SUMMARY

of the doctoral thesis entitled:

RESEARCH ON THE DETECTION OF SOME GENETICALLY MODIFIED ORGANISMS IN ROMANIA

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KEYWORDS: genetically modified plants, legislation, detection, samples, Romania

Genetically modified plants contribute worldwide to general progress, they should form the basis of fighting hunger. Foods obtained from genetically modified plants ensure a high level of health for people, animals, the environment and contribute to innovation in the agri-food system. In recent decades, advances in biotechnology have led to the development of new genomic techniques (NGT), that allow the development of plants with new desired traits, by modifying the DNA of the cells. Agricultural plants represent the benefits of genetic engineering, such as increased yield, resistance to climate change (drought, saline soils), reduced need for pesticides, increased nutrient composition and food quality, resistance to pests and diseases, food security, in the context of a world population bigger and bigger. NGTs have developed rapidly, some NGT products are already on the market outside the EU, and this trend is likely to continue. At the same time there are concerns related to the use of these technologies and the impact on organic agriculture, regarding the right of consumers to information and free choice. Consumer acceptance is essential.

Plants obtained from NGT contribute to the objectives of the European Green Deal, the Farm to Fork and Biodiversity strategies and the United Nations Sustainable Development Goals (SDGs) for a sustainable agri-food system. The EU Court of Justice in 2018 clarified that organisms produced by targeted mutagenesis are GMOs and are subject to the requirements of EU GMO legislation.

Were registered for cultivation 14 genetically modified soybean varieties (GTS - 40-3-2 between 2004 and 2006). Romania has banned the cultivation of genetically modified soybeans, starting from January 1, 2007. In the period 2007-2010, genetically modified maize MON810 on areas of 332.4 ha in 2007, 6130.44 ha in 2008, 822.6 ha in 2010, according to the Ministry of Agriculture, Forestry and Rural Development.

Current legislation is no longer adequate and needs adaptation to scientific and technological progress for some NGTs and their products. The current framework is based on techniques and methods defined by biotechnology as it was understood in

the late 1990s. In addition, the current food traceability authorization and control procedure was not designed to promote sustainability in the agri-food sector. Non-regulation of NGT products would negatively affect innovation and research in agricultural and food biotechnology, operators in the agri-food and feed system, operators in organic farming, commercial partners, national food control authorities on the market, evaluation bodies, consumer organizations.

The doctoral thesis entitled: "R**esearch on the detection of some genetically modified organisms in Romania**" proposes, on the one hand, an analysis of the incidence of genetically modified organisms identified during food and feed control in Romania between 2017 and 2020 and to evaluate the legislation in the field of new genome modification techniques and, on the other on the other hand, to analyze the opinion of consumers by carrying out a public perception questionnaire and a case study regarding genome editing of crop plants (genomic editing techniques) and has the following objectives:

- evaluation for the period 2017 – 2020 of the presence of genetically modified material in soybeans and corn in Romania;
- testing the presence of transgenes in plant material or products derived from sugar beet and papaya;
- critical analysis of new techniques for genetic modification of plants and methods of detection of modifications;
- comparative analysis of the regulations in the market introduction of NGT - GMOs and the debates in order to identify them;
- analysis of the degree of information, perception and acceptance of consumers in Romania for agri-food products, plants and products obtained through new genomic techniques.

The thesis is structured in two parts:

Part I: Bibliographic study;

Part II: Own research.

Part I followed in the first chapter the "review" of transgenic crops worldwide, in the years 2017, 2019 and 2020, showing cultivated areas and countries that adopt their establishment, in continuous and rapid growth. The benefits brought by the use of genetically modified plants in the agri-food sector are presented in the first chapter. Scientists have had opinions for and against their use and cultivation, ever since of the first genetically modified crops. After a period of study, review and reflection, worldwide academies have agreed that there is no danger regarding the safety of food produced by genetically modified crops. This chapter also briefly presents the level of acceptance of biotechnologies worldwide and the methods of qualitative and quantitative detection of DNA. With the help of the real-time PCR method, the amplification and quantification of a DNA sequence is efficiently carried out at the same time, in a single operation performed by automated equipment such as Themocycler.

