Scientific Bulletin. Series F. Biotechnologies, Vol. XXIV, No. 1, 2020
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

# COMPARATIVE STUDY BETWEEN GENETICALLY MODIFIED PRODUCTS OBTAINED BY CONVENTIONAL TRANSGENESIS AND BY NEW TECHNIQUES OF TARGETED MUTAGENESIS

**Luminiţa Raluca SIMIONESCU, Narcisa BĂBEANU, Călina Petruţa CORNEA**

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd., District 1, Bucharest, Romania

Corresponding author email: mihalachioiu.raluca@ansvsa.ro

***Abstract***

*This paper presents a comparative study reguarding similarities and differences between the genetically modified products (food raw materials of vegetable origin) obtained by conventional methods of transgenesis and the product obtained by new techniques of targeted mutagenesis, like CRISPR-Cas9 method. In this article we will present briefly through explanatory drawings the genome of plants obtained by conventional random mutagenesis and targeted mutagenesis CRISPR-Cas9, mutagenesis in EU GMO legislation, the objectives of innovation and multiplication of specific plants which can contribute to a sustainable agriculture and increased food production. The products resulted from small editions, which could also have appeared spontaneously in cultures. In conclusion, CRISPR-Cas9 is the principal used technology for genome editing for simplicity and efficiency. We try to highlight the application and benefits of CRISPR-Cas9 method like a tool genome editing for agriculture and food industry.*

***Key words****: CRISPR-Cas9, genome editing, GMO, mutagenesis.*

## INTRODUCTION

**The product** genetically modified organism (GMO) is an organism, in which the genetic material has been modified in a way that does not occur naturally the recombination, by mating.

**The process** within this definition:

1. Methods for nucleic acid recombination with final scope to obtain a new genetic material by inserting nucleic acid molecules, belonging to an organism (virus, bacterial plasmids or other vectors) and incorporating them into a host organism, in which they are capable of multiple;
2. Techniques by direct introduction of a piece by nucleic acid in an organism, of the genetic material which does not belong the organism, like microinjection, macroinjection and microencapsulation;
3. Cell fusion (protoplast), hybridization methods in which living cells have new combinations of hereditary genetic material formed by the fusion cells through naturally occurring processes.

Conjugation, transduction, transformation, polyploid induction, mutagenesis (irradiation, chemicals - alkylating agents), cell fusion (protoplast) of vegetal origin cells from plants that can change genetic material by conventional methods are not considered genetic modification in light of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (https://eur-lex.europa.eu/legal-content/EN/TXT/ ? qid = 1579766311266 & uri = CELEX: 32001L0018).

**The product** obtained by new genetic engineering techniques: oligonucleotide targeted mutagenesis, zinc finger nucleoside, transcriptional effector nucleases, repeated groups with short intermediate palindromia that were associated with the double-stranded DNA binding protein Cas9, cisgenesis, intragenesis, grafting, agro-infiltration, RNA dependent DNA methylation and reverse multiplication.

**The process** makes specific changes in the DNA of the plants to modify the traits, these changes can be from the modification of a single base, to the insertion or deletion of one or more genes.

The alterations of the DNA sequence produced by genome editing methods are not identified for the changes of the DNA sequence obtained naturally or by conventional mutagenesis. The genome editing could be possible used to change more than two bases into single-site DNA. This are less likely to be a natural or mutagenic process (Jones et al., 2018).

The method of genomic editing system is very used to intermediate short-acting palindromic

groups associated with the binding protein of Cas9 into the specific DNA sequences (CRISPR-Cas9).
The CRISPR-Cas9 technique allows the vegetal origin genome to be precisely modified by removing undesiderated genes or indicating the specific genes can get new functions (Wolt et al., 2016).
This new products obtained by the methods of targeted mutagenesis, like CRISPR-Cas9 system are very similar with the naturally occurring variations.

## MATERIALS AND METHODS

The research methodology used into the paper has the following aspects:
• Bibliographic study by the national and international literature;
• Collecting the information within the researched specific area;
• Order, process and present of the results in a synthetic form;
• Analysis and interpretation of the results, elaboration of conclusions and recommendations.

