of results and formulation of conclusions.

RESULTS AND DISCUSSIONS

The European and national markets allow the marketing and use of genetically modified plants for the production and processing of food and feed for animals (especially soybeans and corn) and in general for the food industry. These foods and feeds are used in various forms, such as cereals, bran, flour, soy isolate, textured. The purpose of this paper is to make public the data on food and feed samples examined for GMO detection.

For a genetically modified plant or part of a plant to be used in the production of food or feed, there must be mandatory conditions such as: it must be authorized for commercialization on the European market by the European Food Safety Authority and not exceed the maximum limit established conventionally at the legislative level, represented by a

percentage of 0,9%, which also requires the appropriate labeling.

It is also mandatory to indicate the presence of genetic modification events in plants, explicitly stating on the label of a food or feed products = "contains GMOs".

These products can be used for the production or processing of finished food or feed and can therefore be marketed on the intra-Community market, therefore they can be found throughout the food chain, "from the fork to the farm".

Traceability of genetic modification events of plants used in the food or feed industry is an essential indicator for human and animal health.

Therefore, the expression "contains GMOs", the content was expressed as a percentage of modification and the specific code of the modified plant species are useful information for the traceability of genetically modified food or feed.

The National Veterinary and Food Safety Authority from Romania is a control authority in the domain of genetically modified food and feed, ensures that only genetically modified foods and feed authorized at the European level are placed on the market and labeled according to the specialized European and national legislation.

There are 8 specialized laboratories for molecular biology and GMOs in Romania, which perform the analysis of food and feed samples taken under the official national control program and the analysis of samples received from economic operators from the food and feed industry in the process of self-control of own unit.

In (Table 1) is the number of food and feed samples examined in 2019 and 2020 (927 samples) the distribution of samples analyzed in the national laboratories to determine genetically modified organisms.

We note that the difference between 2019 and 2020 is 57 more samples in 2019.

In 2019 were examined in total 492 samples and in 2020 the number of samples was 435, with an average of 463.5 samples. The difference comes from the LSVSA Braila, Calarasi, Tulcea, and IDSA laboratories (Figure 1).

Within LSVSA Braila, half of the number of samples from 2019 was analyzed in the 2020 pandemic year. Within the LSVSA Iasi, Salaj,

and Satu Mare, the number of analyzed samples for the two years was maintained. Within LSVSA Suceava and Tulcea, the number of analyzed samples for the pandemic year 2020 was increased, compared to 2019. For LSVSA Tulcea is a resumption of this activity in 2020.

Table 1. Comparative annually samples analyzed for GMOs detection during 2019-2020 and their average

	2019	2020	Total	Average
Braila	97	52	149	74.5
Calarasi	48	37	85	42.5
Iasi	63	64	127	63.5
Satu Mare	10	9	19	9.5
Salaj	158	157	315	157.5
Suceava	11	18	29	14.5
Tulcea	0	16	16	8
IDSA	105	82	187	93.5
Total	492	435	927	463.5

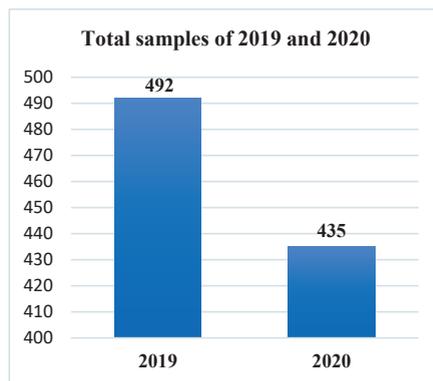


Figure 1. 2019 and 2020 annually total samples/analysis laboratory for GMO identification

The distribution of the total number of samples analyzed during the period 2019-2020 on each laboratory (Figure 2) shows that the laboratories within LSVSA Salaj (Transylvania area), IDSA, and Braila (south Moldova area) examined a lot more samples, indicating increased economic activity in the western area and the center area of Romania. Within LSVSA Salaj, 315 samples were taken for GMO analysis, which is the maximum cumulative for the two years of study.

