GENETICALLY MODIFIED ORGANISMS

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Abstract. *GM technology, enables scientists to insert into a plant's genome a single gene, or a few of them, from another species of plant or even from a bacterium, virus or animal. The kinds of alterations caused by the insertion of genes from other species might be more impactful, more complex or more subtle than those caused by the intraspecies gene swapping of conventional breeding. Alteration of entire packages of genes is a natural process that has been happening in plants for half a billion years and it tends to produce few scary surprises today. Changing a single gene, on the other hand, might turn out to be a more hazardous action, with unexpected effects, including the production of new proteins that might be toxins or allergens. In this review we will discuss about the benefits versus worries of GM foods, validated methods for GMOs detection, also, the authorisation of GMOs in the UE.*

Key words: *GMOs, food safety*

INTRODUCTION

New agricultural technologies are bringing with them structural changes and transitional problems, from their original wild crop cultivars with continous selection and controlled breeding through more productive, pest resistant or a better quality of product than previous ancestral lines. The term of genetically modified organisms (GMOs) has been introduced to describe organisms whose genetic material has been modified in a way that doesn't occur in nature under natural conditions of cross-breeding or natural recombination (PETER R. ET AL. 2011). Applied in crops, the term refers to plants in which a gene or genes from different species have been stably introduced into a host genome using techniques of genetic transfer and where, in most cases, such introduced genes have been shown to produce a gene product. The new genes are translated and the new protein expressed (PETER R. ET AL. 2011). This gives the plant a new characteristic such as resistance to certain insects or tolerance to herbicides.

GMOs when consumed directly or after processing are delivered as genetically modified (GM) food or feed. These foods undergo artificial genetic modification during the phase of raw material production and the most common sources of raw material for GM foods are GM plants (PETER R. ET AL. 2011).

The release of GMOs into the environment and the marketing of GM foods have resulted in a public debate in many parts of the world. This broader debating has raised certain questions, such as whether GM food and feed are safe for human and animal consumption and whether they will have harmful impacts on environment health and biodiversity (ARISTIDIS M. ET. AL. 2017). This debate is likely to continue, probably in the broader context of other uses of biotechnology and their consequences for human societies and clearly need to be addressed by scientific experimentation. In an attempt to minimize such uncertainties, many laws, restrictions, and legislations have emerged, and in most countries legislative procedures for the

approval of any GM crop used for food or feed now exist. (WAIGMANN ET AL., 2012; YAQOOB ET AL., 2016). The use of GMOs, their release into the environment, cultivation, importation and particularly their utilisation as food ingredients, is regulated in the European Union by a set of strict procedures. The first community legal instruments (Council Directive 90/220/EEC and Council Directive 90/219/EEC) were produced in 1990 with the specific scope to protect human and animal health and environment (European Parliament & Council of the European Union, 2001).

ASSOCIATED RISKS

The consequences of cultivating GM plants could have unintended impacts on ecosystem health, such as un-natural gene flow, diminished genetic diversity, effects on non-target species, weediness, reduced pesticide and herbicide efficiency, herbicide and insecticide toxicity, modification of soil and water chemistry and quality and damaging ecosystem complexity by diminishing biodiversity (ARISTIDIS M. ET. AL. 2017). Second, the use of GM plants as human food and animal feed could represent a hazard to health. (FORD ET AL., 2006; HAN ET AL., 2015; YAN ET AL., 2015).

The main concern is the necessity to examine the consequences of transferred gene and the potential toxicity of expressed proteins. However, transfer of gene (nptII) from GM plants to soil bacteria and the detection of *Agrobacterium tumefaciens* genes in sweet potato suggest the interplay of alleles in plants and microorganisms is an established fact and cannot be neglected (KYNDT ET AL., 2015). GM rice, soybean, maize, and wheat, alone or in combination, have been fed to laboratory animal and recorded pathological, hematological, histopathological, serum chemistry, macroscopic, food intake, and reproduction-related characteristics (TYSHKO ET AL., 2014; TYSHKO AND SADYKOVA, 2016).

The first controversy started when, an article published by French molecular biologist Gilles-Eric Seralini, in Food and Chemical Toxicology, reported increased tumor size in rats fed with GM maize and roundup (SERALINI ET.AL., 2012,2013,2014).

A recent report from National Academy of Sciences, USA, (2016) revealed that cultivation of GM crops has had no negative impact on the environment, ecosystems, biodiversity, or health. By growing herbicide- and insect-resistant crops, the amount of pesticide and herbicide has been decreased, whereas yield has been increased.The report further found that statistically significant differences are there between GM and non-GM plants regarding chemical composition and nutrients.

It is also important to bear in mind that humans are exposed to a complex mixture of GM diets rather one single event. Different GM organisms include different genes inserted in different ways. This means that individual GM food and their safety should be assessed on a specific basis and that it isn't possible to make general statements on the safety of all GM foods (FRAITURE M.A., ET. AL. 2017).