Chapter II includes the types and mechanisms of the new genomic techniques and highlights the social and economic benefits brought by the progress of biotechnology applied to plants. They allow the introduction of new traits or to modify

existing traits effectively. NGTs allow researchers to insert or delete entire genes, make small changes to DNA, or alter gene activity. They give researchers more precise tools for breeding plant variations. This chapter presents some applications of NGT.

Techniques for editing the genome by applying site-specific nucleases include cutting and altering the genome during the repair process (ZFN, TALEN and CRISPR-Cas9) and modification of only a few base pairs (ODM). Cisgenesis involves the transfer of a gene from an identical or closely related species. Intragenesis represents the alteration of the genetic material of the same species, by adding new traits. Epigenetic methods change the activity of a gene without changing the DNA. Applying NGT on different matrices of biological material are developed for the agro-food industry. Is mandatory that these products or ingredients can be monitored (Regulation (EU) no. 625/2017 on official controls) throughout the production, processing and marketing chain, through the procedures specific traceability (Regulation (EC) no. 1830/2003 on the traceability and labeling of GMOs) and through the information on the labels (complete and correct).

The challenge brought by the new genomic techniques is represented by the detection of the products developed by these techniques. It is currently unclear how to demonstrate specificity of the method if the mutation could also occur spontaneously or could be introduced by genome editing techniques or random mutagenesis. For the control of the EU agri-food market, it is highly unlikely that laboratories will be able to detect the presence of unauthorized plant products in food or feed entering the EU market, without having prior information on modified DNA sequences. Through the status of the new genomic techniques, evaluated by the European Commission in 2021, it was concluded that there are NGT plants and products for sale on the world market, presented in chapter II, as well as their new induced traits.

The European Food Safety Authority (EFSA) has concluded that plant products with risk profiles similar to those developed by some NGT methods (targeted mutagenesis and cisgenesis) can be obtained with conventional breeding techniques or natural mutations and that they do not present new risks. Examples of NGT regulation in countries outside the EU are included in chapter II.

In **Part II**, the objectives of the research, studies, the methodology used, the results obtained for the own research are presented.

Chapters III, IV, V and VI include comparative data studies for the period 2017-2020, in Romania, regarding the detection of specific genetic modification sequences for soybean, corn, papaya and sugar beet matrices from raw material and products, with a proposal for adaptation of the examination method for these matrices and studies regarding the analyzes carried out in the national laboratories of molecular biology for the detection of GMOs in food and feed sold on the market. Data on the production and imported quantity of soybeans, corn, sugar beet and papaya are also presented.

The study concludes that for soybean production is decreasing (smaller cultivated areas), imports with continuously increasing quantities, in contrast to exports. For corn, we know significantly increasing production and an important export activity (import is insignificant). For sugar beet, there is a continuous and

significant decrease in national production, accompanied by the import of large quantities, following the demand from the animal breeding sector for protein-rich fodder. For papaya we can conclude the existence of imports (exports are insignificant), in small quantities, but with an increasing trend, which may mean the development of consumers' taste for this fruit.

In Romania, there are 8 accredited laboratories involved in the official control for the examination of genetically modified food and feed and the national reference laboratory for GMOs, which performed analyzes on several types of products. For genetically modified soy: grains, flour, meal, protein isolate, textured, milk, fiber, granules, cubes, slices, bread, chocolate, spreads, sauces, salami, sausages, biscuits, cakes, preserved meat, pastry mix and cream . For genetically modified corn: grains, malt, popcorn, puffs, chips, oil, flakes, sweet corn, sorghum, starch, fodder yeast, combined fodder. The identification and quantification methods used are accredited by RENAR: ISO 21569 (qualitative methods), ISO 21570 (quantitative methods) and ISO 21571 (DNA extraction methods). All the samples that had a positive result were below the limit of 0.9%, which proves that the food and feed products in the national market comply with the current legislation in force regarding GMOs. 2.25% of the samples analyzed tested positive for genetically modified soybeans, leading to the conclusion that a small amount of genetically modified feed was imported.