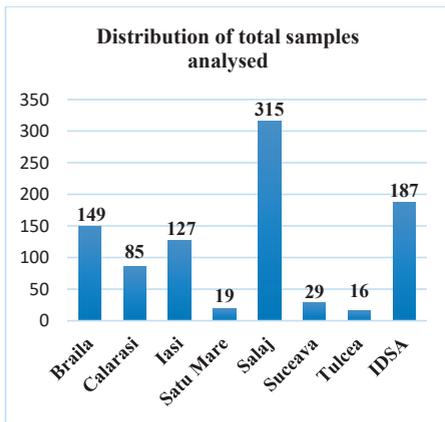


Figure 2. The distribution of total samples/analysis laboratory for GMO identification (2019-2020)

Within LSVSA Braila, Iasi and IDSA we noticed that the number of samples is approximately half, compared to the data provided by LSVSA Salaj for the same period. A much smaller number of samples were taken at LSVSA Satu Mare (19 samples), Suceava (29 samples), and Tulcea (16 samples).

The comparative annually distribution for 2019 and 2020 respectively for each laboratory of the number of samples analyzed for the identification of GMOs (Figure 3), shows a maximum of 158 samples examined in 2019 by LSVSA Salaj laboratory and 0 samples in 2019 on LSVSA Tulcea.

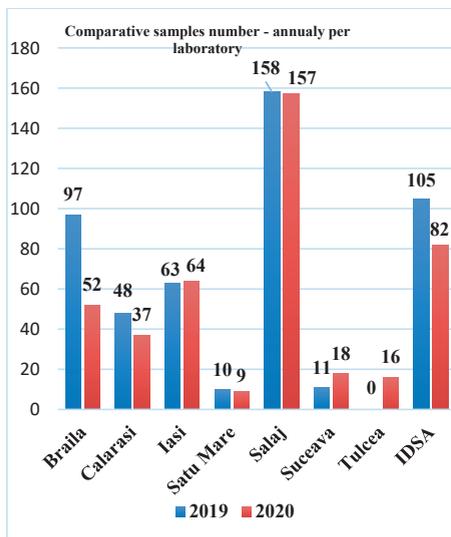


Figure 3. Comparative annually distribution by each laboratory of samples analyzed for GMO identification

Within LSVSA Braila, twice as many samples were processed in 2019 (97 samples), compared to 2020 (52 samples).

A number with 10 fewer samples is observed for LSVSA Calarasi in the pandemic year 2020 (37 samples), compared to 2019 (48 samples).

About a quarter fewer samples were processed at IDSA in the pandemic year 2020 (82 samples), compared to 2019 (105 samples).

The variability of the data regarding the number of examined samples expressed by the measure of their scattering, represented by the standard deviation is $S = 50.58784$, so about half of the data are located on either side of the average (Table 1).

The measure of the degree of statistical correlation between the data of Table 1. is represented in Figure 4. by the linear correlation coefficient. This indicator has values between -1 and 1. For this data the calculated value is $r = 0.06928$ for 2020 and $r = 0.1$ for 2019. Because the value of r is a positive number, the correlation is a direct one, and because the value of r has a small value, the correlation is very weak between 2019 and 2020 (Figure 4).

In the laboratories of molecular biology from Romania is used the PCR method to identify genetically modified soy the GTS 40-3-2 (Roundup Ready) line and the p35S promoter and the tNOS terminator for identify genetically modified corn.

The total number of 541 samples of food and feed analyzed by all laboratories to identify the modified soy line (Table 2) is much higher than the number 391 samples examined for modified corn (Table 3).

For genetically modified soybeans, we noticed that for the eastern part of Romania, a number of 133 samples were examined by the molecular biology and GMO laboratories within the county LSVSA, for the western area a number of 252 samples, and for the southern area a number of 156 samples (the southern area also includes samples examined within IDSA, which is the national reference laboratory for determining genetically modified organisms), according to the data provided by the county LSVSA laboratories and which are included in Table 2.