VALIDATED METHODS FOR GMOs DETECTION

The basis of every type of GMO detection technology is to exploit the difference between the unmodified variety and the transgenic plant. This can be done by detecting the new transgenic DNA that has been inserted, or the new protein expressed, or if the protein acts as an enzyme by using chemical analysis to detect the product of the enzymatic reaction (PETER R. ET AL. 2011).

The first method validated at the EU level was for a standard PCR-based screening method able to detect most of the GMOs presently approved for marketing (LIPP ET. AL , 1999). This method, developed by PIETSCH ET AL. (1997), is based on the detection of the control

sequences flanking the newly introduced gene, namely the *35S promoter* and the *Nos terminator*. It can be extremely sensitive, capable of detecting one or a few copies of a gene or target sequence of interest within an entire organism's genetic material or genome. The need of quantifying the amount of GMO present in a sample led to the development of many PCR-baded protocols, which allow not only a qualitative answer about presence or absence of transgenic line, but also a more precise indication of the relative quantity of GMO present in a given sample (ROSA S. ET. AL. 2016). The two most competitive PCR-based approaces are real-time PCR and digital PCR.

The real-time PCR system monitor the reaction as it actually occurs in real time by detecting the PCR products as they accumulate. In this kind of system the PCR reaction is coupled to the emission of a fluorescent signal being proportional to the amount of PCR product produced in subsequent cycles. This signal increases proportiolally to the amount of PCR product generated in each successive reaction cycle. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during its exponential phase. The first significant increase of fluorescence correlates to the initial amount of target template. (AHMED F.E., 2002, ROSA S. ET. AL. 2016). However, real time PCR has a number of technical limitations including the need for assay calibration with standards that are similar in quality to the samples being evaluated. This can lead to an iterative workflow process and challenges to provide qualified standards for comparison.

Digital PCR (dPCR) is a novel method for precise quantification of nucleic acids. It uses similar assay reagents as used in standard analog measurements, but counts the total number of individual target molecules in a digital format, enabling many applications that require high sensitivity and have restricted sample availability (ROSA S. ET. AL. 2016).

Digital PCR measurements are performed by dividing the sample into a very large number of separate small volume reactions, such that there is either zero or one target molecule present in any individual reaction (POHL ET. AL. 2004, DUBE ET.AL. 2008). This is the fundamental concept for making *digital* measurements. Any target-containing compartments will become brightly fluorescent while compartments without targets will have only background fluorescence. Digital PCR platforms, which divide the sample into a larger number of compartments, will have the highest accuracy, by directly counting single molecules (WHALE A.S. ET AL. 2012, ROSA S. ET. AL. 2016).

A new method for monitoring GMOs on the market uses the Next-generation Sequencing (NGS) technology for massively parallel DNA sequencing of multiple samples, which are differentiable during the subsequent bioinformatics analysis on the basis of unique barcodes that are added to each sample during the library preparation step (BUERMANS, H.P.J. ET. AL. 2014, FRAITURE M.A., ET. AL. 2017).

LEGISLATIVE PROCEDURES

The International Service for the Acquisition of Agri-Biotech Applications (ISAAA) reported a record of 181.5 million hectares of biotech crops grown in 2014 in a total of 28 countries (JAMES, 2014).

The European Union established a strict regulatory framework to trace GMOs and derived products undergo an authorisation process that aims to guarantee safety for human, animal and environmental health. As part of this regulatory framework, a mandatory labelling of any GMO-derived or GMO-containing food or feed has been introduced, intending to ensure consumers' freedom of choice (European Parliament & Council of the European Union, 2001).

In addition, a ''Minimum Required Performance Limit" of 0.1% was established for feed containing GMOs already approved elsewhere and for which an application for

authorisation in the EU had been requested (European Commission, 2011). Based on these regulations, EU control laboratories must be able to detect low amounts of GM materials, evaluate their authorisation status and, when appropriate, quantify the GM content to check the compliance with legal provisions. (European Commission, 2003).

CONCLUSIONS

The applications of more precise, and well-regulated technologies, such as CRISPR (clustered regularly interspaced short palindromic repeats), CRISPR-associated (Cas) genes, and new breeding technologies, will increase in usage as these technologies come under appropriate legislation. Regarding safety assessment and health hazards, remain concerns about long-term usage of GM food and feed.The specific issue of labelling of GM food has been addressed by several legal instruments in order to ensure that the final consumer was informed of any change in the characteristic or food property (European Parliament & Council of the European Union, 2004). Labeling should be mandatory and should be considered as a basic consumer right. The next generation of GM foods will be crops with improved nutritional value to functional foods and nutraceuticals and evaluations will incresingly have to consider the impact of next generation GM on food safety assessment strategies.

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