## RESULTS AND DISCUSSIONS

These new targeted mutagenesis techniques are much faster and cheaper than conventional breeding techniques. There are already several products of vegetable origin obtained by the new techniques, which are near or in the testing phase in crop or marketing.
The main differences between the food products consist in the mechanism of inducing the break on DNA sequence and their efficiency in targeting the desired sequences. Conventional mutagenesis mechanisms produce multiple local mutations of the genome, while CRISPR-Cas9 (targeted mutagenesis) method yields nucleotide point mutations.
Editing the genome through the CRISPR-Cas9 method allows the application of new genetic engineering techniques, several DNA sequences can be targeted at the same time. Are obtained the specific products much faster and at lower costs than conventional methods and is easy to apply to plants. According to the literature, CRISPRs are prokaryotic DNA segments containing short, repetitive base sequences. In a palindromic repeat, the nucleotide sequence is the same in both directions. Each repeat is followed by short segments of the distal DNA from previous exposures to foreign DNA (virus or plasmid).
Which is different from what we normally consider a GMO (Figure 1): conventional mutagenesis products obtained by natural randomisation, targeted mutagenesis products obtained by genome editing method like the CRISPR-Cas9 method and conventional transgenesis GMO obtained with techniques for nucleic acid recombination (Custers R., Flemish Institute for Biotechnology, 2019).

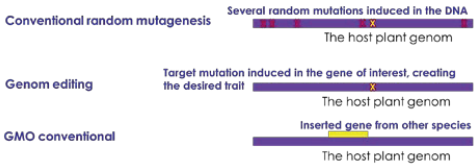


Figure 1. The host plant genome by different methods of mutagenesis and transgenesis
Source: Custers R., 2019

From the Figure 1 graphical representation of the host genome is easy to observe the important changes in the genome of plants in the case of conventional mutagenesis and the transfer of genes of interest by well-known methods of nucleic acid recombination. By comparison, the new methods of targeted mutagenesis and plant genome editing, induce minor modifications, by several nucleotides.
The predictability of phenotypic manifestations is well determined in the products obtained by targeted mutagenesis, because the modifications are minor at the genome level, compared to the conventional methods of obtaining genetically modified plants. Several random mutations are induced in the nucleic acid of the host plant genome by the conventional random mutagenesis method (Figure 2).

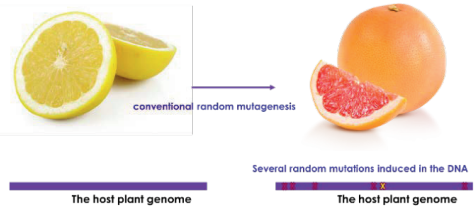


Figure 2. Representation of the conventional random mutagenesis plant genome
Source: Custers R., 2019

Target mutation induced in the gene of interest using methods of genetic engineering like CRISPR-Cas9 create the plants and vegetable food products with precise features, previously desired (Figure 3).

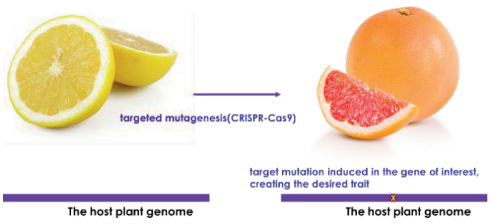


Figure 3. Representation of the targeted mutagenesis plant genome by CRISPR-Cas9
Source: Custers R., 2019

The objectives of the innovation of the products obtained by the targeted mutagenesis and the multiplication of the specific plants, according to the specialized literature are:
- increased production and poverty reduction in areas with salted or dry soils,
- high quality nutritional foods,
- reduced pressure on the soil through less work (fertilizers, pesticides),
- soil bioremediation.

The conventional random mutagenesis and targeted mutagenesis are very different at the genome level, even if phenotypically we identify the same gene expression (Figure 4).
Targeted mutagenesis, having a precise and unique genomic position, as opposed to naturally occurring and then randomized mutagenesis, where other undesirable changes occur, there will be no possibility for future undesirable characters to manifest in future generations of plants or vegetable food.