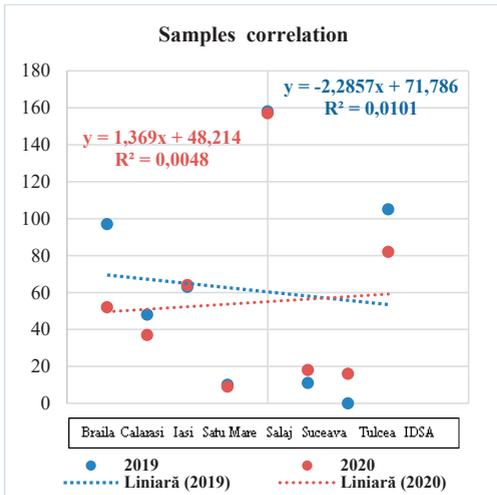


Figure 4. Correlation of samples analyzed for GMO detection, for 2019 and 2020

Table 2. Total samples number of food or feed processed for identification of modified DNA soy line during 2019 - 2020

	Species	Total
Braila	soy	63
Calarasi		85
Iasi		44
Satu Mare		19
Salaj		233
Suceava		26
Tulcea		16
IDSA		55
Total		541

During the study period, LSVSA Salaj examined for identification of genetically modified soy most food and feed samples, a total of 233 samples.

For genetically modified corn, we noticed that for the eastern part of Romania, a number of 177 samples were processed within LSVSA Braila, Iasi, and Suceava.

For the western area within LSVSA Salaj we observed a number of 82 samples and for the southern area within IDSA a number of 132 samples (Table 3) Data on the analysis of food and feed for genetically modified corn were received from 5 molecular biology and GMO laboratories among the 8 county laboratories, data that were included in this study.

Table 3. Total samples number of food or feed processed for identification of modified corn

	Species	Total
Braila	corn	86
Iasi		88
Salaj		82
Suceava		3
IDSA		132
Total		

The graphical representation of the distribution on laboratories of the samples examined during this study shows that all 8 laboratories examined samples for the identification of genetically modified soy (Figure 5) and 4 laboratories for the identification of genetically modified corn (Figure 6).

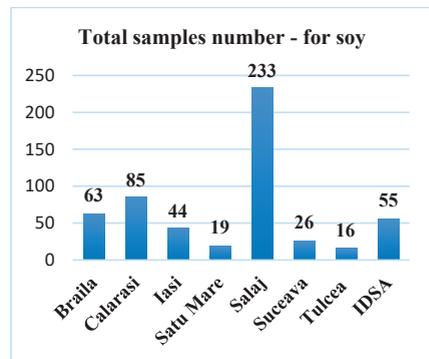


Figure 5. The total samples number analyzed for the identification of the specific modified DNA of line GTS 40-3-2 (Roundup Ready) during 2019-2020

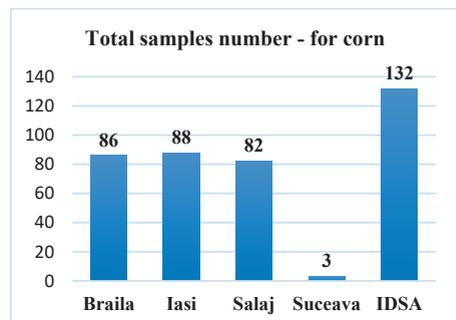


Figure 6. The total samples number analyzed for P-35S and T-NOS identification from corn during 2019-2020

In comparison, for the years 2019 and 2020 (Table 4.) is approximately equal to the annually number of food or feed samples for

which it was examined to identify the genetically modified soybean line, by the PCR detection method to find the p35S and tNOS sequences.

Table 4. Annually samples number of food or feed by soy analyzed in 2019 – 2020 and their average

	Species	2019	2020	Average	
Braila	soy	40	23	31.5	
Calarasi		48	37	42.5	
Iasi		22	22	22	
Satu Mare		10	9	9.5	
Salaj		116	117	116.5	
Suceava		8	18	13	
Tulcea		0	16	8	
IDSA		32	23	27.5	
Total			276	265	270.5

In 2019 (276 samples), they were analyzed with only 10 more samples than in 2020 (265 samples), with an average of 270.5 samples.