For sugar beet and papaya, the raw materials, sample preparation and sampling, DNA extraction, qualitative and quantitative testing by the real-time PCR method with the real-time PCR equipment are detailed. The raw materials analyzed were products of plant origin, which represented 23 samples (6 samples of papaya fruit, seeds, dried and frozen candied cubes and 17 sugar beet samples from different geographical areas of the country (Mureș - Cereal Land, Mureș – Luduș, Sibiu – Aflat, Bistrița Năsăud – Lechința, Cluj – Baba Ioana, Alba – Ariesul, Alba – Sălișteа, Covasna – Târgul Secuiesc – Cultiliș, Covasna – Angheluș, Botoșani – Unteni, Brașov – Tarlungeni, Brașov – Feldioara Suceava, Iași , Neamt, Brăila)). All samples were used for genomic DNA extraction and real-time PCR procedures were applied according to the manufacturers' procedures. Nucleic acid extraction kit: GeneSpin for 50 DNA Extraction (PCR GRADE). Kit for amplification: DIATHEVA-MBK0013, EUROGENTEC primers and probes. All the examined samples had a negative result for GMO testing, which leads to the conclusion that non-genetically modified varieties are present on the national market. The adaptation of the work method requires, for the samples of sugar beet and papaya seeds, during the preparation stage of the samples, more time to be moistened to reduce the risk of heating, prevent the activation of DNases and facilitate the extraction process. The extraction method used is effective for sugar beet and papaya matrices, following spectrophotometric quantification, confirmation of the absence of inhibitory substances and evaluation of the state of fragmentation of DNA molecules.

Chapter VII includes a study on the perception and evaluation of the degree of acceptance of Romanian consumers on agro-food products derived from plants obtained from new genomic techniques, compared to foods derived from conventional plants and the availability to purchase and consume them. Between June 2023 and October 2023, a questionnaire was distributed in the online system.

Chapter VIII mentions the current legislative framework regarding genetically modified organisms, at national and European level. There are debates at the European level for the finalization of the draft Regulation regarding plants obtained by targeted mutagenesis and cisgenesis and their products. The European Network of GMO Laboratories (ENGL) in 2023 identified limitations for the development and validation of event-specific detection methods for plants resulting from targeted mutagenesis or cisgenesis. Products with identical DNA sequences cannot be distinguished by analytical methods and specified whether they have occurred naturally, through conventional breeding or through the use of NGT.

At the end of the doctoral thesis, the conclusions of the studies carried out to achieve the proposed objectives and the perspectives for future studies are presented, in the context of the need for new foods and feeds, which contribute to the objectives of the sustainable European agri-food system according to the European Green Deal and related strategies. Innovation and research in the agri-food system and biotech industries would be encouraged if they benefited from a clear legal framework for NGT plants.

Recommendations and news items

For the first time, a consumer perception and acceptance study on the new genomic techniques and the products obtained from them was carried out in Romania.

In the studies that were the subject of this thesis, for the first time the detection of different types of products or raw materials, such as papaya and sugar beet, considered as new matrices for the work methods currently used in molecular biology and GMO laboratories in our country, which carries out the supervision and monitoring of food and feed on the market, accompanied by the adaptation of the detection method for these matrices.

The study elaborated with data on the productions obtained and the commercial balance for the soybean, corn, sugar beet and papaya species, as well as the study on the detection of GMOs in food and feed, both carried out for the period 2017-2020 on the territory of Romania, provides for the first time the complete picture for this field and can be the starting point for further studies. There were no positive results in the 2020 pandemic year, due to the growing responsibility of operators in the food industry and consumer confidence in the products on the market.

The critical analysis of the criteria proposed by EFSA for the assessment of NGT are for the first time in debate at national and European level, as well as a possible regulatory act of targeted mutagenesis and cisgenesis.