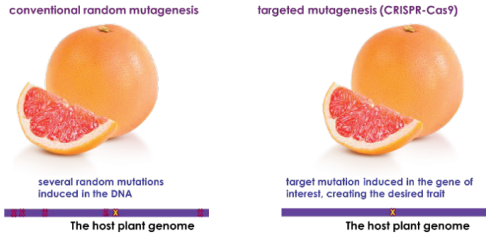


Figure 4. Representation of the comparation of conventional random and targeted mutagenesis
Source: Custers R., 2019

Changing the DNA sequence obtained by genomic editing methods cannot currently be identified by methods known by the laboratory, as compared to changing the DNA sequence obtained by natural processes or conventional mutagenesis. When the method of genome editing is used to introduce more than two base pairs into the single-stranded DNA strand, these being less probable to be natural or mutagenic, may be an exception (Jones et al., 2018).
If not held information about the changes introduced at this moment is impossible to detect these changes. Detection might be possible if there was a reference genome for comparison (Lusser et al., 2011).
On 25 July 2018, the European Court of Justice ruled that organisms obtained by mutagenesis must be considered to be GMOs, exception could be only the organisms obtained by conventional mutagenesis, which have a long safety history. The judgment of the European Court of Justice notes that the organisms obtained by the new genome editing techniques (CRISPR-Cas9 methods) are GMOs by Directive 2001/18/EC. The directive requires for this organism produced by genome editing to be developed specific detection methods within the European national reference laboratories for GMOs.
In accordance with the new legislation mentioned above, products of vegetable origin genetically modified by targeted mutagenesis methods are subject of specific market authorization legislation for genetically modified organisms, as opposed to products obtained by conventional mutagenesis by natural selection, chemical or irradiation methods (Figures 5 and 6).


Figure 5. The conventional mutations products are not subject to GMO specific legislation
Source: www.vib.be


Figure 6. The genome editing products are subject to GMO specific legislation
Source: www.vib.be

EURL - GMFF has developed a report for detection issues and the possible ways to detect these products (Jones et al., 2018).
Creating a database for genomic comparisons would be a huge economic effort for European Union. This was one of the proposals, but on the European Union market are registered 14,442 varieties of bread wheat, Durham wheat, corn, soybeans, barley, Swedish rapeseed, rapeseed and potatoes, according to the European Commission's plant variety database. According to Wikipedia, there are 7,500 varieties of apples and 10,000 varieties of tomatoes.
This would be very costly, impossible to implement and would provide relatively weak evidence. (Jones et al., 2018).

## CONCLUSIONS

Plants with the same modification, obtained by targeted or natural mutagenesis, cannot be precisely identified.
Genome editing is at least as safe as conventional mutagenesis. CRISPR-Cas9 is a tool that can help achieve the objectives in a better oriented and faster way.
CRISPR-Cas9 system, known as genome editing is the most simplest and efficient technique for crop development.
The conventional random mutagenesis and targeted mutagenesis are very different at the genome level, even if phenotypically we identify the same gene expression:
- Several random mutations are induced in the host nucleic acid of the plant genome by the conventional random mutagenesis method;
- Target mutation induced in the gene of interest using methods of genetic engineering like CRISPR-Cas9 create the plants and vegetable food products with precise features, previously desired.
From the studies done so far, it appears that the CRISPR-Cas9 method for obtaining vegetable products is going to change the course of the agricultural and food industry.

## REFERENCES

Custers, R. (2019). NBTs and the ECJ Ruling C-528/16, Flemish Institute for Biotechnology
Directive 2001/18/EC of the EuropeanParliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Off.J.Eur. Comm. L106:1-38.
European Court of Justice, C-528/16 - Judgement of 25 July 2018. See: http://curia.europa.eu/juris/document/document.jsf?docid=204387&mode=req&pageIndex=1&dir=&occ=first&part=1&text=&doclang=EN&cid=515140.
Jones, E., Bell, R., Bryce, S., Adams I. (2018). Technical notes on the 'detectability problem' of GE, Fera Science.
Lusser, M., Parisi, C., Plan, D., Rodríguez-Cerezo, E. (2011). New plant breeding techniques. State-of-the-art and prospects for commercial development. Luxembourg, Publications Off. Eur. Union, 184 p. (https://publications.europa.eu/en/publication-detail/-/publication/12988d6d-c6a4-41b2-8dbd-760eeac044a7/language-en).
Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Off. J. Eur. Union L268:1-23.
Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. Off.J.Eur.Union L268:24-28
Wolt, J.D., Wang, K., Yang, B. (2015). The Regulatory Status of Genome-edited Crops. 2016; 4, 510-518. DOI: 10.1111/pbi.12444
www.vib.be, January 2020
www.eur-lex.europa.eu