In the LSVSA Tulcea, we noticed that no samples were analyzed for the identification of genetically modified soybeans in 2019, but in 2020 the analyzes for this species were resumed in food and feed products and we observed a number of 16 samples examined in this molecular biology and GMOs laboratory.

All 8 county laboratories for the determination of genetically modified organisms examined samples of food and feed to analyze the genetically modified soybean line.

We observe from the following graph (Figure 7) that we have a maximum of 117 samples examined in 2020 at LSVSA Salaj and a minimum of 8 samples examined in 2019 at LSVSA Suceava.

For genetically modified soybean, in the LSVSA Braila, Calarasi, Satu Mare, and IDSA a smaller number of samples were analyzed in the pandemic year 2020 (Figure 7).

In the LSVSA Iasi and Salaj were analyzed an approximately equal number of samples for the two years of study. In the LSVSA Suceava and Tulcea, the number of analyzed samples was increasing in 2020, compared to 2019. For LSVSA Suceava there is a doubling of the number of samples, and for LSVSA Tulcea we can say that the laboratory resumed the determination of GMOs (16 samples) after

what the year 2019 meant the lack of this activity (Figure 7).

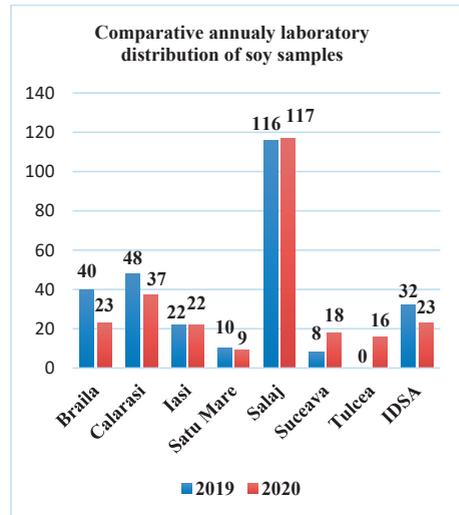


Figure 7. Comparative laboratory distribution of the samples number of food or feed containing soy

The number of samples examined to identify the genetically modified corn (Table 5) presents a maximum of 73 samples in 2019 at the IDSA laboratory.

In 2019, 221 food and feed samples were taken for the determination of genetically modified corn, compared to 170 samples in 2020, with about a quarter fewer samples observed for the pandemic year 2020 and an average of 195.5 samples (Table 5).

The variability of the data on the number of food or feed samples examined to identify the genetically modified soybean line is the standard deviation $S = 34.64144$, so about one-third part of the data is located on both sides of the average (Table 4).

The rank of statistical correlation for the data from Table 4 is represented by the linear correlation coefficient.

For this data, the calculated value is $r = 0.18357$ for 2019. The linear correlation coefficient $r = 0.00006$ for 2020 and the correlation is the direct correlation.

Because the value of r for 2019 is a positive number, the correlation is a direct correlation, and because the value of r has a small value, the correlation is very weak (Figure 8).

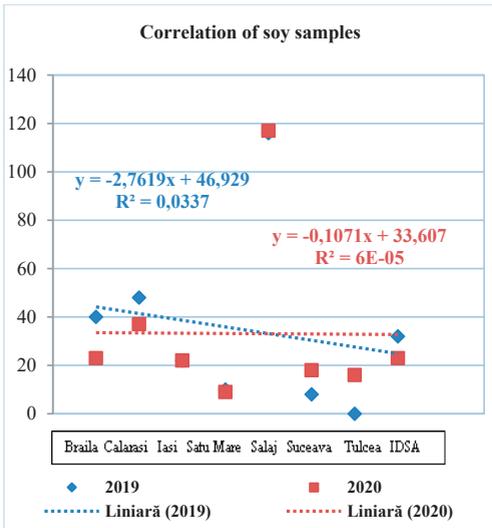


Figure 8. Correlation of samples of food or feed by soy analyzed for GMO detection, for 2019 and 2020

Table 5. Annually samples of food or feed by corn analyzed in 2019 – 2020 and their average

	Species	2019	2020	Average
Braila	corn	57	29	43
Iasi		46	42	44
Salaj		42	40	41
Suceava		3	0	1.5
IDSA		73	59	66
Total		221	170	195.5

Within LSVSA Suceava (Moldova area), no samples were analyzed for genetically modified corn in 2020.

Within LSVSA Braila, approximately half of food and feed samples were processed in 2020 (29 samples), compared to 2019 (57 samples). In the LSVSA Iasi were analyzed an approximately equal number for the two years of study, 46 samples in 2019 and 42 samples in 2020.

The same for LSVSA Salaj was analyzed an approximately equal number for the two years of study, 42 samples in 2019 and 40 samples in 2020.

The laboratory within IDSA examined a smaller number with 14 samples in 2020, compared to the previous study year.

The following graphical representation also shows that in 2019 and 2020, the laboratory within IDSA had a capacity and received for

analyzing much more samples than the other laboratories (Figure 9).

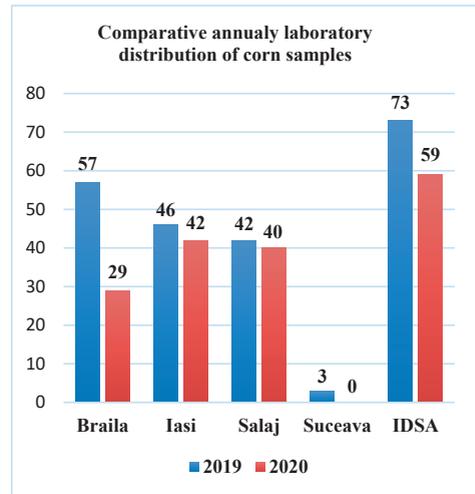


Figure 9. Comparative laboratory distribution of the samples number of food or feed containing corn

The variability of the data on the number of food or feed samples examined to identify the genetically modified corn is the standard deviation $S = 23.24961$, so about one-fifth part of the data is located on both sides of the average (Table 5).

The statistical correlation coefficient for the data contained in Table 5. is represented graphically by Figure 10.

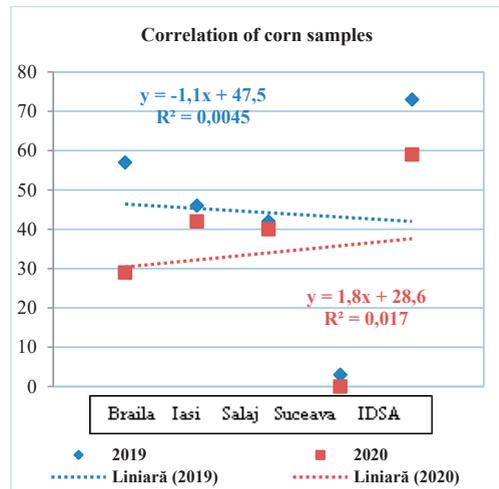


Figure 10. Correlation of samples of food or feed by soy analyzed for GMO detection, for 2019 and 2020

For this data the calculated the correlation coefficient value is $r = 0.13038$ for 2020 and $r = 0.06708$ for 2019.

Because the value of r for 2019 and 2020 is a positive number, the correlation is a direct correlation.

Because the value of r has a small value, the correlation is very weak between the two years of the study (Figure 10).

During the study period 2019 – 2020, there were 0 positive results for GMO determination for genetically modified soy and corn.

Were examined samples from food or feed products and the samples were collected from the marketing, processing, production, warehousing units.

Sampling is carried out according to specific procedures regarding the size of the lots, the packaging or bulk products, the diversity of the products and the observance of national and European hygiene norms, and prevention of direct or indirect contamination of these food or feed samples.

These results in fact without positive samples for the determination of GMOs are by the data provided by the 8 laboratories of molecular biology and the determination of genetically modified organisms in Romania accredited for performing these analyzes.

The purpose of this paper is to make public the data on food and feed samples examined for GMO detection within the molecular biology and GMOs laboratories in Romania during 2019 - 2020 and to be a starting point for future specialized studies.

The European Commission considers that legislation needs to be amended to strike a stable balance between maintaining the European Union licensing system based on scientific health and environmental risk assessment and the need to give Member States the freedom to address specific national or local issues raised by cultivation Genetically Modified Plants.

This approach is expected to respond to the demands of several Member States and to receive public support while maintaining the European Union authorization system for Genetically Modified Organisms, as well as the free movement and import of food, feed, and modified genetic seeds.

CONCLUSIONS

During 2019-2020, eight molecular biology laboratories processed 927 samples of food and feed products of plant origin to identify specific genetic modification sequences of soy or corn genetically modified 492 samples in 2019 and 435 samples in 2020.

The number of samples processed for identifying genetically modified soybeans (541 samples) is higher than the number of samples processed to identify genetically modified corn (391 samples).

The total number of samples analyzed during 2019 – 2020 show that the laboratories within LSVSA Salaj (Transylvania area), IDSA, and Braila (south Moldova area) examined a lot more samples.

For the analysis of GMOs, LSVSA Salaj examined the largest number of samples for each of the two years of study. In comparison, LSVSA Braila, Iasi, and IDSA the number of samples is approximately half.

For the identification of genetically modified soybeans: the LSVSA Salaj laboratory (116 samples in 2019 and 117 samples in 2020) processed the largest number of samples in comparison with all the other laboratories, whereas the LSVSA Iasi and Satu Mare examined an approximately equal number in each of the study years.

Within the LSVSA Braila, Calarasi, Satu Mare, and IDSA a smaller number of samples were analyzed in the pandemic year 2020.

In 2019, 221 food and feed samples were taken for the determination of genetically modified corn, compared to 170 samples in 2020. The samples processed to identify genetically modified corn have been processed mostly by the IDSA laboratory (73 samples in 2019 and 59 samples in 2020).

Within LSVSA Braila, approximately half of samples were processed in 2020, compared to 2019, and in the LSVSA Iasi and Salaj were analyzed an approximately equal number of samples for the two years of study.

During 2019 – 2020, the study period there were no positive results for genetically modified soy and corn determination, according to the data provided by the laboratories of molecular biology and determination of genetically modified organisms from Romania.

From a statistical point of view, several statistical indicators were calculated, such as: arithmetic average, variability by the standard deviation (S), and correlation by correlation coefficient (r).

The average was calculated for:

- the total samples examined (463.5 samples);
- the total sample examined by each laboratory;
- the total samples examined for the determination of the genetically modified soy (270.5 samples);
- samples examined for the determination of genetically modified soy by each laboratory;
- the total samples examined for the determination of genetically modified corn (195.5 samples);
- sample examined for the determination of genetically modified corn by each laboratory.

The standard deviation was calculated for:

- the total samples examined for the two years of study ($S = 50.58784$);
- samples examined for the determination of genetically modified soy ($S = 34.64144$);
- samples examined for the determination of genetically modified corn ($S = 23.24961$).

The correlation coefficient was calculated for:

- samples examined in 2019 and 2020 ($r = 0.06928$ for 2020 and $r = 0.1$ for 2019);
- samples examined for the determination of genetically modified soybeans ($r = 0.00006$ for 2020 and $r = 0.18357$ for 2019);
- samples examined for the determination of genetically modified maize ($r = 0.13038$ for 2020 and $r = 0.06708$ for 2019).

This is a direct correlation because r is a positive number and is very weak correlations because is a number between 0 and 0.2.

ACKNOWLEDGEMENTS

This study was made possible by good collaboration with the Faculty of Biotechnologies within the University of Agronomic Sciences and Veterinary Medicine in Bucharest, the Institute of Diagnosis and Animal Health, Sanitary Veterinary Laboratories and Food Safety Laboratories in Braila, Calarasi, Iasi, Satu Mare, Salaj, Suceava and Tulcea.

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